PhD Thesis

Epidemiological relationships between physical activity, fitness and adiposity with cardiometabolic risk factors in youth

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Preface

This thesis is based on work conducted at the Research Unit for Exercise Epidemiology and Centre for Research in Childhood Health at the Department of Sports Science and Clinical Biomechanics, University of Southern Denmark and at the Medical Research Council Epidemiology Unit, University of Cambridge from August 2014 to November 2017.

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Work included in this thesis was conducted under the supervision of Associate Professor, PhD Niels Christian Møller and Assistant Professor, PhD Anna Bugge.

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The work considered in the thesis is based on data presented in the following original manuscripts

Study 1:

Tarp J, Brønd JC, Andersen LB, Møller NC, Froberg K, Grøntved A. **Physical activity, Sedentary behavior, And long-term cardiovascular risk in young people: A review and discussion of methodology in prospective studies.** Journal of Sport and Health Science 2016; 5(2): 145-150.

Study 2:

Tarp J, Bugge A, Andersen LB, Sardinha LB, Ekelund U, Brage S et al. **Does adiposity mediate the relationship between physical activity and biological risk factors in youth?: a cross-sectional study from the International Children's Accelerometry Database (ICAD).** International Journal of Obesity (Lond) 2017 Oct 3. doi: 10.1038/ijo.2017.241. [Epub ahead of print]

Study 3:

Tarp J & Brage S. **Physical activity intensity, bout-duration and cardiometabolic risk markers in children and adolescents.** Revised version in submission *International Journal of Obesity*

Study 4:

Tarp J, Grøntved A, Møller NC, Klakk H, Rexen CT, Bugge A, Wedderkopp N. **Muscle-fitness changes during childhood associates with improvements in cardiometabolic risk factors: A prospective study.** In submission *Journal of Physical activity and Health.*

Study 5:

Tarp J, Jespersen E, Møller NC, Klakk H, Wessner B, Wedderkopp N, Bugge A. **Long-term follow-up on biological risk factors, adiposity, and cardiorespiratory fitness development in a physical education intervention: a natural experiment (CHAMPS-study DK)**. Accepted for publication *BMC Public Health*.

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Dansk Resumé

Introduktion: Grundlaget for en fysisk aktiv tilværelse gennem hele livet starter i barndommen. En fysisk aktiv tilværelse er nødvendigt for at bevare en fordelagtig kardiometabolisk profil. Flere detaljer i sammenhængen mellem fysisk aktivitet og indikatorer for fedme, samt i sammenhængen mellem fysisk aktivitet og biologiske risikofaktorer (glykæmisk kontrol, lipider og blodtryk) er ikke tilstrækkeligt forstået og beskrevet. En større forståelse af disse detaljer kan være relevant for primær forebyggelse på populationsniveau samt for målrettet intervention. Potentialet for forebyggende populationsgevinster ved skolebaserede fysisk aktivitetsindsatser er ukendt.

Metode: Undersøgelserne inkluderede deltagere i alderen 4 – 18 år. Prospektive studier der undersøger sammenhængen mellem fysisk aktivitet eller stillesiddende adfærd med kardiometaboliske risikofaktorer blev identificeret og samlet i et narrativt review. Fra International Children's Accelerometry Database (ICAD) blev der anvendt data til 2 tværsnitsundersøgelser. Disse undersøgte kilder til variation i sammenhængen mellem fysisk aktivitet og den kardiometaboliske profil ved at fokusere på; 1) hvor meget af sammenhængen mellem fysisk aktivitet og den metaboliske profil der kunne forklares ved sammenhængen mellem fysisk aktivitet og en lavere taljeomkreds (n=3412), og 2) betydningen af intensitet og udførelse af aktivitet i kortvarige eller længerevarende perioder samt kombinationer af disse (n=4338 til 29 734). To studier var baseret på data fra CHAMPS-study DK som inkluderede børn i alderen 6-11 år. En af disse undersøgte langtidsforskelle (6,5 år) i den kardiometaboliske profil mellem børn der gik på interventionsskoler (n=217) og børn på kontrolskoler (n=95). Interventionen bestod af en tredobling af idrætsundervisningen fra børnehaveklasse til 6. klasse. Det andet CHAMPS-study DK manuskript kiggede på sammenhængen mellem ændringer i muskel-fitness og den kardiometaboliske profil over 2 år (n=512). Lineære regressions-modeller med kontrol for tilgængelige demografiske, biologiske og genetisk betingede formodede confounder-variable blev anvendt i alle originale datasæt. En samlet risiko-score bestående af de biologiske variable og en fedme-markør blev beregnet som indikator for den underliggende metaboliske risiko-profil. En lavere risiko-score indikeret en mere fordelagtig profil.

Resultater: Det narrative review fandt prospektive sammenhænge mellem fysisk aktivitet og mindst én biologisk risikofaktor i 10 af 13 identificerede studier. Ligeledes blev der fundet en sammenhæng mellem fysisk aktivitet og en fedme-indikator i 10 af 15 studier. Sammenhængen mellem fysisk aktivitet og fedme-markørerne var særligt stærk for aktivitet af mindst moderat intensitet og i studier med en direkte måling af kropskomposition. Modsatrettede resultater blev også identificeret. Sammenhængene var mindre tydelige for stillesiddende adfærd. I data fra ICAD blev det estimeret at opnåelse af WHO's aktivitetsanbefalinger på 60 daglige minutter af minimum moderat intensitet var forbundet med en mere fordelagtig risikoprofil (-0,31 (95% CI: -0,39 to -0,22) standardafgivelser), hvoraf blot 22 % af denne sammenhæng kunne tilskrives sammenhængen mellem fysisk aktivitet og taljeomkreds. I den anden ICAD undersøgelse var hver 1000 counts/min øgning af intensitetstærskelværdien (indenfor 500 til 3000 counts/min) forbundet med en -0,026 (95% CI: -0,039 to -0,014) standardafvigelse lavere risiko-score. Der blev ikke fundet indikationer

på at varigheden af hver enkelt aktivitetsperiode var af betydning efter forskelle i total fysisk aktivitet var håndteret. I CHAMPS-study DK var den prospektive sammenhæng mellem ændringer i muskel-fitness og risikoprofilen forklaret af sammenhængen mellem muskel-fitness og kardiorespiratorisk fitness blandt drenge (std. β = -0,03 (95% CI: -0,19 to 0,14)), men ikke blandt piger (std. β = -0,20 (95% CI: -0,39 to -0,03)). Den observerede sammenhæng blandt piger var ikke bevaret efter justering for ændringer i taljeomkreds. Større fremgang i muskel-fitness var forbundet med mindre øgning af taljeomkredsen blandt drenge (std. β = -0,23 (95% CI: -0,32 to -0,13)) og piger (-0,21 (95% CI: -0,37 to -0,05)). Deltagelse i interventionen i CHAMPS-study DK var ikke forbundet med statistisk signifikante forskelle i risiko-scoren (std. β = -0,07 (95% CI: -0,32 to 0,18)) i forhold til kontrolgruppen. På opfølgningstidspunktet var der ingen forskel i det fysiske aktivitetsniveau mellem interventions- og kontrolskoler (p-værdier ≥0,13).

Konklusion: Børn og unge der er mere fysisk aktive har en mere fordelagtig kardiometabolisk risikoprofiler, men aktivitet af højere intensitet er forbundet med yderligere fordele. Derimod har varigheden af hver enkelt aktivitets-periode ikke umiddelbart betydning ved hverken lave eller høje intensiteter. Aktiviteter der styrker den muskulære fitness bør promoveres som supplement til aerobe aktiviteter for at opnå yderligere forbedring af den kardiometaboliske profil. En femtedel af sammenhængen mellem opnåelse af WHO's aktivitetsanbefalinger var forklaret ved bidraget til regulering af energibalance. Dette antyder at øget deltagelse i fysisk aktivitet vil medføre forbedret glykæmisk-, lipid- og blodtryksregulering uanset eventuelt vægttab. Nuværende skolebaserede fysisk aktivitetsinterventioner målrettet idrætsundervisningen ser ikke ud til at medføre langtidsholdbare forbedringer af folkesundheden. Tiltag mod sikring af vedvarende deltagelse i fysisk aktivitet efter interventionsindsatsen er ophørt ser ud til at være påkrævet. Resultaterne i denne afhandling bør fortolkes set i lyset af inddragelse af tværsnitsundersøgelser, muligheden for ufuldstændig håndtering af confounding, samt det høje frafald i evalueringen af den skolebaserede intervention.

English Summary

Introduction: The promotion of lifelong engagement in physical activity and optimal management of established cardiometabolic risk markers starts in youth. Parts of the physical activity-adiposity and physical activity-biological risk marker (glycaemic control, lipids, and blood pressure) associations are inadequately detailed. Elucidation of these details may be useful for population-wide and targeted intervention. The long-term benefits of school-based physical education intervention are unknown.

Methods: Studies were restricted to young people 4 - 18 years of age. A review considering prospective studies investigating the association between physical activity or sedentary time with cardiometabolic risk factors was performed. Results were presented in a narrative format. The International Children's Accelerometry Database (ICAD) provided data for 2 cross-sectional studies. These studies explored mechanisms of associations between physical activity and cardiometabolic risk factors focusing on; 1) the role of physical activity in maintaining favourable energy balance using waist-circumference as a marker of excess adipose tissue accumulation (n=3412), and 2) patterns of intensity, bout-duration, and their combinations (n=4338 to 29 734). Two studies were based on data from the CHAMPS-study DK (children 6-11 years old at baseline). One of these evaluated long-term (6.5 years) differences in cardiometabolic risk markers between children attending intervention schools (n=217) as compared to children attending control schools (n=95). The intervention provided a trebling of curricular physical education through kindergarten to grade 6. The second CHAMPS-study DK manuscript evaluated prospective associations between changes in muscular fitness and cardiometabolic risk markers over 2 years (n=512). Linear regression modelling with control for available demographic, biological, and genetically determined putative confounding variables was applied in all original datasets. Composite risk scores including biological risk factors and adiposity were calculated as outcomes to maximize information on the latent cardiometabolic profile. A lower risk-score indicates a more favourable profile.

Results: The review identified prospective associations between physical activity and a biological risk marker in 10 of 13 studies and between physical activity and an index of adiposity in 10 of 15 studies. The association between physical activity and adiposity was particularly strong for moderate-to-vigorous physical activity and in studies using direct assessment of adiposity, however conflicting results were also observed. Sedentary time was less clearly associated with the outcomes. In the ICAD, adherence to the WHO physical activity guideline of 60 daily minutes of MVPA was associated with a favourable metabolic profile (-0.31 (95% CI: -0.39 to -0.22) standard deviation lower composite risk score) of which 22 % of the association was explained by the association between physical activity and waist-circumference. In the second ICAD study, each 1000 counts/min increase in intensity cut-point (range 500 to 3000 counts/min) was associated with a -0.026 (95% CI: -0.039 to -0.014) standard deviation lower composite risk score. Accumulating physical activity in longer bout-durations at either low or high intensities did not add additional benefits when adjusted for total physical activity. In the CHAMPS-study DK, the prospective association between muscular-fitness changes and cardiometabolic risk factors was explained by

cardiorespiratory fitness in boys (std. β =-0.03 (95% CI: -0.19 to 0.14)), but not in girls (std. β = -0.20 (95% CI: -0.39 to -0.03)). The association in girls did not persist after controlling for changes in waist-circumference. Larger muscular fitness changes were highly associated with lower gains in waist-circumference in boys (std. β = -0.23 (95% CI: -0.32 to -0.13)) and in girls (std. β = -0.21 (95% CI: -0.37 to -0.05)) after controlling for cardiorespiratory fitness. The CHAMPS-study DK intervention was not associated with statistically significant differences in composite risk score (std. β = -0.07 (95% CI: -0.32 to 0.18)) as compared to control. There were no statistically significant differences in physical activity levels between intervention and control at follow-up (p-values ≥0.13).

Conclusions: Higher physical activity levels, especially when performed at relatively higher intensities, are associated with favourable cardiometabolic risk marker levels. Bout-duration did not appear of importance suggesting any accumulation pattern provides benefit. Engagement in activity of sufficient intensity and frequency to elicit muscle-fitness adaptations is important to obtain maximal cardiometabolic benefit. One fifth of the association between meeting the 60 minutes MVPA/day guideline and the composite risk score was explained by the role of physical activity in regulation of energy balance suggesting increased physical activity levels will result in beneficial metabolic control irrespective of any concomitant weight-loss. Physical education interventions in public schools may not produce sustainable population health benefits in isolation or under current intervention models. Other initiates to facilitate continuous engagement in physical activity after intervention discontinuation may be needed. The results of this thesis should be interpreted in the light of the cross-sectional nature of some of the studies, the possibility of residual and unmeasured confounding, and the high attrition in the evaluation of the school-based intervention.

Thesis at a glance

Study	Study design	Sample and Exposure	Methods	Aim	Conclusion
1	Literature review	Prospective studies with ≥2 years follow-up in young people, objective PA or SED and a cardiometabolic risk factor as outcome	Search in PubMed with qualitative synthesis of retrieved studies	Qualitative synthesis of evidence for a link between PA and SED with adiposity and biological risk factors over time	Ten of 15 studies supported an association between PA and adiposity. Ten of 13 studies supported an association between PA and a biological risk factor. There was little evidence supporting the role of SED.
2	Cross-sectional	Six ICAD studies from Denmark, Portugal, Estonia, and the U.S. (n=3412). Participants mean (SD) age was 12.1 (3.4) years Accelerometry assessed PA dichotomized into meeting or not meeting PA guidelines	Composite and single biological risk factors as outcome 2-stage regression analysis allowing for exposure-mediator interaction Controlled for available co-variables and estimates aggregated by meta-analysis	Decompose total effect of meeting PA guidelines on the risk score into a direct and an indirect association, using waist-circumference as the mediator	The total effect was a -0.31 (-0.39, -0.23) SD lower risk score. The direct was -0.24 (-0.32, -0.16) SD, and the indirect effect was -0.07 (-0.11, -0.02) SD, suggesting 22 % of the association was attributable to the indirect effect
3	Cross-sectional	Twenty ICAD studies (n=38 306 observations). Mean (SD) age was 11.7 (2.7) years Accelerometry assessed PA summarized as 16 bout/intensity combinations (≥500cpm to ≥3000cpm and ≥1 to ≥10 minutes)	Composite and single cardiometabolic risk factors as outcome. Mixed linear regression models controlling for age, sex, wear-time and study Isotemporal substitution of short to longer bouts	Investigate the role of physical activity intensity, bout-duration, and their combinations, in modulating cardiometabolic risk factors	Physical activity intensity, but not bout-duration appeared the major source of variation in the risk factors. Isotemporal substitution models produced an irregular pattern of association

Study	Study design	Sample and Exposure	Methods	Aim	Conclusion
4	Prospective	Children (n=512) from the CHAMPS-study DK. Mean (SD) age 8.4 (1.4) years followed for 2 years Muscular strength, power, agility, and a composite index of these	Composite and single cardiometabolic risk factors as outcome Sequential mixed linear regression models controlling for age, sex, sexual maturity, intervention status, demographic factors, cardiorespiratory fitness, and waist-circumference	Analyse associations between changes in single- and composite muscle-fitness indices and the risk score	Associations between single-and composite muscle-fitness with the risk factors were largely explained by cardiorespiratory fitness and waist-circumference. Associations more pronounced in girls than in boys
5	Controlled intervention (natural experiment)	Children from the CHAMPS-study DK (n=312) with a mean (SD) age of 7.8 (1.3) at baseline followed for 6.5 years 270 (intervention) vs. 90 (control) weekly minutes of physical education	Composite and single cardiometabolic risk factors as outcome Mixed linear regression models controlling for age, sex, sexual maturity, birthweight and demographic factors	Analyse differences in risk score between children attending intervention and control schools	No statistically significant differences between intervention and control schools observed. Direction of association favoured intervention

PA; physical activity, SED; sedentary time, ICAD; International Children's Accelerometry Database, CHAMPS-study DK; Childhood Health And Motor Performance School study Denmark, SD; standard deviation, MICE; multiple imputation by chained equations, Counts/min; cpm.

Abbreviations

BMI; body-mass index

CHAMPS-study DK; Childhood Health And Motor Performance School Study Denmark

CI; confidence interval

CVD; cardiovascular disease

DXA; Dual-energy X-ray absorptiometry

EYHS; European Youth Heart Study

NHANES; National Health and Nutrition Examination Survey

HOMA-IR; homeostasis model assessment of insulin resistance

ICAD; International Children´s Accelerometry Database

IOTF; International Obesity Task Force

MetS; metabolic syndrome

MICE; multiple imputation by chained equations

MVPA; moderate-to-vigorous physical activity

NCD; non-communicable disease

WHO; World Health Organization

Introduction

The Global Burden of Disease Study estimated non-communicable diseases (NCDs) such as cardiovascular disease (CVD), type 2 diabetes, cancer, chronic respiratory diseases and neurological disorders caused 72.3 % of global deaths in 2016[1]. CVD alone accounted for 19.6 million or 32 % of all deaths, and thus remain the largest preventable cause of premature mortality. Further, on a global scale an estimated 422 million individuals lived with diabetes in 2014 (90-95 % type 2[2]). This is an estimated increase of 391 % since 1980 and albeit this development appears to have been slowed in some high-income countries, on a global scale there is little support for a reversal or even plateauing of the trajectory[3]. In highly obesity-affected countries such as the U.S. and the U.K, 1 in 10 and 1 in 14 individuals have diabetes[2, 4] with ≈ 24 % of these cases being undiagnosed. However, about 40 % of global diabetics reside in China and India[3] underlining diabetes as a disease affecting developing as well as developed countries. Further, global trends in blood pressure and obesity mirrors diabetes trends with a decline or plateau in high-income countries, while continuing to rise in developing and low-income countries[5, 6]. Despite an aging global population and substantial demographic transition in many countries, global progress to reduce the burden of CVD has been achieved as the number of age-standardized disability-adjusted life-years lost to CVD decreased by 29 % from 1990 to 2016. However, the loss of healthy years of life from diabetes has increased by 15 % over the same period[7]. Type 2 diabetes is typically diagnosed from the 5th decade of life[4] and requires continuous self-monitoring to avoid complications such as CVD, renal insufficiency, blindness, motor neuropathy, and lower extremity amputations[8]. In Denmark, a high-income country with a (in an international context) low prevalence of obesity, the costs of diabetes were estimated as 4.27 billion EUR in 2011[9]. An economic burden of this extent will be unbearable for many countries. Declining age-adjusted death rates from CVD are partly attributable to a combination of successful risk factor management such as reducing smoking, lower population

levels of blood cholesterol[10], blood pressure[5], and improvements in medical treatment. The simultaneously increase in age-standardized type 2 diabetes rates is to some extent explained by increased awareness but shifts in population risk-behaviours over time are also important. The risk of type 2 diabetes in obese vs normal-weight adults is increased by 719 %[11], while the same contrast for coronary heart disease produces a 60 % risk-increase[12]. Of note, the strength of the obesity-diabetes association is not far from the 9-fold increased risk of lung-cancer with smoking[13]. During the last 40 years the proportion of total energy intake from dietary fat has declined and largely been replaced by a higher intake of carbohydrates[14]. This change is most likely neutral for CVD (potentially detrimental if carbohydrates replaces polyunsaturated fatty acids), but high intake of particularly refined and added sugars increases the risk of weight-gain and type 2 diabetes[14, 15]. Concomitant with changes in dietary quality, shifts in the population distribution of physical activity[16] favour an obesogenic environment[17]. One in 10 men and 1 in 7 women in the world are now obese[6].

The failing global efforts towards reducing the NCD burden has prompted the World Health Organization (WHO) to re-launch their global action plan on physical activity and calling for "comprehensive and multisectoral approaches"[18]. The evidence base to support this call is strong as meta-analysis of observational studies show higher levels of physical activity, cardiorespiratory- and muscle-fitness are, in a relative well-described dose-response manner, strongly protective of CVD[19-21], type 2 diabetes[20, 22, 23], and some forms of cancer[20, 24]. Further, life-style intervention including nutritional counselling, physical activity and weight-loss reduces the risk of progression from pre-diabetes to diabetes[25] and substantially lowers the need for pharmacological regulation of blood glucose in type 2 diabetics[26]. Indeed, conservative estimates suggest physical inactivity accounts for 6 – 10 % of global deaths from coronary heart disease, type 2 diabetes, breast cancer, and colon cancer[27]. Despite some individuals being genetically more susceptible to CVD or type 2

diabetes[28], overt disease represents years of accumulated risk-exposure which is reflected by elevated levels of marker of disease risk (cardiometabolic risk factors) during a substantial subclinical phase. Management of cardiometabolic risk factors to within recommended levels will reduce the risk of progression to disease[29]. Key modifiable variables for risk-stratification are the biological risk factors; triacylglycerol, cholesterol-fractions, and blood pressure[29], glycaemic control, and excess adipose tissue accumulation[30]. Together, these five risk-markers constitute the metabolic syndrome (MetS)[30]. A meta-analysis of 160 randomized controlled studies, demonstrated that exercise favourably modulates the cardiometabolic risk factors in healthy adults[31]. Further, physical activity is the largest modifiable component of total energy expenditure, making it relevant for the prevention of excessive weight-gain[32]. It is thus imperative to identify and promote physical activity behaviours and initiatives in order to improve the health of individuals and populations.

A strategy for prevention

Echoing the WHO global action plan on physical activity, projections of improvements in NCD management based on trend data suggests radical policy actions are needed[33], that primordial and primary prevention of NCDs should be a priority throughout life, and should be starting in childhood. Examples of radical policies which are/could be implemented by nations include 1) increased taxation on sugar-sweetened beverages[34] and/or non-essential food products with high energy density[35], 2) universal screening for elevated lipids in 9-11 year old children[36], or 3) intensive camp-based physical activity and diet interventions for overweight children[37]. However, such monetary or individual-agency based strategies may disproportionately impact segments of the population[17, 38] which may or may not be were the largest absolute health-effects would be achieved. Further, screening programs are based on intervening in high-risk individuals and will,

even if effective for the individual, not prevent the majority of cases in the population[39]. This is illustrated by 70 % of obese adults not being obese in their youth[40], suggesting a high-risk approach during youth will have unsatisfactory population effectiveness. Preventive strategies are further appealing because childhood obesity, as adult obesity, is notoriously difficult to treat and an achieved weight-loss is hard to maintain over time[41]. School-based approaches have the potential to reach near population-wide coverage as school attendance is mandatory, thus representing an ideal setting for large-scale primordial prevention based on a comprehensive and structural strategy. Population-based behavioural interventions initiated in schools have shown positive benefits on cardiometabolic risk markers in young people when evaluated immediately post-intervention[42-44]. Physical education led by teachers trained in class-management and provision of quality physical education lessons is associated with relatively high physical activity levels during class[45, 46]. Accordingly, provision of additional physical education led by professionals could be a viable option for increasing physical activity levels of school-aged children. However, there is a scarcity of data on long-term or sustained benefits[47], rendering the data suboptimal for public health decisions on NCD prevention. Long-term and sustainability evaluation is needed to create evidence-informed practice.

Data on temporal trends in the physical activity levels of young people is limited[48]. Information on temporal trends is needed to adequately understand and potentially intervene on unfavourable trajectories. Trend data is particularly lacking for (overall) habitual physical activity but is more available for physical activity segments[48]. A complete picture of physical activity levels, and not only trends in isolated behaviours such as sports-participation and physical education, is however needed as opposing trends in different segments may cancel out. Stabile levels of e.g. physical education would also not reveal if a substantial drop in leisure-time activity has occurred. There was little indication of a substantial decline in self-reported participation in vigorous physical activity

between 1986 and 2002 in the Health Behaviour in School-Aged Children Survey. However, repeated cross-sectional surveys in randomly selected samples indicate potential declining activity levels within the last decade, at least in the Scandinavian countries[49, 50]. Importantly, updated trends informed by objective monitoring are virtually non-existent. Updated data is urgently needed given recent technological developments allowing for near-universal access to portable screen-behaviours. More data is available on cardiorespiratory fitness, indicating a global deterioration of aerobic capacity over 30 years with the data-series ending in 2003. The downwards trend may have continued[51], although not all studies agree[52]. Trends for muscular-strength produce unclear findings across studies[52-54]. The latter may relate to true differences in muscle volume and quality trends between populations over time, to variation in the quality of data between studies, or to variation in the balance between opposing muscular strength correlates such as obesity (higher absolute strength) but lower engagement in activities of sufficient intensity and frequency to elicit muscular strength adaptations. What is clear is that a substantial proportion of contemporary children do not accumulate sufficient physical activity to obtain satisfactory cardiometabolic benefits[48, 55, 56], suggesting a substantial potential for improvement in population health. As physical activity levels demonstrate stability (tracking) over time[57] inactive children are more likely to become inactive adults, suggesting additional left-shifts of the population activity distribution may occur if not acted upon.

Physical activity for health in young people: a look at the evidence

Physical activity is a highly complex behaviour often defined as "any bodily movement produced by skeletal muscles that results in energy expenditure"[58]. Physical activity may be subdivided by features such as; domain (e.g. transportation, work/school, leisure), type (e.g. aerobic, resistance,

activities of the daily living), intensity (e.g. light, moderate, vigorous), and context (e.g. socialization, recreation, unstructured). National and international physical activity guidelines recommend young people aged 5-18 years engage in minimum 60 minutes of daily physical activity of moderate-to-vigorous physical activity (MVPA) *"to improve cardiorespiratory and muscular fitness, bone health, and cardiovascular and metabolic health biomarkers*[59-61]. The choice of 60 daily minutes is largely arbitrary as there is no physiological or evidence-based cut-off behind this recommendation and the guidelines note that additional physical activity is likely to produce greater benefits. A substantial amount of experimental and epidemiological evidence corroborates adult data and supports the link between physical activity and cardiometabolic risk markers in young people[62-70]. However, the quality of the evidence-base was rated as "very low" in a recent systematic review which served as the foundation of the Canadian 24-hour movement guidelines for young people[66].

A pivotal difference in the quality of evidence behind population physical activity recommendations for adults and young people is the absence of data over time in the latter. Particularly, data over time using a non-subjective methodology is important. This is because the validity of self-reported physical activity in young people is particularly hampered by issues such as social desirability and recall bias which introduce substantial non-differential measurement error and potentially differential measurement error. Further, cross-sectional studies, where exposure and outcome are measured at the same point in time (i.e. no time resolution), are unable to elucidate the direction of causality, hence inference is at particular risk of reverse causation bias. Accordingly, public health recommendations and policy should be grounded in higher quality evidence when possible. Prospective studies, in which the same individuals are followed and data collected over time, have the potential to reduce (but not eliminate) the risk of reserve causation bias by collecting data in the appropriate temporal sequence. If, however, in a prospective study the sample is re-examined

without allowing for sufficient time for the outcome to change/occur, the analysis will be uninformative. Defining "sufficient" follow-up time may be particularly problematic when considering adiposity indices as outcomes[32].

Recent consensus suggests high levels of sedentary time is a risk behaviour distinct from low physical activity and not just the other end of the continuum[71]. In adults, sedentary time is as an independent predictor of mortality[72]. However, in young people, the role of sedentary time might be less clear[73], possible because of engagement in larger amounts of physical activity. A critical appraisal of evidence for an association between physical activity and sedentary time with cardiometabolic risk markers from prospective studies with a meaningful follow-up period is therefore needed.

In adults, the dose-response relation between leisure-time physical activity and mortality and morbidity is highly curvilinear[20, 22]. The steepest slope is observed with an increase in physical activity from the lower end of the activity spectrum[74], and some authors have suggested more than one-third of the benefits on mortality are achieved by just 15 daily minutes of leisure-time activity[74]. This amount would be considered inactive by most guidelines. The estimate was not explained by prevalent chronic disease or deaths within the first 3 years of follow-up. In contrast, the shape of the dose-response association remains relatively undescribed for many outcomes in the paediatric population. It is largely considered to be linear throughout the entire spectrum of physical activity[75]. In the seminal paper by Andersen and colleagues in 2006 it was suggested 90 daily minutes of MVPA should be obtained for optimal cardiometabolic benefits in 9- and 15 year old European children[76] as a steep decrease from the fourth to the fifth quintile of physical activity was observed. A stronger association between MVPA and waist-circumference at higher adiposity levels has also been reported[77]. Another cross-sectional analysis of 5261 to 20 871 participants from the International Children's Accelerometry Database (ICAD) demonstrated that MVPA, but not

sedentary time, was favourably associated with fasting insulin, triacylglycerol, HDL-cholesterol, waist-circumference, and systolic blood pressure when mutually adjusted for MVPA/sedentary time in addition to waist-circumference[78]. When exploring appearance of a graded association in tertiles of MVPA and sedentary time, the relationship with MVPA appeared linear within all tertiles of sedentary time, although with some variation between metabolic phenotypes (e.g. 71 % and 27 % difference in fasting insulin and triglyceride between extreme tertiles of MVPA, respectively). Trends for sedentary time were substantially less pronounced. A limitation of this analysis was the lack of specific details at high activity levels as the most active tertile comprised individuals ranging from 20.6 to 185.0 daily minutes of MVPA. Importantly, no statistically significant longitudinal association between MVPA and waist-circumference was observed in the 6313 participants who provided this information. In agreement, Wilks and colleagues meta-analysed 6 prospective studies using objective methodology to quantify whole-day physical activity and found no evidence of an association[79]. However, a substantial amount of data has become available since that work which was only able to include just shy of 600 participants.

In a systematic review of randomized controlled trials conducted in 6 - 19 years old participants, exercise effects on glycaemic regulation did not reach statistical significance in 24 of 32 retrieved estimates[63]. Importantly, the review was restricted to intervention condition without concomitant dietary manipulation and with a non-treated control condition. The pooled effect-size suggested improved insulin-sensitivity with exercise, but 82 % of the variation in effect-size between studies could not be explained by sampling error suggesting a need for further understanding of the details linking physical activity with insulin-sensitivity. While experimental studies have the ability to reduce selection bias and confounding of the exposure-outcome relation as a consequence of randomization they are usually conducted in highly motived (often inactive, high-risk) individuals and consider differences between one or more experimental condition versus control under very

specific circumstances. Concerning physical activity, this could be e.g. the effect of 75, 150, and 225 weekly minutes of aerobic physical activity versus control in sedentary, overweight or obese postmenopausal women with elevated blood pressure[80]. Additionally, because of the experimental manipulation, there may be compensatory changes in other energy-balance behaviours which are difficult to fully control. Epidemiological studies provide an important adjunct to the randomized trials because they provide information on the natural variation in physical activity in a population and not only the activity imposed by a trial which will be in addition to unquantified activity (the "baseline" level such as domestic chores, walking from the bus, play, etc.). As such, epidemiological data is needed to investigate the importance of the full range of activity behaviours in populations. By obtaining high-resolution and time-stamped data formulation of physical activity guidelines may be based on a whole-day assessment of activity rather than solely on experimental conditions or self-reported leisure-time activities. The application of non-invasive objective methods in large-scale epidemiological studies[76, 81, 82] has greatly enhanced these possibilities. Advancement of the collective inference from randomized and observational evidence is needed to provide insights which may optimize the promotion of physical activity and markers of health in young people.

How should physical activity be performed?

In contrast to the predominant guideline for adults[59], most countries do not specify whether certain patterns of activity (aside from intensity) are recommended to achieve maximal cardiometabolic benefits in youth. The guidelines thereby convey the message that irrespective of accumulation pattern, health adaptations will be similar as long as total volume is matched. One exception is Denmark which specifies that activity should be accumulated in consecutive bouts of at least 10

minutes duration, and that vigorous activity of at least 30 minutes duration should be included at least three times per week [83]. This interpretation of the evidence is likely to modify the perception of what type of behaviour is needed compared to e.g. WHO, U.K., and U.S. recommendations. The adaptation is therefore unlikely trivial. The number of studies investigating potential additional benefits of bouted activity in young people is scarce, provides mixed conclusions, and the studies are discordant in their analytical approach for handling correlations with total physical activity[76, 81, 84-89]. Accordingly, a systematic evaluation of cardiometabolic risk marker associations with different bout-durations is warranted. Further, while the vigorous activity domain has previously been systematically investigated in relation to cardiometabolic biomarkers[90], comparisons of intensity thresholds within the light-to-moderate domain are lacking. Currently, comparisons are made between studies which include additional sources of heterogeneity such as random and systematic sample variation (e.g. age, sex, overweight prevalence). Even when applying the same measurement device, variation in accelerometer data-reduction (e.g. sampling frequency, non-wear, and "valid" day definition) will still have an impact.

What type of physical activity should be performed?

Vigorous activity at least three times per week is recommended in the guidelines for young people to increase muscle and bone strength. Cardiorespiratory fitness has been the focus of many studies, is a potent predictor of all-cause and CVD mortality in adults[19], and is prospectively associated with lower cardiometabolic risk markers in youth[91]. Recently, engagement in other exercise modalities than aerobic activities such as resistance-type activities[92] has been identified as a potential target of NCD prevention. Low muscle-fitness is a strong marker of mortality and morbidity in both young[23, 93] and middle-aged adults[94], and the predictive capability of handgrip strength for CVD mortality

was identical to that of systolic blood pressure in a large multi-national cohort of middle-aged men and women spanning the full spectrum of low- to high income countries[94]. Importantly, current data suggests muscle-fitness has a CVD risk pattern which is distinct and independent from that of cardiorespiratory fitness[21, 95]. In children and adolescents, cardiometabolic risk markers also appear favourably modulated by higher muscle-fitness and engagement in resistance-type activities[96, 97]. However, there are a number of limitations to this literature 1) beneficial adaptation in glycaemic, lipid, or blood pressure control were only observed in 5 of 13 randomized controlled trials[96], 2) available randomized studies may have low population generalizability as they include primarily high-risk adolescents[96], 3) experimental studies provides an exercise modality which may not reflect the type of activities most pre-adolescent children engage in, 4) muscle-fitness encompasses distinct phenotypes such as strength, power, and endurance which may have unique metabolic roles, and 5) observational evidence is primarily cross-sectional with only 2 prospective studies providing data in pre-adolescent children[91, 98]. Pre-adolescent children exhibit unique adaptations to resistance-type exercise[99] which may have implications for the potential for health benefits with higher muscle-fitness.

Is body-weight the answer?

Whether physical activity confers health benefits irrespective of any potential effects owing to regulation of energy-balance is of immense aetiological, public health, and scientific interest. Support for a non-adiposity dependent effect of physical activity is often sought by adding adiposity to a regression model with a cardiometabolic risk factor as the dependent variable and physical activity as the independent variable. Persistence of a statistically significant association with physical activity in this model is interpreted as evidence of a non-adiposity dependent (direct)

association[78, 100]. Noticeably, the relative importance of the direct and adiposity-related (indirect) effect is infrequently estimated and measures of uncertainty such as confidence intervals (CI) are lacking. A direct effect of physical activity would have substantial public health relevance as weight-loss is notoriously difficult to achieve and to maintain[41]. There is also a substantial and mostly unexplained variation in weight-loss between individuals despite substantial and identical volumes of exercise[101]. A recent meta-analysis suggested 46 % and 76 % of the effect of body-mass index (BMI) on coronary heart disease and stroke, respectively, was mediated through blood pressure, cholesterol and glucose levels underpinning the importance of maintaining metabolic homeostasis[102]. A direct effect would suggest focus could be shifted towards activity patterns and away from a potentially difficult to achieve weight-loss. If however, the major constituent of the link between physical activity and metabolic homeostasis is through a potential regulation of excess tissue accumulation, this implies interventions would need to ensure weight-loss to be successful. For the general population, the message that unfavourable metabolic control occurs with inactivity irrespective of a healthy weight-status would be a strong impetus to take up or maintain exercise. Further, it is not biologically implausible that physical activity would have a larger impact on e.g. glycaemic regulation in the presence of excess adipose tissue than in lean individuals (i.e. a physical activity-by-adiposity interaction) as glucose uptake into the muscle is inhibited by high levels of circulating free-fatty acids[103, 104]. Conversely, large amount of physical activity are usually needed to elicit weight-loss. Physical activity-by-adiposity interactions[105] and non-linear associations between physical activity and adiposity indices[77] have been noted previously. Information on the relative roles of direct/indirect effects and a potentially modulated balance between these thus holds importance for constructing population policy and for development of targeted interventions.

Epidemiology of NCD risk factors in the paediatric population

In developed countries the prevalence (based on International Obesity Task Force (IOTF) definition) of paediatric overweight/obesity has increased from 17 % in 1980 to almost 1 in 4 in 2013 in[106], and developing countries are following this trend with a 2013 prevalence of about 13 %[106]. The prevalence of youth overweight/obesity is estimated as 29 % in North America and 23 % in Western Europe, with only minor difference between boys and girls[106]. This has resulted in type 2 diabetes no longer being confined to the adult population and the first paediatric type 2 diabetic in the U.K being diagnosed in 2000[2]. In the U.S., 5300 children and adolescents aged 10 - 19 years are diagnosed with type 2 diabetes every year[4]. Moreover, data from the nationally representative U.S. National Health and Nutrition Examination Survey (NHANES) suggests a pre-diabetic condition (defined by HbA1c, fasting plasma glucose, or oral glucose-tolerance test) is not uncommon in 12 - 19 years old adolescents with an estimated prevalence of 18 % (95% CI: 16 – 20)[107]. These sobering numbers are mirrored by an Australian population-based study with more than 25 % of 12-year olds having reduced insulin sensitivity (defined as homeostasis model assessment of insulin-resistance (HOMA-IR) >3.0). Other population-based studies show insulin-resistance prevalence rates ranging from 3 % to 44 %[108]. Dyslipidaemia is also not uncommon in young people of the general population with 1 in 5[109] of 8 - 17 year old participants in the NHANES 11/12 having at least 1 of total cholesterol, HDL-cholesterol or non-HDL-cholesterol levels exceeding National Heart, Lung, and Blood Institute cut-offs for initiation of dietary management and lifestyle intervention. Slightly more promising figures come from a population-based Danish sample of 6 - 19 year olds, with 6.4 % exceeding the 2003 American Heart Association cut-off for dyslipidemia[110]. The discrepancy is likely due to differences in the BMI distribution in these populations, but may also relate to the slightly stricter cut-point for elevated HDL-cholesterol in the Danish sample (below 40 vs 35 mmol/l). Low HDL-cholesterol was the most prevalent condition in the NHANES sample.

Prediabetes, dyslipidaemia, and high blood-pressure was more prevalent in overweight and obese individuals in both the U.S. and Danish samples which supports deteriorated glycaemic, lipids and blood pressure regulation in overweight and obesity affected children and adolescents[111] and thus the key role of excess adipose tissue accumulation in NCD aetiology[112]. Deleterious biological effects aside, overweight and obesity involves stigmatization and predicts involvement in bullying behaviours in youth[113].

Childhood risk factors, adult risk factors, and the difficulties of shaking the habit

There are a number of arguments as to why primordial NCD prevention in young people should be prioritized in conjunction with other preventive strategies; 1) CVD pathology is anatomically and functionally detectable in youth, 2) cardiometabolic risk factors in youth are associated with morbidity and mortality in adulthood, and 3) cardiometabolic risk factors in youth are associated with cardiometabolic risk factors in adulthood. These arguments are clearly intertwined but they are not mutually exclusive and the specific roles of these mediators are far from fully understood. In the Bogalusa Heart Study, Berenson et al., (1998) used autopsies of individuals aged 2 - 39 years to show the progression of atherosclerotic CVD with age, as indicated by increased presence of fatty streaks and fibrous plague[114]. These observations are consistent with the aetiology of CVD as a largely subclinical disease developing over decades. Importantly, the amount of infected tissue increased in proportion with the number of elevated cardiometabolic risk factors. Further, changes in anatomy[115] and function[116] of major arteries linked with atherosclerotic and arteriosclerotic processes have been observed in youth.

Concurrent with these observations, having a high BMI in childhood has been associated with higher all-cause[117] and CVD mortality[118] and a higher risk of a type 2 diabetes and hypertension in

adulthood[119]. Further, in a pooling of 4 highly influential cohort studies (Cardiovascular Risk in Young Finns, Bogalusa Heart Study, Princeton Lipids Research Study, Minnesota Insulin Study) elevated levels of the biological risk factors (operationalized by a composite score including standardized values of BMI, systolic blood pressure, triacylglycerol, HDL-cholesterol, and glucose) were linked with incident MetS, type 2 diabetes and advanced atherosclerosis (carotid-intima thickness >90th percentile) in young adulthood after a median of 22 years of follow-up. A 1 standard deviation increase in the score was associated with a substantial roughly 3-fold increase in the risk for MetS and type 2 diabetes, and an around 2-fold increased risk of advanced atherosclerosis[120]. The association was consistently observed, but varied in magnitude according to age of childhood risk assessment. An interesting observation with substantial clinical relevance is that elevated BMI in youth may have similar predictive capabilities to elevated levels of 3 or more of the biological risk factors[120, 121]. However, the risk of type 2 diabetes and CVD in young adulthood also appears to increase with advanced "clustering" of the risk factors in youth irrespective of the combination of its constituent parts[114, 121]. Even though these studies have followed cohorts of young people over an impressive amount of time, these individuals are still fairly young (mean age in adulthood ranges from 31 to 42 years in the pooled analysis[120]) and the collective size of the cohorts is relatively small which limits analytical possibilities. Therefore, understanding of the link between biological risk factors in youth and adult clinical end-points is still limited[122].

A pivotal question is whether associations between childhood risk markers and adult outcomes are evident because childhood represents a particularly sensitive period of life and deviation from the "natural" growth pattern will cause damage throughout life? Current epidemiological data does not support this notion as individuals with high-risk conditions in childhood, which are resolved by adulthood, do generally not present elevated disease risk as compared with peers with consistently favourable levels[119, 123]. These observations are supported by associations between childhood BMI

and cardiovascular risk in adulthood being removed or attenuated when controlling for adult BMI[124-126]. This supports the notion that the increase in risk is because of tracking of the risk factors over time. The relative stability (correlation coefficient) is usually low-to-moderate for systolic blood pressure (r=0.38)[127]. The Bogalusa Heart Study and the Cardiovascular Risk in Young Finns Study observed slightly higher tracking for cholesterol fractions (r ≈ 0.40 to 0.50) but a lower degree of tracking for triacylglycerol (r ≈ 0.11 to 0.30)[128, 129]. Similar to the association between the risk factors and MetS, type 2 diabetes and advanced atherosclerosis, tracking-coefficients increases with age at the first data-point[127-129]. This is largely an example of the "horse racing effect"[130] and effective intervention at any time-point is likely meaningful. Another pivotal factor in determining the degree of tracking is the sum of measurement error in the variable, (including device/analytical error and day-to-day variability in risk factor levels) with greater measurement error resulting in the appearance of lower tracking. Tracking does suggest identification and effective lifestyle intervention in high-risk youth would reduce or delay the need for intervention or clinical manifestation of disease when these individuals reach adulthood. Conversely, levels within the lower end of the spectrum will tend to remain in the recommended range in adulthood. Because of genetic influence some stability over time is expected. However, tracking is likely also explained by stability of behavioural factors over time. BMI demonstrates a strong pattern of tracking over time with tracking coefficients ranging from 0.27 to 0.47 after 30 years[131]. Further, in meta-analysis of 200 777 participants from 15 cohorts, the risk of obesity in young adulthood was increased 5-fold when obesity was prevalent in childhood[40].

Purpose and aim

To sum up, childhood physical activity strategies should be employed because individuals who are more physically activity in their youth are more likely to be physically active as adults. Further, leading sources of NCD morbidity and mortality are characterized by a substantial pre-clinical

phase spanning years or decades. It may also be speculated that if lifestyle intervention in adulthood is prescribed then having a previous positive experience with being physically activity may increase chances of successful behavioural modification. Finally, an environment promoting a physically active lifestyle throughout all stages of life may be more likely to foster a population of physically active adults and elderly. Taken together, this makes childhood a pivotal foundation for promoting lifelong engagement in physical activity and for the maximization of risk marker management throughout life.

The aim of this thesis is to increasing understanding of the link between physical activity, sedentary time, and augmented physical education in schools with cardiometabolic risk markers in young people by examining:

1) Associations between volume and patterns of physical activity and cardiometabolic risk markers
2) Associations between sedentary time and cardiometabolic risk markers
3) Associations between changes in single- and composite muscle-fitness phenotypes and cardiometabolic risk markers
4) Adiposity as a mediator of the association between accumulating 60 daily minutes of MVPA and biological risk markers
5) The potential for population-wide primordial prevention by increased curricular physical education in public schools

The purpose of this thesis is to facilitate primordial- and primary prevention in individuals, communities, and populations by providing epidemiological data for evidence-informed policy and individual decisions.

Methodology

This section presents an abridged overview of methodology employed in studies 1 – 5. The reader is referred to the appendix for full details.

Study 1

Aim

To provide a qualitative synthesize of evidence from prospective observational studies linking physical activity and sedentary time with adiposity and biological risk factors in young people.

Data and Methods

A search for relevant literature was conducted in the PubMed database in February 2016. The following search terms were used: ("physical activity" OR acceleromet* OR sedentary OR objective) AND (metabolic OR cardiometabolic OR overweight OR obesity) AND (prospective OR longitudinal OR long-term). The results were filtered to human studies. The search terms were restricted to title/abstract identification. A single author (JT) identified relevant studies and extracted data (specified below). In addition, JT searched the references of relevant studies and all authors went through their personal records. For this thesis, studies published between February 2016 and October 2017 or studies which were missed in the original search, were included to update the review. The following inclusion criteria were applied:

- Included an objective measure to quantify whole-day physical activity or sedentary time at baseline

- Observational study including healthy, population-based samples of children and adolescents aged ≤18 years at baseline and followed the same individuals for a period of ≥2 years
- Related an exposure to any form of the conventional biological risk factors (recommended for use in CVD risk stratification among asymptomatic adults[29]) with the addition of indices of insulin-resistance. All forms of adiposity outcomes were considered.
- Building on the work of previous reviews[68, 132] only studies published after 29 October 2009 were considered for adiposity outcomes. No time-restrictions were enforced on data considering the biological risk factors.

From identified studies, the following information was extracted: author, country, cohort, baseline age of participants, number of included participants, whether boys and girls were considered, follow-up time, exposures used, outcomes considerd, type of analysis, and included co-variables.

Study 2 and 3

Aims

Study 2 aimed to decompose the total association between achieving 60 minutes of MVPA/day and composite (and single) biological risk factors into a direct and an indirect component using waist-circumference as the mediator. Study 3 sought to investigate how physical activity accumulated in different intensities and bout-durations, and their combinations, relate to cardiometabolic risk markers in young people.

Sample and data source

Data for these studies was based on the ICAD[133]. The ICAD is a consortium including 20 unique samples spanning 4 continents and 11 countries with physical activity assessed by ActiGraph accelerometers (ActiGraph, LLC, Pensacola, Florida, USA) in young people aged 3 - 18 years. Collaborators were identified through a pragmatic search of large (>400 participants) studies including population-based samples of young people and through personal contacts. Data from original studies was collected between 1997 and 2009 using waist-worn ActiGraph models: 7164 (former CSA and MTI), 71256, and GT1M. Only data from ICAD 1.0 was available for the present thesis. Data was analysed cross-sectionally. For study 2, participants had to provide the following information to be included; 1) the variables HOMA-IR, triacylglycerol, systolic blood pressure, and HDL-cholesterol based on fasting blood samples, 2) at least 3 days of at least 500 minutes of wear-time, and 3) a measurement of waist-circumference and stature. In case data from the same participant was available from multiple time-points the first observation was used. In study 3 participants were included if they satisfied; 1) data on anyone of fasting insulin, glucose, triacylglycerol, or HDL-cholesterol, or diastolic blood pressure, waist-circumference, or BMI, 2) at least 3 days of at least 500 minutes of wear-time, and 3) aged 4 – 18 years. All studies applied standardized (but not identical) procedures for ascertainment and handling of biological risk factors, adiposity indices, physical activity and any additional variables included in ICAD (e.g. demographic or biological indicators). A total of 3412 participants (51 % girls) with a median (25^{th} – 75^{th} percentile) age of 12.1 (9.6 – 15.4) years from 6 samples were included in study 2. Using IOTF cut-points, the prevalence of overweight and obesity was 14.8 % and 6.6 %, respectively. Study 3 included from 4338 to 29 734 (dependent on outcome) participants (63 % girls) from 38 306 eligible observations. All ICAD samples were included in study 3. Median (25^{th} - 75^{th} percentile) age of participants was 11.7 (11.1 – 13.6) years. The overweight and obesity prevalence

were 18.2 % and 8.2 %, respectively. In analysed participants, cohort mean count/min ranged from 428 to 744 in study 2, and from 384 to 733 in study 3.

Exposure data

All accelerometry data included in ICAD were provided by the original study investigators as unprocessed files (as stored by device). The files were centrally cleaned, reprocessed and harmonized by a common protocol using commercially available software (KineSoft v3.3.20, Loughborough, UK or (Python, Python Software Foundation, Delaware, United U.S.). All files were analysed using a 60-second epoch due to lack of availability of shorter epochs in older studies. Accelerometer data-processing details are provided in Table 1.

Table 1. Accelerometer data-reduction settings in study 2 and 3

	Study 2	Study 3
Epoch	60 seconds	60 seconds
Days required	3 days	3 days
Valid day definition	≥8.33 hours	≥8.33 hours
Data-window	7 am to midnight	7 am to midnight
Non-wear definition	60 consecutive minutes of zero-counts strings, allowing for up to 2 non-zero interruptions	60 consecutive minutes of identical count-values, no breaks allowed.
ICAD "flagged days" removed	Yes	Yes
"Extreme" count values removed	No	Yes (≥30 000 counts/min considered non-wear)
"Extreme" days removed before summation	No	<0.1st percentile (36 counts/min) or >99.9th percentile (2125 counts/min)
Cut-point(s) applied (counts/min)	≥2296	≥500, ≥1000, ≥2000, ≥3000
Bouts of activity considered (minutes)	No	≥2, ≥5, ≥10, 1-4, 5-9
Summation of activity	Average of valid days	Average of valid days

In study 2, a cut-point of ≥2296 counts/min was used to define MVPA[134]. The average time spent above this cut-point was calculated and dichotomized into meeting or not meeting the 60 minutes of MVPA/day recommendation. This analysis was supplemented by dichotomizations (meeting versus not meeting) at 30 minutes of MVPA/day and 90 minutes of MVPA/day. Finally, contrasts comparing < 30 versus ≥ 90 minutes of MVPA/day and < 60 versus ≥ 90 minutes of MVPA/day were applied. Study 3 used a range of increasing cut-points (≥500, ≥1000, ≥2000, and ≥3000 counts/min) and combined these with activity accumulated in bouts of ≥1, ≥2, ≥5, and ≥10 minute durations leading to 16 combinations of intensity and bout-duration. No interruptions in the time-series were allowed when summarizing bouts. The following minute-by-minute accelerometer sequence 0-3000-3000-3000-3000-3000-500-500-3000-3000-3000-3000-3000-0 would therefore be summarized as 5+5 = 10 minutes spent in ≥5 minute bouts ≥3000 counts/min but zero minutes spent in ≥10-min bouts (and similar for ≥2000 and ≥1000 counts/min intensities), whereas there would be 12 minutes accumulated in all the 500 counts/min bout variables. Whether "breaks" should be allowed in bout-accumulation[84, 86], and if so, how they should be defined appears a matter of opinion. The no-break criterion here may be considered conservative when using a biomechanical physical activity indicator such as accelerometry as compared to a physiological data-signal from e.g. heart-rate. Results in study 3 are presented as difference in outcome per 10 min/day positive difference above the intensity cut-point.

Outcome data

The main outcome of study 2 was a composite risk score consisting of HOMA-IR (calculated as (insulin x glucose)/22.5)[135], triacylglycerol, HDL-cholesterol, and systolic blood pressure. The main outcome of study 3 was a composite score including HOMA-IR, triacylglycerol, HDL-cholesterol, mean arterial pressure (calculated as 1/3*systolic blood pressure + 2/3*diastolic blood pressure),

and BMI. In both studies, composite outcomes were calculated by summing age- and sex-standardized residuals (z-scores) from linear regression models with logarithmic transformation of variables applied if appropriate. Blood pressure variables are additionally standardized for stature (standardizing for stature-squared providing only minimal improvement in variance explained). HDL-cholesterol is inverted before standardizing. These residuals are subsequently averaged and the score re-standardized (mean 0 and standard deviation of 1). Results for individual risk factors (and a non-adiposity composite score in study 2) are also presented.

Statistical approach

In Study 2, a 2-stage regression approach to decompose the total effect on the respective outcome into a natural direct effect and a natural indirect effect, while allowing for exposure-mediator interaction was applied. As study 2 is cross-sectional, the use of "effects" is inappropriate as it implies a causal relationship which cannot be concluded based on a cross-sectional association. To be consistent with terminology in the mediation framework, "effect" has been used in reference to the model and its decomposition, while "association" is used in relation to interpretation. The waist-circumference to stature ratio was used as mediator with a higher ratio implying relatively greater levels of excess adipose tissue. For these effects to be identified in the presence of exposure-mediator interaction it is necessary to estimate the change from one fixed level of exposure to another (exact definitions given below)[136, 137]. The counterfactual approach was used to apply the decomposition using the following statistical models (omitting error terms)[138, 139].

$$E[Y|a,m,c] = \beta_0 + \beta_1 a + \beta_2 m + \beta_3 am + \beta_i c_i \qquad (1)$$

$$E[M|a,c] = \theta_0 + \theta_1 a + \theta_i c_i \qquad (2)$$

Using the counterfactual framework, the direct effect can be interpreted as the contrast between achieving the activity target and not achieving the activity target, while for each individual fixing

the mediator to the level it would have assumed if the activity target had not been achieved. In other words, the direct effect estimates the effect of the activity target on the composite risk score not acting through abdominal obesity. Similarly, the indirect effect can be interpreted as the contrast between fixing the mediator to the level it would have assumed had the activity target been achieved versus the level it would have assumed had the activity target not been achieved, while setting the activity target to not achieved. That is, the indirect effect is the effect of the activity target acting on the composite risk score by the activity target influencing abdominal obesity which, in turns, affects the composite risk score[140]. An index of abdominal obesity was used as mediator because it is included in the definition of the MetS[30].

Total, direct, and indirect effects for the physical activity contrasts were estimated separately for each study using a bootstrap procedure (1000 repetitions with replacement) to derive the study specific 95% bias-corrected CI for the indirect effect as the 2.5th and the 97.5th percentiles. A priori selected putative confounders of the exposure-outcome, exposure-mediator or mediator-outcome relations, which were available from ICAD, were used as covariates; these included age, sex, ethnicity (White or not), birthweight (continuous), mother's BMI (continuous), sexual maturity (Tanner stages) and mothers education (high/medium/low) from European Youth Heart Study (EYHS) studies. Available from NHANES was age, sex, ethnicity (White, Black, Asian, and Hispanic) and household income (quartiles). Age and sex was available from the Copenhagen School Child Intervention Study. Study-specific estimates were pooled in meta-analysis. In meta-analysis the study weights are usually given as the inverse of the variance, however, as the standard errors here are derived from a bootstrap procedure (in contrast to standard deviation/√N), the standard errors of the total, direct and indirect effects will not necessarily be identical if using inverse variance weighting. This would result in failure of the decomposition to sum to the total effect in the meta-analysis, which is counterintuitive. Therefore, study weights were given as

(√study sample size) / (√total sample size)[141]. Specifying the weights as such precluded using a random-effects meta-analysis. The consequence of using a fixed rather than a random effects approach is that relatively more weight will be given to larger studies. The Q-statistic and I-squared were used to assess between-study heterogeneity. The proportion of the total effect which can be attributed to the indirect effect was calculated as (indirect effect/total effect) x 100. To assess the robustness of results, a line of sensitivity analyses were applied of which 3 are highlighted in the thesis; 1) omitting, in turn, one study from the meta-analysis, 2) using log-log regression to calculate a waist-circumference to stature exponent to make waist-circumference independent of stature[142], and 3) defining MVPA as ≥1999 counts/min.

In study 3, data from studies was pooled into 1 dataset and separate multivariable linear mixed regression models were used to analyse associations between the 9 outcomes and 16 combinations of intensity and bout-durations while including the co-variates age, sex, and wear-time. Participants and studies were modelled as "random-effects". Models of diastolic blood pressure and waist-circumference were additionally adjusted for stature. Waist-circumference was harmonized using a correction formula[143] as NHANES studies had followed a different protocol. This correction was applied in a sensitivity analysis in study 2. The non-adiposity composite score, insulin, glucose, triglycerides, HDL-cholesterol, and diastolic blood pressure were additionally controlled for BMI in secondary models. To directly model whether physical activity spent in medium or long bouts confers an additional health benefit over an identical amount of shorter bouts of physical activity, an isotemporal substitution approach[144] was applied. These models took the form (omitting error term):

$$Y = \beta_0 + \beta_1 \text{Physical Activity}_{\geq 5\text{-}9 \text{ minute bouts at intensity}} + \beta_2 \text{Physical Activity}_{\geq 10 \text{ minute bouts at intensity}} + \beta_3 \text{Total Physical Activity}_{\text{at intensity}} + \beta_4 \text{Wear-time} + \beta_5 \text{Age} + \beta_6 \text{Sex} + \zeta_1 \text{Study} + \zeta_2 \text{Participant} \quad (3)$$

This model constraints total physical activity above the intensity threshold, thereby allowing for investigation of its composition[145]. The coefficients β_1 and β_2 thus represents the effect of substituting time spent in physical activity of 1-4 minutes duration (short bout-duration) with an equal amount of time spent in medium- (5-9 minutes) or long-bouted (≥10 minutes) MVPA[144]. Quintiles of residual variation in medium- and long-bouted physical activity after controlling for total activity volume (≥1 minute bouts) in addition to sex, age and wear-time where calculated to assess whether there would be meaningful variation in the exposure-variables after cancelling out total physical activity. The difference between quintile 1 and 5 was 56.0 minutes for ≥10 minute bouts above 500 counts/min and 10.9 minutes for ≥10 minute bouts above 3000 counts/min. This suggest reasonable contrasts for intervention-targets were available within the data (e.g. increase total physical activity by 10 minutes of bouted activity >3000 counts/min per day without reducing other physical activities). Meta-regression was used to explore trends in the influence of intensity and bout-duration on the outcomes. An intensity-by-bout-duration interaction term was added in a separate meta-regression model to explore potential heterogeneity in effects across bout-duration/intensity combinations. Estimates for bout-durations of ≥3 and ≥7 minutes were added to the meta-regression to increase information. CIs in meta-regression were adapted to account for non-independence of coefficients by recalculating the standard error as: (√(number of coefficients (20) – 1)) x the standard error obtained from the meta-regression model.

Appropriate model diagnostics were applied. Regression coefficients and 95 % CIs are presented. Analyses were conducted using PARAMED and METAN modules in Stata IC v.14.1 or v.15.0 (StataCorp, College Station, Texas, USA). Significance tests were 2-sided, and *p* values less than 0.05 or CIs (all 95 %) not straddling zero were considered statistically significant. Neither study included adjustment for multiple testing.

Study 4 and 5

Aims

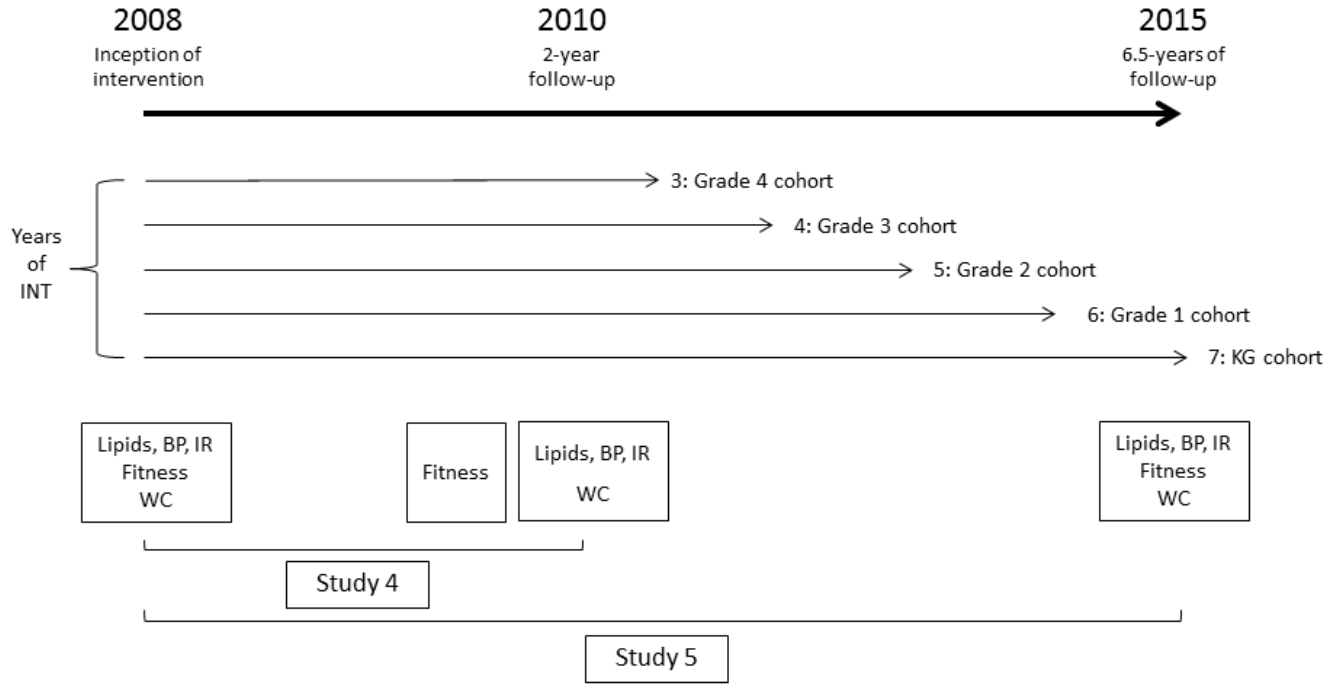
Study 4 aimed to analyse the association between gains in single- and composite muscle-fitness phenotypes and changes in cardiometabolic risk factors, focusing on whether associations are independent of cardiorespiratory fitness and waist-circumference. Study 5 evaluated long-term (6.5 years) differences in cardiometabolic risk factors between children exposed to intervention or control conditions in the Childhood Health And Motor Performance School study Denmark (CHAMPS-study DK).

Sample and data source

Data for study 4 and 5 originates from the CHAMPS-study DK, a prospective controlled intervention evaluated as a natural experiment[146] with continuous data-collection from 2008 to 2015. For this thesis, data from 2008 to 2010 is analysed as an observational study (prospective cohort, disregarding intervention or control status of participants) in study 4, while study 5 includes follow-up from baseline to 2015 (controlled intervention study). A temporal overview of exposure and outcome data used for study 4 and 5 is presented in figure 1 (figure 1 additionally including age-cohort specific exposure to the intervention within the intervention group which is detailed below). In 2008, the municipality of Svendborg, Denmark decided to treble weekly curricular physical education from the national curriculum 90 minutes to 270 minutes in the 5 lowest grades of the public school system (U.S. equivalents are kindergarten to grade 4). Children were from 5 – 12 years old at inception of the study. Six of 19 schools in the municipality (covering urban and rural schools) accepted to fund additional physical education and became intervention schools. Four schools were matched on size, rural/urban and socioeconomic uptake area and agreed to serve as controls[147]. A total of 1507 children were invited to participate in the study of which parents or legal

guardians of 1209 consented (total of 80 % of the sample, with 90 % at intervention and 71 % at control schools). Due to practical reasons, the "baseline" assessment was conducted during August-October 2008 with the additional physical education already being implemented. The first phase of the CHAMPS-study DK assessed anthropometrics and cardiovascular- and muscle-fitness characteristics every 6 months until spring 2011. Biochemical markers were obtained at "baseline" and in fall 2010 (2 years later). In 2015, an additional follow-up of the cohort was initiated with 1278 adolescents attending 6th to 9th grade at the now 9 schools (due to merging of schools) invited to participate. Recruitment in 2015 was based on handouts at schools, postal mail of study material to parents, and telephone calls by study staff. Additionally, 10th grade students with prior participation in the study (n=179) were approached via postal mail and telephone calls. The 2015 data-collection included information on anthropometry, fitness, and biological risk factors. Of consenting participants, 959 provided a fasting blood sample in 2008 (64 % of invited). Participants included in study 4 are those providing information on; 1) 2-year changes in biological risk factors, 2) 1.5-year changes in muscle-fitness, 3) changes in cardiorespiratory fitness, and 4) putative demographic and biological confounding variables. These restrictions left 512 participants for analysis (analytical sample: 34 % of invited and 53 % of those providing fasting blood samples in 2008). Participants were 51 % girls, mean (standard deviation) age 8.4 (1.4) years. The prevalence of overweight and obesity (using IOTF definition) was 8.8 and 1.0 %, respectively. For study, 5 participants were included if they had information on; 1) a fasting blood sample at baseline and long-term follow-up and 2) self-reported sexual maturity at both these time-points, leaving 312 participants for analysis (analytical sample: 21 % of invited and 33 % of those who obtained fasting blood samples in 2008). Characteristics of the sample for included in study 5 are presented in the results section.

Figure 1. School-years with exposure to augmented physical education in the intervention group and a temporal overview of exposure/outcome data included in study 4 and 5 (CHAMPS-study DK).



INT; intervention, KG; kindergarten, BP; blood pressure, IR: insulin resistance, WC; waist-circumference

Exposure data

In study 4, single- and composite indices of muscle-fitness (strength, power, and agility) were used as exposures. Upper body muscular force (strength) was measured as the highest maximum voluntary contraction (in kilograms) of the dominant hand following 2 attempts on an analogue handgrip dynamometer (Smedley´s dynamometer, Scandidact, Odder, Denmark) while standing. The result was divided by body-weight for analysis because this index is frequently used. Muscular power was measured by a vertical jump test as the highest vertical displacement (in centimetres) in a minimum of 3 attempts, with additional attempts prompted if continuous improvement was observed. Participants were asked to perform a maximal jump with allowance of countermovement.

Muscular agility was measured using the 50-meter short shuttle-run. It was performed as 10 laps on a 5-meter lane and measured in seconds. Less elapsed time reflects higher muscular agility. A standardized composite muscle-fitness score was constructed by standardizing and averaging each muscle-fitness phenotype (using inverted shuttle-run). Handgrip strength is a valid and reliable marker of upper body and global muscular strength[148-150], while validity and reliability of the vertical jump and short-shuttle tests as markers of muscular power and agility are less described and usually with lower test-retest and criterion-measure correlations than is observed for handgrip strength[148, 149, 151]. In a subsample (n=94) of the CHAMPS-study DK, the standard error of the mean (as percentage of mean) for same-day repeated tests were 6.6, 7.6, and 2.5 % for handgrip strength, vertical jump and the short shuttle-run, respectively[152].

Study 5 compared long-term differences in cardiometabolic risk factors between children attending intervention and control schools. The additional physical education at intervention schools was implemented from the start of the school year in August 2008. In addition to increased physical education, all physical education teachers (in Denmark physical education is mainly taught by physical education specialists) attended a 40-lessons skill developing course based on an Age-related Training Concept developed by the Danish organization for elite sports (Team Denmark)[153]. The purpose of this program is to augment development of body and motor skills in children and adolescents by considering their physical, physiological, mental and social development. Shortly put, the program is based on play, exercise and games with an increased focus on technical and coordinative skills in adolescence. After the first 3 years of the study, the 6 intervention schools have maintained additional physical education from kindergarten to the 6th grade (children approximately 12 – 14 years old). Thus, from 7th to 9th grade (final mandatory school year in Denmark) the "standard" 90 minutes of physical education per week were provided at both

intervention and control schools. Hence, intervention-school participants could receive from 3 (4th grade in 2008) to 7 (Kindergarten in 2008) years of additional physical. Classes serving as controls have maintained national guidelines.

Outcome data

Outcome data was collected in 2010 for study 4 and in 2015 for study 5. All data, except for 10th graders, was collected during school hours usually within 8 am to 13 pm. Data-collection was conducted by trained research staff following a standardized protocol but staff was not blinded to intervention/control condition. The main outcome of study 4 and 5 are composite scores consisting of HOMA-IR, triacylglycerol, HDL-cholesterol, systolic blood pressure, waist-circumference, with the composite score in study 5 additionally including cardiorespiratory fitness to facilitate comparison with the 2-year follow-up of the study[154]. The scores were calculated by summing age- and sex-standardized residuals (z-scores) from linear regression models with logarithmic transformation of variables applied if appropriate. Blood pressure and waist-circumference are additionally standardized for stature (standardizing for stature-squared providing only minimal improvement in variance explained). Biochemical markers in 2015 were additionally standardized for week-day of ascertainment[155], but this information was not available in 2008 or 2010. HDL-cholesterol and cardiorespiratory fitness are inverted before standardization. These residuals are subsequently averaged and the score re-standardized (mean 0 and standard deviation of 1). Individual risk factors were included in standardized form as secondary outcomes in study 4 and 5. Blood samples were collected after an overnight fast (minimum 8 hours required) by trained biomedical laboratory scientists at all time-points. Fasting status was confirmed by the participant. Blood samples were collected between 8 am and 10 am and stored within 4 hours. Samples for analysis of insulin were kept on ice until storage. Samples were stored at –80 °C until analysed at a

certified routine laboratory associated with the University of Vienna, Austria. Total cholesterol, triacylglycerol, HDL-cholesterol, and glucose were analysed by quantitative determination using enzymatic, colorimetric method on Roche/Hitachi cobas c system (Roche, Mannheim, Germany), while insulin was analysed using solid phase enzyme-labelled chemiluminescent immunometric assay (Access® Ultrasensitive Insulin (Beckman Coulter GmbH, Vienna, Austria). Intermediate precision was determined using human samples and controls in an internal protocol according to the manufacturers. Coefficients of variation for the controls and samples ranged from 0.6 to 0.8 % for TC, from 0.6 to 0.9 % for triglycerides, from 0.5 to 0.8 % for HDL-cholesterol, from 0.5 to 0.8 % for glucose, 3.1 to 5.6 % for insulin. Resting blood pressure was measured using appropriate sized cuffs by a Vital Signs Monitor 300 series with Flexiport™ Blood Pressure *(*Welch Allyn, New York, NY, USA) in 2008 and 2010. In 2015 the Omron 705IT (Omron, Kyoto, Japan) was used. The Omron 705IT oscillometric monitor has been recommended in young people[156], while there is no validation data supporting the use of the Welch Allyn Vital Signs monitor in the paediatric population. Participants sat resting in the sitting position for 5 minutes before monitoring. At least 5 subsequent values were recorded with 1-minute intervals until the last 3 values had become stable. The mean of the last 3 recordings of systolic blood pressure was used. Waist-circumference was measured by a measurement band (Seca 201, Seca Corporation, Hamburg, Germany) to the nearest 0.5 centimetre across the umbilical cord following a gentle expiration. At least 2 measurements were performed with a third undertaken if the 2 differed by more than 1 centimetre. Cardiorespiratory fitness was assessed using a field-test (Andersen-test) lasting 10 minutes with 15 seconds of intermittent running and pausing. Total distance covered was used to represent cardiorespiratory fitness. Criterion validity (r-squared approximately 0.5 against directly measured maximal oxygen uptake)[157, 158] and test-retest reliability (r-squared approximately 0.7 – 0.8)[158, 159] of the Andersen-test are acceptable and have been validated in a subsample of the cohort[158]. Study 5

also included physical activity levels assessed by questionnaires and accelerometry in 2015. These data are presented as participation in structured leisure-time physical activity (yes/no), % MVPA/day, and mean counts/minute.

Other variables

Body mass was measured to the nearest 0.1 kg on an electronic scale (Tanita BWB-800S, Tanita Corporation, Tokyo, Japan) with participants wearing light clothes. Stature was measured to the nearest 0.5 cm using a portable stadiometer (Seca 214, Seca Corporation, Hamburg, Germany or Harpenden stadiometer (West Sussex, UK)). Both measures were conducted barefoot. Sexual maturity was self-reported by indicating resemblance on 5 drawings (progressive rating 1 – 5) of secondary sex characteristics as described by Tanner[160]. Pubic hair was used in boys and breast development in girls. Using this approach, young people 7 - 15 years old are able to correctly self-report their pubertal status within +/- 1 category as compared with paediatrician assessment[161]. Parents of participants returned mailed questionnaires in 2008 and in 2015. Questionnaires inquired on the educational attainment, stature, and body-weight of the parents or legal guardians, birthweight of the child, and any history of CVD, hypertension or diabetes (any type) in (biological) sibling, parents, or grandparents. The educational level was indexed in an abbreviated 7-level instrument based on a Danish adaptation of the International Standard Classification of Education 2011. Study 4 only used data from the 2008 questionnaire, while study 5 used the updated 2015 data, but in case of non-response or missing answers to the 2015 questionnaire the 2008 data was carried forward. The Pearson's correlation between birthweight as reported in 2008 and 2015 in 222 participants with information at both time-points was 0.96 with a small mean bias of -0.14 grams (95% limits of agreement -378 - 349 grams). Agreement between educational attainment of the mother or female guardian in 2008 and 2015 (n=264) was 81 % (kappa coefficient 0.6), with only 2

% reporting lower attainment in 2015. Agreement for family history of CVD, hypertension, or diabetes (n=270) was 76 % (kappa coefficient 0.5), with 10% of parents who indicated family history of NCD in 2008 reporting no CVD, hypertension, or diabetes in 2015. Discrepancies between educational attainment and family history of NCDs is likely due to a combination of 1) true changes over time, 2) genuine misclassification because of e.g. memory (recall bias) or comprehension of the question item, and 3) questionnaire being filled out by different persons.

Statistical approach

Study 4 derived, for single and composite muscle-fitness phenotype, a series of multivariable mixed effects linear regression models to analyse 1.5-year changes in these with 2-year changes in individual and composite cardiometabolic risk factors. The following covariates from the questionnaire were used in all models; mother's BMI (continuous) mother´s educational attainment (low, medium, or high), history of CVD, diabetes or hypertension in the nearest family (yes/no). Model 1: including age, sex, sexual maturity (stage 1 or 2-5), intervention status (intervention/control) and a random intercept to account for school class membership. Model 2: as model 1, but including cardiorespiratory fitness. Model 3: as model 2, but including waist-circumference as a covariable (using a non-adiposity composite score, when analysing this outcome). Models took the form; $Y_2 = \beta cons + \beta X_2 + \beta X_1 + \beta Y_1 + \beta_i C_i$, where Y_2 and Y_1 represent the respective outcome at time 1 and 2, X_2 and X_1 are the muscle-fitness phenotype of interest at time 1 and 2, and C_i represents the set of relevant covariates. The term βX_2 from this model can be interpreted as the association between changes in the muscle-fitness phenotype and changes in the composite risk score, controlled for their baseline values. The models included time-variant covariates at the child level (sexual maturity, cardiorespiratory fitness, and waist-circumference) at baseline and follow-up. A sex-by-composite muscle-fitness interaction term was added in a separate

model to statistically evaluate evidence of sex-specific associations and data was re-analysed stratified by sex if p-values for the interaction term were ≤0.10. Two sensitivity analyses using other normalizations for body-weight were applied. One used body-weight$^{2/3}$ (theoretical scaling constant)[162] and one used body-weight^{p} where p represents a sample-, time- and sex-specific power to make handgrip strength independent of body-weight (empirical scaling constants by log-log regression)[142]. Muscular power or agility were not scaled to body-weight as these tests control for weight by design[162]. Correlations for the muscle-fitness phenotypes with body-weight and cardiorespiratory fitness are presented in Table 2. Sensitivity analysis using a multiple imputation procedure (multiple imputation using chained equations (MICE)) to include all consenting participants at baseline was performed. Estimates and beta-coefficients were based on 20 imputed datasets. MICE is based on the assumption of data being missing at random conditional on observed covariables. Re-analysing the data as a cross-sectional study and prospectively but using baseline-fitness only were pursued in secondary analyses.

Table 2. Partial correlations (controlled for age and sex) between single- and composite muscle-fitness indices, body-weight, and cardiorespiratory fitness stratified by time-point.

	Body-weight		Cardiorespiratory fitness	
	Baseline	Follow-up	Baseline	Follow-up
Muscular strength (kg/kg body-weight)	-0.33*	-0.33*	0.23*	0.38*
Muscular power (cm)	0.01	-0.02	0.22*	0.25*
Muscular agility (seconds)	0.10*	0.17*	-0.42*	-0.56*
Composite muscle-fitness[a]	-0.22*	-0.25*	0.41*	0.55*
Cardiorespiratory fitness (meters)	-0.27*	-0.37*	-	-

*p<0.05. [a]Including kg/kg body-weight as muscular strength

Study 5 applied multivariable mixed effects linear regression models to contrast differences in composite- and single cardiometabolic risk factors between intervention and control school membership at baseline (using control as reference). Models were controlled for age, sex, sexual maturity (stage 1 or 2-5 in 2008 and stage 1-3, 4 or 5 in 2015), mother´s educational attainment (low or high), history of CVD, diabetes or hypertension in the nearest family (yes/no), the child's birthweight, and a random intercept for school-class membership at baseline. Random intercepts for baseline school (1 % explained), follow-up school-class (<1 % explained), or follow-up school (<1 % explained) were not included as little additional variance was explained by these terms. To explore if augmented physical education had a distinct effect among those with the least favourable metabolic profiles, participants at control and intervention schools were stratified at the baseline median and these were analysed separately in secondary analyses. Stratification was performed for each outcome. As follow-up data is collected as an extension of the original study, no power calculations were performed prior to participant recruitment. Missing values (n=3 to 33) of variables other than blood chemistry and sexual maturity were imputed using MICE in 20 datasets. Data on sexual maturity could not be imputed due to non-convergence of models. Baseline comparisons between the intervention and control group was conducted using an unpaired t-test for normal distributed continuous data or Wilcoxon rank-sum test for non-normal distributed data. A chi-squared test was used for categorical data.

In study 4, comparisons were made between the analytical sample and those lost to follow-up using data obtained in 2008, while study 5 used data obtained in 2008, 2010, 2012, 2013, and 2015. Appropriate model diagnostics were applied in both studies. Regression coefficients and 95 % CIs are presented. Analyses were conducted using Stata IC v.14.1 and v.15.0 (StataCorp, College Station, Texas, USA). Significance tests were 2-sided, and *p* values less than 0.05 were considered statistically significant. Neither study included adjustment for multiple testing.

Ethical and participants considerations

The ICAD included studies with appropriate ethical consent/approval from 1) participant and/or legal guardians if participants are under the age of 18, and 2) institutional ethics board to share data in an international collaboration. The CHAMPS-study DK obtained parental consent and child assent before collecting any data. A series of public meetings was organized to inform parents about details, procedures and outcomes of participation in the study. The possibility of peer-pressure was reduced by allowing children to participate in data-collection activities irrespective of consent/assent status. The CHAMPS-study DK was approved by the ethics committee of the region of southern Denmark (S-20080047 and S-20140105). No original data was included in study 1.

Results

The main findings from studies 1 to 5 are presented below. The reader is referred to the appendix for additional details.

Study 1

A total of 22 unique publications (adding 5 to the original report) were identified from the literature search and authors' personal records, yielding 37 associations of physical activity or sedentary time with adiposity or the biological risk factors (adding 6 to the original report). Five additional studies on the biological risk factors [163-167] and 1 study on adiposity[50] were added to the review. Updated data on biological risk factors was included from 2 cohorts[168, 169]. No additional data was identified for sedentary time. The median (25^{th} – 75^{th} percentile) duration of follow-up was 2.3 years (2 – 5).

The studies included samples aged 4-18 years at baseline, with a median (25^{th} – 75^{th} percentile) sample-size of 466 (315 to 813). All but 2 cohorts used physical activity derived from waist-worn accelerometry.

In the updated review, 10 of 15 (67 %) prospective studies including ≥2 years of follow-up reported an inverse association between physical activity and an adiposity index, suggesting higher levels of physical activity were associated with lower adiposity levels. Interestingly, reports of significant positive associations, i.e. higher activity levels associated with higher levels of adiposity, were also observed. Physical activity intensity appeared to modulate associations as light- and moderate physical activity were generally not associated with lower levels of adiposity while associations with MVPA were consistently observed. However, vigorous activity only did not produce consistent associations. Additionally, the method for outcome ascertainment appeared important with physical activity consistently presenting an inverse association when adiposity was assessed by more precise instruments, albeit the association was lost in the study by Metcalf and colleagues[170] when baseline-values of the outcome were appropriately included in models. Table 3 presents an overview of data on the prospective association between physical activity and adiposity indices. Only 2 of 9 studies (22%) reported a significant association between time spent sedentary and higher levels of adiposity. Both of these studies reported a positive association, suggesting higher levels of sedentary time were associated with higher levels of adiposity.

For the biological risk factors 10 of 13 (77 %) of studies in the updated review reported a beneficial association between physical activity and a biological risk factor, suggesting higher levels of physical activity was associated with a more favourable metabolic profile. Beneficial associations were generally observed for insulin sensitivity (6 of 7 studies), triacylglycerol (2 of 3 studies), blood pressure (5 of 6 studies), HDL-cholesterol (2 of 3 studies), and for composite outcomes (3 of 4 studies). No studies reported a significant association between physical activity and total

cholesterol, LDL-cholesterol or glucose (study overview available in Table 4). MVPA was significantly associated with any biological risk factor in 6 of 8 studies. Only 4 studies gave results for different intensities[168, 171-173]. Two of these studies demonstrated that vigorous physical activity, but not lower intensities[171, 172], was significantly associated with the risk factors. Four studies[163, 166, 171, 172] provided categorical analysis with more than 1 comparison group, thereby allowing for evaluation of potential graded associations. From the EYHS vigorous physical activity presented, in a linear fashion, a graded inverse association with a composite risk score across 5 strata of mean activity exposure over 6 years. The analysis covered from 2.8 to 15.7 minutes of >5200 counts/min per day[172]. A linear graded trend was also observed for systolic blood pressure with vigorous activity by Carson and colleagues. However, when stratified by sex the trend was only apparent in boys with the 2nd most active quartile presenting the lowest blood pressure levels[171]. No trend across quartiles of MVPA on insulin-resistance was observed in 1042 pre-adolescent children from the IDEFICS study. The 2nd most active quartile presented the most favourable risk profile in analysis covering below 27 to above 55 minutes of >2296 counts/min[166]. The study from the Pelotas birth cohort did not indicate any trend in the association between mean counts/min and glycated haemoglobin[163]. Finally, one study considered sedentary time but found no significant association with any of the biological risk factors before or after adjustment for MVPA.

Table 3. The impact of exposure and outcome on the prospective association between physical activity and adiposity indices (studies with ≥2 years of follow-up).

	Exposure					*Outcome*					
Publication	CPM	LPA	MPA	VPA	MVPA	OW/OB	WC	BMI[a]	Bio-impedance	SSF	Reference methods
Treuth 2009[174]		O				O		O		O	
Metcalf 2011[170]	+				+		O	O			+ (DXA)
Ekelund 2012[78]					O		O				
White 2012[175]	+					+				+	
Jáuregui 2012[176]					+/-			-			+ (air-dis)
Basterfield 2012[177]	O				+			+	+		
Kwon 2013[178]					+						+ (DXA)
Mitchel 2013[179]					+			+			
Carson 2014[171]		-	+	+		O	+	+			
Collings 2015[180]	-	O	-	-					-		
Lätt 2015[181]			O	+	+	+					
Stamatakis 2015[182]					+/-		-	-			+ (DXA)
Van Sluijs 2016[183]			O	O		O	O		O		
Henderson 2016[b,165]					+						+ (DXA)
Dalene 2017[b,50]		O	O	O			O	O			
Inverse (+) association reported	2 / 4	0 / 4	1 / 5	2 / 5	8 / 9	3 / 5	1 / 6	3 / 8	1 / 3	1 / 2	5 / 5

A "+" denotes any statistically significant inverse (expected) association observed. A "-" denotes any statistically significant positive association observed. "O" denotes no statistically significant associations observed. Maximally adjusted models are evaluated. [a]Also includes zBMI. [b]Not included in original report. CPM; counts/min, LPA; light physical activity, MPA; moderate physical activity, VPA; vigorous physical activity, OW; overweight, OB; obesity, WC; waist-circumference, SSF; sum of skinfolds, DXA: dual-energy X-ray absorptiometry, air-dis; air displacement plethysmography.

Table 4. Summary table of the prospective association between physical activity and biological risk factors, stratified by risk factor

Publication	Insulin sensitivity	Glucose	Cholesterol (non-HDL)	HDL-cholesterol[a]	Triacylglycerol	Blood pressure[b]	Composite risk score
Jago 2008[173]	+	O					
Andersen 2011[184]							O
Telford 2012[c,169]	+						
Carson 2014[171]						+	
Ried-Larsen 2014[172]							+
Ried-Larsen 2015[115]	+	O		+	+	+	+
Metcalf 2015[c, 168]	+	O	O	O	+	+	
Stamatakis 2015[182]	+	O	O	+	O	O	+
Knowles 2013[d,164]						+	
de Moraes 2015[d, 167]						+	
Peplies 2016[d,166]	+						
Henderson 2016[d,165]	O						
Nakamura 2017[d,163]		O					
Beneficial association reported	6 / 7	0 / 5	0 / 2	2 / 3	2 / 3	5 / 6	3 / 4

A "+" denotes any statistically significant inverse (expected) association observed. A "-" denotes any statistically significant positive association observed. "O" denotes no statistically significant associations observed. Maximally adjusted models are evaluated irrespective of adjustment for body-weight or changes herein. [a]Or HDL-cholesterol including variables such as total cholesterol/HDL-cholesterol ratio. [b]Any measure, [c]Data updated from original report. [d]Study not included in original report.

Study 2

The median (25^{th} – 75^{th} percentile) number of valid days in the analysed sample was 4 (4 – 5). Adherence to the WHO 60 minutes of MVPA/day guidelines was achieved by 31.1 % of the sample (between-study range from 13.0 % to 44.4 %). Meeting the activity target of 60 min of MVPA/day, versus not meeting it, was associated with a -0.31 (95% CI: -0.39 to -0.23) standard deviation lower composite risk score. This effect was composed of a direct effect of -0.24 (95% CI: -0.32 to -0.16) standard deviation and an indirect effect of -0.07 (95% CI: -0.11 to -0.02) standard deviation suggesting that 22 % of the effect was attributable to the mediator. Tests of between-study heterogeneity were highly significant for total, direct and indirect effect (all p-values <0.01). The I-squared indicated that 78 % to 91 % of the total variation was due to heterogeneity. Meeting the activity target of 30 min of MVPA/day, versus not meeting it, was associated with a total effect on the risk score of -0.24 (95% CI: -0.32 to -0.16) standard deviation, a direct effect of -0.17 (95% CI: -0.25 to -0.10) standard deviation and an indirect effect of -0.07 (95% CI: -0.10 to -0.03) standard deviation, i.e. 28 % of the total effect was attributable to the mediator. Similarly, meeting the 90 min of MVPA/day target versus not meeting the target resulted in a total effect on the risk score of -0.39 (95% CI: -0.51 to -0.27) standard deviation, a direct effect of -0.33 (95% CI: -0.44 to -0.24) standard deviation and an indirect effect of -0.06 (95% CI: -0.13 to 0.01) standard deviation hence 15 % of the total effect was attributable to the mediator. The CI for the indirect effect included zero. When exploring the larger MVPA contrasts, larger total effects were explained primarily by larger direct effects. Excluding one study at a time resulted in little variation of the total effect, but the composition changed substantially with estimates explained by the mediator varying from 15 % to 35 %. Calculating stature-independent waist-circumference indices or lowering the MVPA cut-point resulted in trivial changes in the total, direct and indirect effect estimates.

Study 3

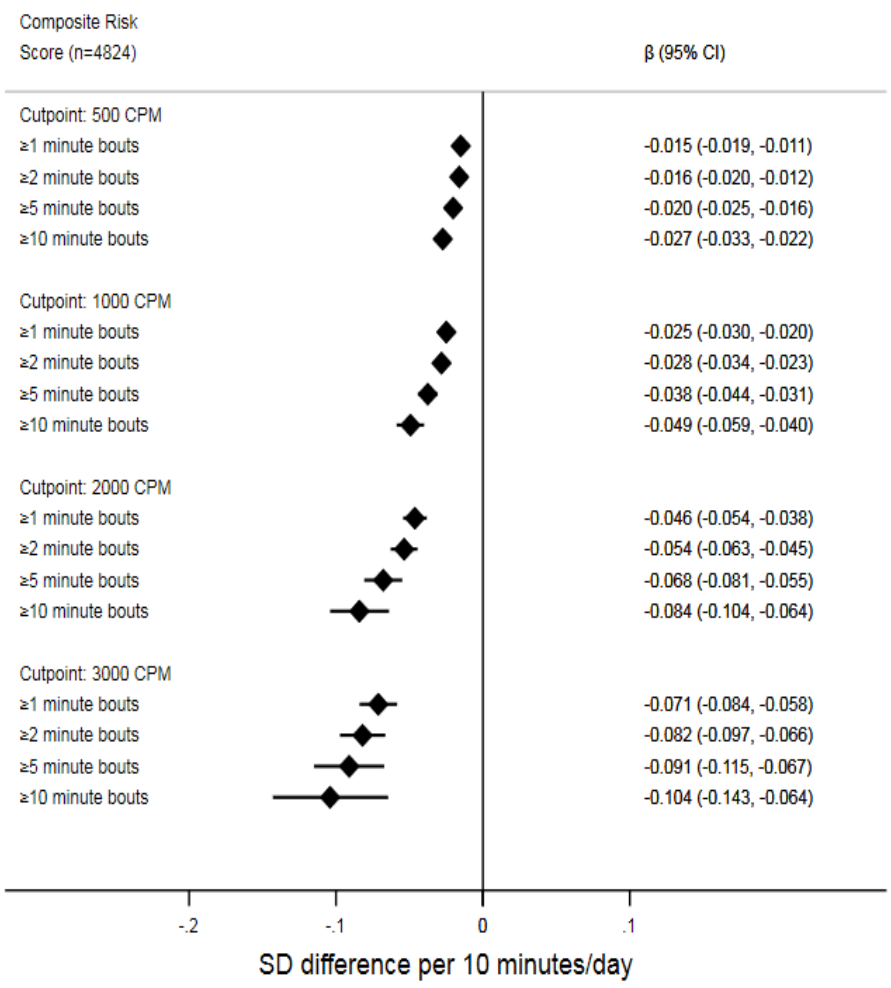
The median (25^{th} – 75^{th} percentile) number of valid days per observation was 6 (4 – 6). The median percentage of wear-time ≥500 counts/min, ≥1000 counts/min, ≥2000 counts/min, and ≥3000 counts/min were 26.2 (20.6 – 32.9), 15.7 (11.6 – 20.7), 6.9 (4.6 – 9.8), and 3.1 (1.8 – 4.9) %, respectively. The median percentage of wear-time accumulated in ≥10 minute bout-durations was 6.5 (3.3 – 10.9), and 0.0 (0.0 – 0.5) for ≥500 and ≥3000 counts/min, respectively. The partial correlations (controlling for age, sex, wear-time, and study) between bouts of ≥1 and ≥2 minute durations were ≥ 0.98 irrespective of intensity thresholds. Correlations for bout ≥1 minute with ≥5 and ≥10 minute bout-durations were ≥ 0.84 and ≥ 0.62, respectively.

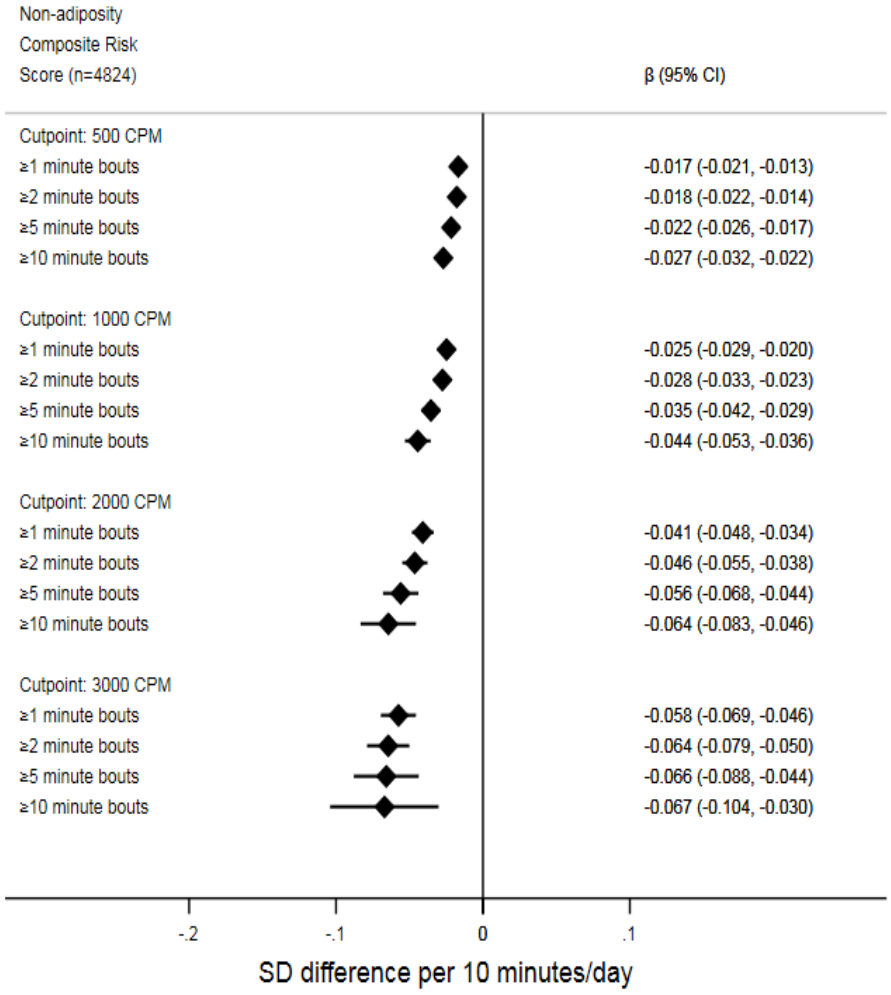
Forest plots of bout/intensity combinations and their associations with the composite risk score, non-adiposity composite risk score, and BMI are shown in figure 2 and 3. Results for insulin, glucose, triglyceride, HDL-cholesterol, diastolic blood pressure and waist-circumference are presented in the appendix (study 3). Intensity/bout combinations were negatively associated with the cardiometabolic risk factors suggesting participants with higher activity levels had more favourable risk profiles irrespective of intensity threshold and bout-duration in the range examined. Additional control for BMI attenuated effect-sizes, with attenuation appearing greater (absolute and relative) at higher intensities. Overall, the data suggested a pattern of increasing effect-sizes with activity accumulated at higher cut-points and in longer bout-durations. Effect-sizes for glucose and triglycerides followed a more irregular pattern. Meta-regression suggested independent contributions of intensity for all outcomes except for glucose (95% CI: -0.003 (-0.009 to 0.003) mmol/l per 1000 counts/min threhold) and triglyceride (95% CI: -0.004 (-0.009 to 0.0005) mmol/l per 1000 counts/min threhold). No statistical support for independent effects of bout-duration on any outcome was found in meta-regression. Each 1000 counts/min increase in the activity threshold was

associated with a statistically significant -0.026 (-0.039 to -0.014) standard deviations and a statistically significant -0.064 (-0.09 to -0.038) kg/m^2 difference in the beta-coefficient for the composite score and BMI, respectively. When adding the intensity-by-bout duration interaction terms, these did not reach statistical significance for any outcome.

Replacing 10 minutes/day of activity accumulated in short bouts with an identical amount of same intensity time accumulated in medium or long bouts in isotemporal substitution models produced mixed associations with the cardiometabolic risk factors. Substituting short bout activity above 500 counts/min with physical activity accumulated in long bouts was associated with a statistically significant -0.032 (95% CI: -0.047 to -0.018) standard deviation *lower* composite score, but substituting 10 minutes of short bout activity above 3000 counts/min with the same amount of activity accumulated in long bouts was associated with a statistically significant 0.066 (95% CI: 0.013 to 0.118) standard deviation *higher* composite score. For BMI, the reverse pattern appeared as substituting short bout physical activity at ≥1000 and ≥2000 counts/min for long bouts of activity was associated with a *lower* BMI, while substituting short bout for medium duration activity with 500 counts/min as the cut-point, was associated with a *higher* BMI.

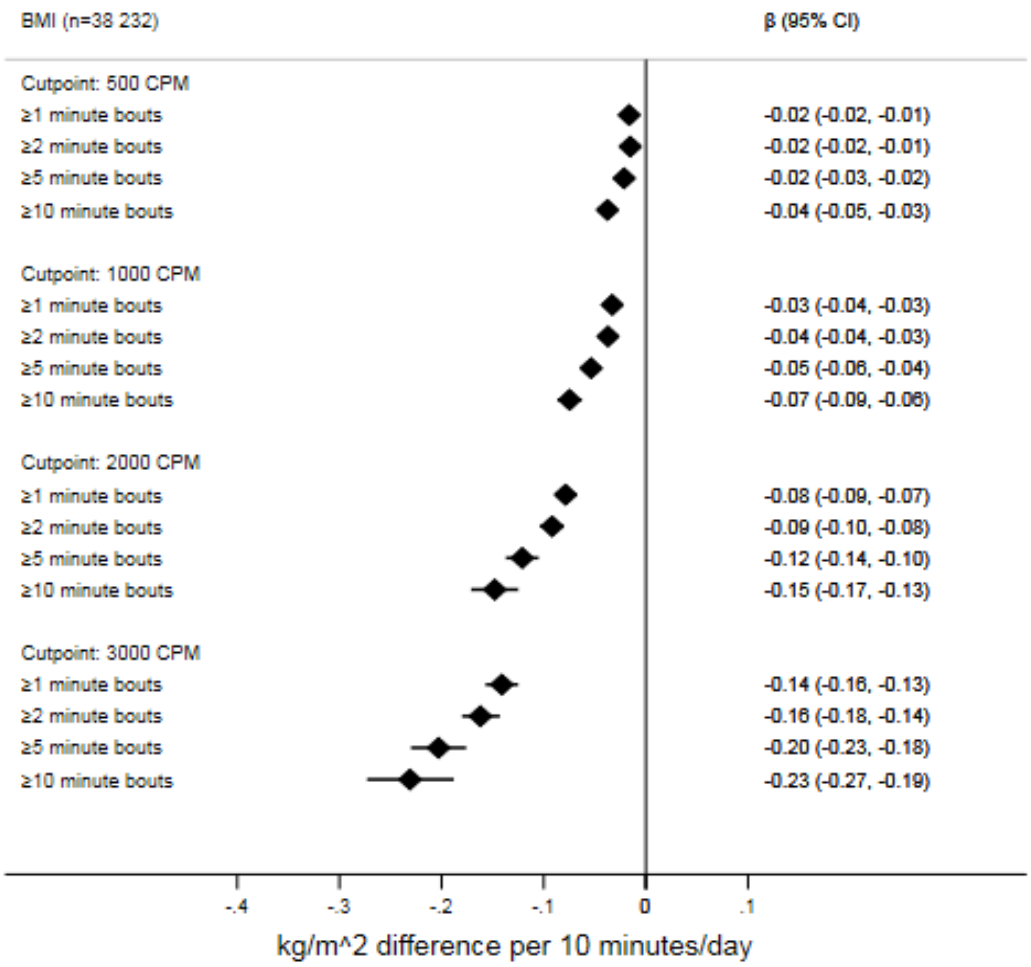
Figure 2. Forest plot of associations between intensity/bout combinations and composite risk scores





Beta-coefficients and 95 % CI from linear mixed regression models controlled for age, sex, wear-time including study and participant as "random-effects". Non-adiposity composite risk score additionally controlled for BMI. The figure is included from study 3 in the appendix.

Figure 3. Forest plot of associations between intensity/bout combinations and BMI



Beta-coefficients and 95 % CI from linear mixed regression models controlled for age, sex, wear-time including study and participant as "random-effects". The figure is included from study 3 in the appendix.

Study 4

Changes in the composite risk score were significantly (p-values ≤0.002) associated with changes in the composite muscle-fitness score (-0.19 (95% CI: -0.30 to -0.07) standard deviation), muscular strength (-0.15 (95% CI: -0.26 to -0.06) standard deviation), and agility (0.14 (95% CI: 0.04 to 0.23) standard deviation), but not muscular power (-0.06 (95% CI: -0.14 to 0.03) standard deviation, p=0.17). Following addition of changes in cardiorespiratory fitness to models, only muscular strength remained significantly associated with the composite risk score (-0.10 (95% CI: -0.19 to -0.003) standard deviation, p=0.04), but this association was lost completely when removing waist-circumference from the composite risk score and including changes in waist-circumference to the model (0.01 (95% CI: -0.10 to 0.11) standard deviation, p=0.93). In gender-stratified models, the composite risk score, triglyceride, total cholesterol/HDL-cholesterol ratio and waist-circumference were significantly and inversely associated with composite muscle-fitness (p-values <0.02) in girls with standardized betas ranging from -0.22 (95% CI: -0.40 to -0.04) for triglyceride to -0.28 (95% CI: -0.45 to -0.12) for the composite risk score. Only the association between total cholesterol/HDL-cholesterol ratio and composite muscle-fitness (-0.21 (95% CI: -0.33 to -0.09) standard deviation, p=0.001) remained statistically significant when adding cardiorespiratory fitness and waist-circumference to models. In boys, prospective associations with composite muscle-fitness were significant for waist-circumference (-0.29 (95% CI: -0.40 to -0.18) standard deviation, p<0.001) only. Despite more favourable baseline characteristics in analysed participants compared to those lost to follow-up, results from imputed data yielded similar conclusions as non-imputed data. Using other scalings for body-weight generally resulted in attenuation of associations which were most pronounced in cross-sectional analysis. The theoretical scaling approach yielded smaller effect-sizes compared to the empirically based approach. In re-analysis of associations using baseline muscle-fitness only, muscular agility (p=0.02) but not muscular strength (p=0.62) was

associated with 2-year changes in the composite risk score. This association was only slightly attenuated after additional control for baseline cardiorespiratory fitness (p=0.04). Further, the composite muscle-fitness score was not associated with waist-circumference at follow-up (p=0.07).

Study 5

Despite more favourable clinical characteristics in the 312 analysed participants compared to the 897 CHAMPS-study DK participants not available for long-term follow-up, no indication of differential characteristics between those lost to follow-up at intervention and control schools were found for anthropometric variables, the composite risk score, or cardiorespiratory fitness at any time-point (p-values >0.34). However, indications of non-identical missingness characteristics were observed for some demographic variables and for sexual maturity in 2012.

Baseline (2008) characteristics of the analytical sample are shown in Table 5. At baseline, participants at intervention schools had a 1.79 (0.06 to 3.53) mmHg higher systolic blood pressure, but a 19 % lower HOMA-IR score. The prevalence of overweight or obesity was 8.4 % in 2008 and 6.8 % in 2015 with no statistical difference between intervention and control schools (p-values ≥0.21).

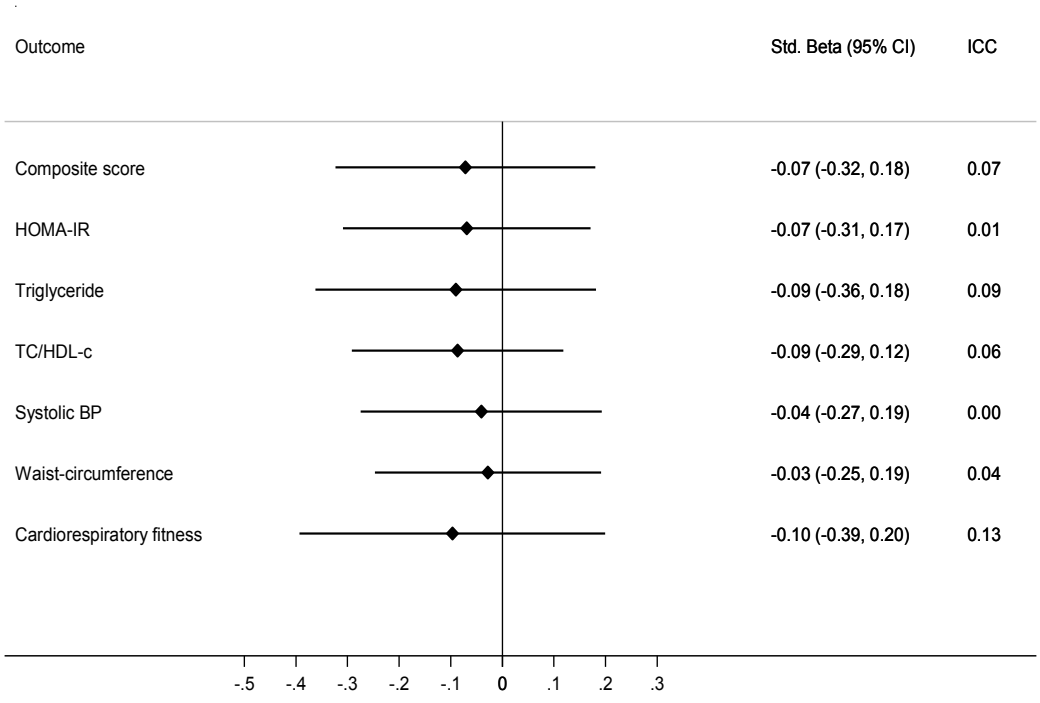
Table 5. Baseline characteristics of participants

	n	Intervention	n	Control	p-value for between school-type difference
Age (years)	217	7.8 (1.3)	95	7.8 (1.3)	0.98
Sex (% girls)	217	56	95	44	0.06
Stature (cm)	216	129.2 (8.8)	92	128.8 (9.7)	0.71
Body weight (kg)[a]	217	26.8 (5.4)	92	26.9 (5.9)	0.94
Sexual maturity (% tanner stage 1)	217	75	95	79	0.38
Mothers educational attainment (% any tertiary)	210	65	92	58	0.24
Family history of NCDs (% yes)	211	50	92	53	0.58
Birthweight (gram)	298	3495 (711)	91	3481 (550)	0.87
Composite score (z-sores)	200	-0.07 (0.97)	89	0.17 (1.05)	0.06
Cardiorespiratory fitness (meters)	203	887 (101)	91	885 (107)	0.84
Systolic blood pressure (mmHg)	214	100.1 (6.5)	90	98.3 (8.2)	0.04
Waist-circumference (cm)[a]	217	55.0 (52.5 - 58)	92	56.5 (53 - 61.3)	0.08
HOMA-IR[a]	217	0.54 (0.37 - 0.75)	95	0.65 (0.47 - 0.89)	0.004
Triacylglycerol (mmol/l)[a]	217	0.58 (0.46 – 0.71)	95	0.56 (0.47 – 0.71)	0.79
TC:HDL-cholesterol-ratio[a]	217	2.6 (2.3 - 3.0)	95	2.6 (2.4 - 3.0)	0.51

Mean (standard deviation) is given unless otherwise noted. [a]median (25^{th} – 75^{th} percentile). NCD= non-communicable diseases. HOMA-IR= homeostasis model assessment of insulin resistance. TC= total cholesterol. HDL-cholesterol= High-density lipoprotein cholesterol. The table is included from study 5 in the appendix.

Figure 4 presents differences in composite and individual risk factors at follow-up between intervention and control schools. The difference of -0.07 (95% CI: -0.32 to 0.18) standard deviation did not reach statistical significance. Likewise, differences were non-significant for the individual risk factors (p-values ≥0.41) with standardized betas ranging from -0.10 (95% CI: -0.39 to 0.20) for cardiorespiratory fitness to -0.03 (95% CI: -0.25 to 0.19) for waist-circumference. The direction of associations, although not significant, favoured intervention schools except for cardiorespiratory fitness.

Figure 4. Long-term difference in risk factors between intervention/control schools



Estimates are standardized mean differences at follow-up (in z-scores with 95% CI), using control schools as the reference. Negative values are in favour of intervention schools except for cardiorespiratory fitness where a positive value favours intervention. ICC= intra-class correlation coefficient, BP = blood pressure; TC = total cholesterol, HDL-c = high-density-lipoprotein cholesterol. The figure is included from study 5 in the appendix.

In analyses with outcomes stratified at the median baseline-level, the least favourable half at intervention schools presented, compared to the least favourable half at control schools, a -0.17 (95% CI: -0.55 to 0.20) standard deviation lower composite score at follow-up albeit the difference did not reach statistical significance. No statistically significant differences were observed for the individual risk factors (p-values $\geq$ 0.07). When analysing physical activity levels as assessed in 2015 from n=495, neither participation in structured leisure-time physical activity (odds ratio: 0.79 (95% CI: 0.46 to 1.36)), %MVPA/day (unstandardized beta: -0.17 (95% CI: -0.67 to 0.33)), or mean counts/minute (unstandardized beta: -25 (95% CI: -58 to 8)) reached a statistically significant difference between intervention and control schools.

Discussion

The studies included in this thesis have contributed to understanding the role of physical activity, sedentary time, and muscle-fitness in modulating cardiometabolic risk factors in young people. Additionally, the potential for an augmented physical education program during school as a strategy for primordial prevention was evaluated. Three different study designs were utilized in the original studies for the thesis; a cross-sectional design, a prospective observational design, and a non-randomized controlled intervention design. These different study designs present some overlapping sources of bias, while some are distinct. Key methodological considerations pertinent to the studies included in the thesis are discussed in the ensuing sections. The section will end with a comparison of results in relation to other studies and a consideration of underlying biological explanations.

Information bias

Information bias relates to systematic or random error in the assessment of exposure, outcome or other variables. Systematic (differential) error in exposure or outcome assessment may produce spurious associations. As an example, physical activity may be over- or underreported from subjective methodology in obese children because of social desirability bias. Random (non-differential) error in (continuous) exposure variables in a linear regression model will cause regression-dilution leading to attenuated effect-sizes. Conversely, non-differential measurement error in outcome assessment produces larger CIs and may thus be ameliorated if an infinite sample-size is available[185]. Compared to the gold-standard doubly labelled water method, accelerometry offers low-to-moderate criterion-related validity of free-living physical activity energy expenditure in children and adolescents. Variance explained is about 26 %, but with substantial between-monitor and between-study variation[186]. In the ActiGraph 7164, average counts/min assessed over a 14-day period for a mean of 13 hours/day explained 29 % of the variance in physical activity energy expenditure in 26 9-year old children[187]. Importantly, Bland-Altman plots did not suggest a graded mean bias, indicating absence of systematic bias by activity level. More relevant to studies included in this thesis is the ability to discriminate activities performed at distinct intensity levels. In study 2, a cut-point of ≥2296 counts/min was used to define MVPA[134]. This cut-off corresponds to the acceleration of the hip/waist during walking[134, 188, 189] and thus appears a reasonable cut-off for the lower spectrum of the moderate intensity domain, although 2000 counts/min has also been used[76]. Other studies again have suggested MVPA should be defined in the range of 3000 to 3600 counts/min[188, 190]. Walking is a reasonable reference-point for health-enhancing physical activity because it is accessable, contributes a substantial proportion of adult leisure time physical activity, and has well-established health-benefits[191]. In study 2, a sensitivity analysis re-defining MVPA as ≥2000 counts/min did not alter conclusions. Re-analysis using ≥3000 counts/min as the cut-point

was not performed as only 6.5 % of participants achieved 60 minutes/day above this cut-point rendering CIs uninformative. The ≥2296 counts/min cut-point was applied in an external validation study (U.S) of the ActiGraph GT1M and showed the highest classification accuracy for MVPA (specificity at 88 % and sensitivity at 92 %) in a sample of 5 - 15 year old boys and girls when compared with 4 other common cut-points[188]. Another validation study (of the ActiGraph GT3X+), also in 5 - 15 year old boys and girls (Brazil), identified a very similar cut-point at 2428 counts/min as the optimal balance between specificity and sensitivity of MVPA (specificity at 94 % and sensitivity at 97 %)[189]. However, these validation studies are conducted under strictly controlled settings and using selected activities in a laboratory so the discriminatory capability is likely lower during free-living activities. A substantial part of the variation between suggested MVPA cut-points is explained by a combination of differences in laboratory activities and participant characteristics. Waist-mounted accelerometry suffers from substantial underestimation of activity during cycling[192], weight-bearing activities, graded locomotion, and upper-body movements. Older units, as used for the studies in the thesis, are also not water-proof resulting in inability to capture e.g. swimming. The result is a systematic underestimation of physical activity of individuals performing large amounts of these activities. The direction of bias would likely be an attenuation of effect-sizes, however the magnitude of bias is difficult to ascertain and will depend on the amount of engagement in these activities in a given population. It may be more pronounced in study 2 which dichotomized an absolute value of MVPA/day.

The major source of error in accelerometry is likely to be non-differential. A one-off assessment of physical activity explained 46 % and 64 % of the 1-year variation in MVPA in 12 year old British[190] and 10 year old Danish[193] boys and girls, respectively. This suggests the regression coefficients obtained in study 3 and evaluated in original studies included in study 1, are substantially attenuated and should be nearly doubled. This is however based on the assumption that all variation is a result

of measurement error, which is unlikely to hold because of true variation in physical activity over time. Measurement error, flooring, and regression to the mean effects have been suggested explanations of the counterintuitive association between higher physical activity levels and higher levels of adiposity observed in some prospective studies. Similarly to MVPA, there is no well-established validated cut-off point to define sedentary time from waist-worn accelerometry. The <100 counts/min appears to have prevailed and is employed in most studies. The amount of variation explained by a one-off assessment of sedentary time is roughly 60%[190, 193]. The definition of <100 counts/min does however not provide a distinction between standing (which may have positive health-benefits)[194] and a sitting or reclining position, and is thus not directly compatible with recent consensus definitions of sedentary time[71].

The use of a harmonized data-reduction protocol across 20 studies in ICAD is a substantial strength of the thesis, reducing between-study variability as a result of different post data-collection decisions. It is well-know that non-comparability across studies because of data-reduction heterogeneity represents a major concern in the interpretation of absolute physical activity levels from accelerometry[195]. Some post data-collection decisions such as short non-wear definitions may also disproportionately (systematically) penalize overweight individuals[196] potentially causing issues with selection bias (discussed later). A limitation of the ICAD is the 60-second epoch as a consequence of including mostly older studies. The activity patterns of particularly pre-adolescent children is sporadic[48] which may not be reflected by averaging over 60 seconds. Further, activity accumulated at higher intensities may be downwards biased[197]. These potential limitations in exposure operationalization are likely to impact study 3 in particular.

In study 5, assessors were not blinded to participant intervention/control status which may influence outcome ascertainment (ascertainment bias) of the cardiorespiratory fitness test by disproportionately encouraging intervention school children to perform better. Analysis in study 4

would most likely not be affected by ascertainment bias as the cardiometabolic profile was unknown to research staff (but it may have been speculated based on adiposity status) and analysis were controlled for intervention/control status. Standardized protocols and training of research staff were employed to minimize this potential bias.

In studies were 1 or more intervention group is being compared to a control condition valid inference from the experimental contrast is dependent on actual differences in exposure. In many trials, the achieved contrast is substantially smaller than intended because of compliance issues in intervention/ and or control groups. That is, intervention participants do not perform the intended tasks or control participants engage in the experimental task. As the intervention in study 5 was delivered as part of compulsory school curriculum, bias due to insufficient *intervention exposure* is unlikely. A causal interpretation of the effects of delivering additional 180 minutes weekly minutes of physical activity through augmented physical education may however still be biased if physical activity of the intervention group is also effected outside of the intervention (e.g. reduced), or control participants increase their activity as a consequence of involvement with the study (which was widely disseminated). This would render the contrast in *total exposure* smaller than intended. Despite intervention children being more physically active during school hours there was no difference in overall physical activity between intervention and control participants in 2010[45] or when evaluated at the long-term follow-up in study 5 (noting that suboptimal data was available at both these time-points). Thereby, some concern with establishment of a valid total physical activity contrast appears justified. Assuming the no-difference in overall physical activity between intervention and control schools is correct, the nature of this potential bias has substantial and far-reaching consequences. If control participants increased their activity in response to being monitored, the causal effect of the intervention might may beneficial and we would fail to identify a potentially meaningful public health strategy. On the contrary, if intervention participants lowered

their activity outside of the intervention the strategy would likely not be of preventive relevance and this question would be appropriately addressed by the contrast. The direction of these unintended and uncontrolled effects is speculative but lower leisure-time sport participation in girls at intervention schools suggest some unintended intervention effects could be in place[45]. Formal quantification would require action in the preparation phase such as employing a Solomon four-group design[198].

Both upper- and lower body muscle strength increases with engagement in progressive resistance-training in pre-adolescent and adolescent children irrespective of weight-status[99, 199, 200]. This suggests high muscle-fitness is a valid model for engagement in muscle-strengthening activities. Handgrip strength is associated with global muscle-fitness[150] and usually produces higher reliability coefficients than lower body jump tests[149] when a standard test-retest procedure is employed. The reliability of muscular agility tests is not well-described[149]. Lower reliability would suggest larger issues with non-differential measurement error, potentially explaining apparent muscle-fitness phenotype specific associations with the cardiometabolic risk markers. Standing vertical displacement allowing for countermovement requires, in addition to rapid muscle-contractions, technical skills such as efficient agonist-antagonist co-activation and upper- and lower body limb coordination when performing the stretch-shortening cycle. Likewise, the 50-meter short shuttle-run is a highly complex task including acceleration, deceleration, and reversing movement direction and thus taps multiple motor- and muscle skills. In contrast, handgrip strength involves contraction of an isolated body-segment. An additional issue is how to correct muscle-fitness indices for differences in body-size (discussed in greater detail in the confounding section).

Even in a fasting state the absolute levels of the biochemical markers are highly variable between days[155], and with additional variation across ICAD studies due to study-level differences (e.g. analysis kits and protocols). Further, blood pressure measurements are only moderately reliable.

The effect of these measurement issues is likely non-differential misclassification at the individual level, but could also introduce additional study-level variation if not appropriately modelled. In study 2, meta-analysis was used to pool study-specific estimates while study 3 allowed for variation between studies by modelling study-specific intercepts. To reduce the impact of measurement error in blood pressure readings a minimum of 3 consecutive stabile blood pressure readings were required in the CHAMPS-study DK. To maximize information on the latent cardiometabolic profile, a composite continuous risk score was created as main outcome variable in studies all studies, with individual risk factors additionally analysed to assess their relative contribution to the summed score and for aetiological inquiry. In study 5, the biochemical risk factors were standardized to week-day to minimize irrelevant extraneous sources of variation. However, while composite risk scores in youth are associated with morbidity in young adulthood, the effect size[201] and associations with physical activity and cardiorespiratory fitness[202] varies between score-definitions leaving the optimal composition of variables unidentified[203]. HOMA-IR was calculated from fasting blood samples as an indicator for hepatic insulin sensitivity. HOMA-IR has previously been validated against the gold-standard for insulin sensitivity assessment the euglycemic-hyperinsulinemic clamp. In a study including a sample of about 300 U.S. adolescents, Schwartz and colleagues reported that HOMA-IR explained only 24 % of the variance in clamp-derived insulin sensitivity[204]. They also reported that more variance was explained in participants with a BMI >85th percentile. In another U.S. study including pre-adolescent children, normal-weight adolescent, and obese adolescents (n=156), the variance explained was 83 %[205]. Thus, HOMA-IR appears a reasonable, although imperfect, indicator of insulin sensitivity in young people of varying ages and degrees of adiposity. Consistent with the data from Schwartz and colleagues[204], the slope of the adiposity-risk factor association may be non-linear throughout the adiposity spectrum[206] potentially causing some misspecification of the mediation model in study 2. This will likely result in

regression dilution because of the intercept being pulled too high at the low end of the adiposity spectrum and to low at the high end of the spectrum. The same could hold for physical activity[77] but would probably be to a lower extent.

Studies 2 to 5 used indirect anthropometrical assessments of general (BMI) and central (waist-circumference) adiposity and not a criterion-related measurement such as dual-energy X-ray absorptiometry (DXA), displacement plethysmography or magnetic resonance imaging. Using anthropometrical indicators offer substantial advantages in feasibility in epidemiological studies. BMI and waist-circumference/stature ratio offers indicators of excess weight for stature and excess girth for stature, respectively. By squaring and dividing with stature the association with stature is reduced (but not eliminated, and the correlation may vary with age and sexual maturity[207, 208]) which may be of particular concern in growing children with larger variation in stature than adults. These procedures serve first of all to remove variation in weight and girth owing to age and growth (which would produce confounding), but as stature may itself (irrespective of any age or sexual maturity effects) be a marker of elevated risk levels[209], the appropriate elimination of body-size related associations may be needed to appreciate the isolated effect of excess adipose tissue. Alas, the optimal approach remains unidentified. The degree of measurement inaccuracy in BMI is likely greater at the lower end of the adiposity spectrum (with larger relative influence from lean mass) compared to the children with high levels of adiposity where an additional 1 unit increase in BMI will reflect adipose tissue to a larger extent. This would result in differential measurement error, likely pulling the association towards the null. A sensitivity analysis in study 2 using a stature-independent (by log-log regression) waist-circumference index as mediator produced virtually no change in estimates. Further, the correlations between DXA (% fat mass), BMI and waist-circumference in children are high (variance explained ≥ 64 %)[210, 211], the (< 6 years) tracking-coefficients are similar[210, 211], and associations with health outcomes are nearly identical[210-212]. This

suggests conclusions from the 2 cross-sectional studies would not be substantially altered by including a more precise adiposity assessment. High body fat from DXA do however in some, but not all studies[211], add to the identification of high-risk children over elevated BMI[213] and, as suggested from study 1, the inability of BMI to distinguish lean mass from adipose tissue may have substantial implications for inference from prospective studies of physical activity and weight-gain in growing children. This could potentially have implications for study 4, causing underestimation of muscle-fitness related benefits on waist-circumference.

Selection bias

Study 4 and 5 included a substantial loss to follow-up and analysed participants presented with slightly more favourable cardiometabolic risk profiles than those not included in the studies. This was most consistent for higher cardiorespiratory fitness in the analysed samples. A key issue is whether the exposure-outcome association is expected to be substantially different between analysed and non-analysed individuals. Because participants were recruited from a general population of healthy individuals, the most likely impact is a reduction in exposure/outcome variation within the sample, potentially leading to attenuated regression coefficients. In study 4 it was possible to re-analyse muscle-fitness associations with the cardiometabolic risk factors using MICE to keep all 1209 consenting participants in the analysis. Despite small variations, the overall pattern remained unchanged suggesting selection bias did not explain the results. This analysis does however not account for the about 300 children who were invited but did not consent to provide any data for the study. MICE is also based on the assumption of data being missing at random (MAR) conditional on variables included in the imputation step, however this assumption cannot be empirically tested. In study 5 selection bias by attrition of individuals presenting the least

favourable cardiometabolic profiles could have reduced the potential for beneficial intervention-related adaptations to occur (bias towards the null). This is because the 2-year evaluation of the study found particularly strong effects in the half presenting the least favourable metabolic profile[154]. No evidence of differential missingness characteristics in these variables across intervention and control participants was observed, but the substantial attrition suggests the analysed sample is highly selected. A cautions interpretation of the estimates from study 5 is therefore warranted. The prevalence of overweight/obesity in analysed participants in study 4 and 5 was fairly low but this is the case for the general CHAMPS-study DK cohort (prevalence was 11.3 % for the full cohort at baseline).

Relevant to study 2 and 3 ICAD does not hold information on participants from original studies not providing physical activity data. This makes it impossible to evaluate the possibility of selection bias. It is highly likely that participants included in ICAD are generally healthier, more physically active and come from families with higher socioeconomic possibilities than those not included. Some of the ICAD cohorts (e.g. EYHS and NHANES) are based on representative or randomly selected individuals, while other studies are based on convenience samples. True representativeness is however not possible because of non-response and unavailability of sampling frames or other information to allow appropriate weighting of individuals. With overweight/obesity prevalence at 21.4 % the ICAD at least covers a wide spectrum of cardiometabolic risk profiles. Concerns with selection bias apply to studies included in study 1 as well (attrition was 50 % in some studies) and are amplified by the relatively modest sample-sizes of included studies.

Despite the advantageous of ICAD there is still a need to make decisions on what constitutes a "valid" day and what defines a "valid" measurement period. Clearly, up to a point more days including more time will present a better representation of an individual's habitual physical activity[214]. However, this decision should be balanced against the substantial loss of eligible

participants when applying a progressively stricter criteria and the ensuing possibility of selection bias. It could be argued that 1 "valid" day in a new individual will ultimately provide more information about the population than 1 additional day in an individual already having 2 or 3 "valid" days. However, this will only be the case assuming the added individual has not specifically selected to wear the monitor on this particular day. The impact of different valid-day and valid measurement period definition was assessed in study 2, resulting in only minor changes.

Confounding

Common to observational studies and the controlled intervention in study 5 is that exposure status is not randomly assigned but self-selected by individuals/schools. Accordingly, the contrasts in the studies are all susceptible to bias (confounding) by other potentially self-selected exposures if information on these variables is not carefully collected and included in analysis. Putative confounding variables such as sex, age, sexual maturity, birthweight, demographic indicators, BMI of parents, and family history of NCDs were included in analysis when possible. However, besides age and sex, these variables are only crude indicators of biological, sociodemographic and genetic indicators and are completely incapable of refuting the possibility of residual confounding from these sources in the observed associations. Information on other energy-balance related behaviours was not available leaving the additional possibility of unmeasured confounding by e.g. diet quality and quantity[32] or from sleep quality and quantity[215]. Other potentially unknown confounding variables also represent potential sources of bias. Imperfectly measured or operationalized (e.g. collapsing categories because of insufficient data) and unmeasured confounding variables may lead to an over- or underestimation of the strength of associations. Insulin levels fluctuate substantially throughout puberty in both boys and girls but it is very unlikely that self-reported tanner stage

provides an adequate reflection of these biological variations. Original studies included in study 1 varied substantially in their ability/efforts in including putative demographic, biological, energy-balance related and time-varying confounders in their models highlighting the limitations in making causal inference from the literature. When omitting 1 study at a time in study 2, some variation in the effect-decomposition was present. As re-analysis using only age and sex as control variables resulted in nearly identical estimates as in the analysis applying maximal control. This implies the variation in estimates across samples owes to other sources of heterogeneity (potentially unmeasured) than available control variables. It also suggests that either; 1) confounding of the physical activity – cardiometabolic risk association is unproblematic or 2) that available variables are far from optimal. I would lean against the latter. The decision to apply restrictive inclusion criteria to maximize possibility of confounding control in study 2 was made a priori. Non-analysed participants presented as less physically activity and with less favourable cardiometabolic risk profiles, reiterating the possibility of selection bias by restriction. Study 3 included a minimal amount of control variables because the dataset was pooled to maximize the possibility of focusing on the intensity/bout-duration contrasts.

Participation in the intervention condition in the CHAMPS-study DK was self-selected by schools. Control schools were matched on size, socioeconomic uptake area, and urban/rural location in attempt to provide an unbiased contrast. Based on summary statistics from Statistics Denmark, there were no differences in the mean household income or parental educational level between intervention or control schools[216]. Several individual-level variables were included in regression models, but confounding at the school level cannot be refuted, although school-level random-intercepts suggested variation at this level was negligible after accounting for school-class membership. Self-selection of participants into intervention or control schools was avoided by not disclosing the intervention to parents prior to commencing the school-year.

The optimal expression of muscle-fitness is complicated because of associations with body size. Larger individuals will be stronger necessitating some normalization but the optimal approach remains unidentified and may depend on the specific research agenda and muscle-fitness phenotype. Often, as in the present thesis, division by body-weight is employed for handgrip strength but as seen in study 4 this will not remove the association entirely. Thereby, associations between muscle-fitness and health-outcomes may be confounded by adiposity. The direction of bias will likely be away from the null (association appears stronger) if estimates are confounded by adiposity as suggested by the negative correlation between per kg body-weight normalized muscular fitness and body-weight. As was seen in study 4 the choice of normalization affected particularly the cross-sectional results while prospective analyses were affected to a lesser extent but were not unaffected. As has been recommend earlier muscular power and agility were not normalized because these tests do to some extent control for body-weight by design[162] which is indicated by correlations with body-weight being fairly weak. These observations suggest at least some of the association between higher muscle-fitness (particularly muscular strength) and cardiometabolic risk factors is under the influence of adiposity-related confounding. A substantial strength of study 4 was the ability to control for changes in cardiorespiratory fitness. The impact of controlling for cardiorespiratory fitness on effect-sizes was strong for boys in particular. Thereby, confounding from engagement in aerobically demanding activities would be reduced as the correlations between cardio- and muscle-fitness indices were moderate. Apart from growth, physical activity is the only modifiable factor behind variation in muscle-fitness in healthy young individuals but fitness also has a strong genetic component[217]. A genetic profile predisposing to higher muscle-fitness could also be linked with e.g. higher enzymatic capacity or other favourable metabolic adaptations. This could potentially confound any fitness-metabolic control association.

Reverse causation bias

As study 2 and 3 were cross-sectional the causal direction of effect is impossible to discern from these studies alone. While it is less intuitive (but not impossible) how slightly higher levels of biochemical risk markers in healthy children should negatively impact physical activity levels, there is well-supported evidence from prospective studies that higher levels of adiposity predispose to lower physical activity and elevated sedentary time[78, 170, 218]. This may come from the excess body-weight causing decreased joy or inability to compete with peers, resulting in abstainment from engagement in exercise or play and potentially replaced with time spent sedentary. Decreased physical activity levels may then lead to further weight-gain causing a vicious circle in a potentially bi-directional association. These observational data are supported by the results of Mendelian Randomization analysis were children carrying larger amounts of genetic variation predisposing to higher BMI also had lower physical activity levels[219] and more sedentary time[219, 220] indicating a causal direction of effects. While it is quite difficult to completely ignore the role of physical activity as part of energy balance, randomized controlled trials in adults have shown substantial variation in the amount of weight-loss following highly controlled exercise modalities of up to 2000 kcal/week[101]. Some individuals even gain weight despite excellent trial adherence. This suggests physical activity is not the major source of variation in body-weight and that an increase in physical activity may in some individuals be regulated by decreased non-exercise activity in other domains[221] and/or by concomitant increases in total energy intake[101]. Importantly, physical activity was associated with the biological risk factors after control for BMI and waist-circumference in study 2 and 3 and with the total cholesterol/HDL-cholesterol ratio in girls in study 4, suggesting reverse causality from adiposity on physical activity energy expenditure would not explain these beneficial associations.

Study 4 was prospective which strengthens a causal argument as information on exposure was assessed before the outcome. However, reverse causality from adiposity is not eliminated by this design as the child may already have been overweight or on an unfavourable weight-trajectory resulting in low muscle-fitness. This would require a pre-baseline measure of adiposity to counter[139, 222]. Many of the original studies in study 1 used a one-off baseline assessment to predict subsequent adiposity or risk markers levels. When adiposity is the outcome this approach may not adequately reflect the aetiology of excess adipose tissue accumulation as (assuming this is the causal direction) the effect of physical activity may already have influenced the weight-trajectory. Thereby, a zero-association with subsequent body-weight when controlled for baseline body-weight is observed. Excess weight-gain under stabile activity patterns would not continue as a new energy-balance will ultimately be achieved as a result of increased body-weight causing both higher resting and activity energy expenditure. This also makes it relevant to consider what would constitute "sufficient" follow-up time. A minimum 2 year inclusion criteria was chosen in study 1 under the assumption that physical activity would influence excess tissue accumulation by a very slight, but sustained, positive energy balance thereby requiring a long period to accumulate to a detectable degree. This decision will inevitably be somewhat arbitrary. When considering 5 identified studies on adiposity relevant to study 1, but with between 0.6 and 1.7 years of follow-up[218, 223-226], only 2 of these reported any significant association between an adiposity index (as compared with 10 of 15 for the ≥2 year studies) indicating some relevance of allowing for a "not to short" follow-up duration. However, the apparent difference may also relate to outcome measure as only 1 of these studies used a criterion-method for adiposity assessment[223]. The correct model for excess adipose tissue accumulation in young people remains to be established and is a difficult issue because of natural growth. For the biological risk markers which are more readily influenced by very recent behaviour, a one-off assessment of physical activity as a marker of habitual physical activity may be

more representative of aetiology. As an alternative to the baseline only model, a change vs. change model was considered in study 4 under the rationale that changes in muscle-fitness would likely result in a lowering of adiposity levels and improvement in the biological risk factors. This is somewhat analogous to what would be induced in an experimental setting, but has also been criticised for being a "masked" cross-sectional study[132]. A noticeable strength is that it was possible to analyse follow-up outcome variables 6 months after assessment of changes in the muscle-fitness phenotypes. Applying a temporal lag increases the likelihood that it is changes in muscle-fitness which causes changes in adiposity and the risk markers and not vice-versa. The importance of time-resolution in model-inference was underpinned in re-analysis of muscle-fitness associations using baseline exposure only. These models showed a phenotype-specific pattern of results which was largely incompatible with inference from observations when modelling changes in exposure.

Systematic evidence synthesis

The methodology applied in identifying and retrieving original studies for the review in study 1 did not conform with best-practice recommendations such as those specified in the PRISMA statement[227]. E.g. a single reviewer identified relevant studies from a search in a single database. A systematic review should search multiple databases and studies/data be retrieved by >1 researcher. This could have resulted in relevant studies being missed and/or study information being incorrectly transferred. Further, the risk of bias in an across studies was not considered. A beneficial association was noted in a study irrespective of effect-size or the number of non-significant associations also observed. This "vote-counting" procedure does not provide data on clinical significance, is over-inclusive (optimistic) and increases the likelihood of false positives. Finally, there was no attempt to synthesize harmonizable data in meta-analysis or evaluate indications of

publication bias. As retrieved studies were fairly small, random variation in effect-estimates may be non-trivial and an estimate from meta-analysis informative. However, reporting was heterogeneous and others have failed to perform meta-analysis on this account[66]. Some participants will be analysed >1 time in original studies included in study 1 as they could be included in analysis from their original cohort and in the ICAD publication[78]. In fact, and individual may be included in this thesis as much as four times (2 times in study 1, in study 2, and in study 3). The effective number of unique individuals providing data is thus lower than the sum of available sample-sizes. Optimally, such dependence between observations is handled. Potential source of bias in original studies included in the review are discussed above.

Effect decomposition

Application of effect-decomposition models inevitably implies the assumption of exposure being causality related to the outcome of interest. Several stringent and often untestable assumptions such as temporality, no unmeasured or residual confounding of the exposure-mediator, exposure-outcome, or mediator-outcome associations, no mediator-outcome confounder which is affected by the exposure, no measurement error, and correct specification of statistical models[139] are needed for estimates to represent causal effects. The effect-decomposition in study 2 clearly violates the first assumption by being a cross-sectional study (potential implications discussed under section considering reverse causation bias). Additionally, the possibilities for confounding control were limited and heterogeneous across studies (potential implications discussed under confounding section). In the presence of a mediator-outcome confounder which is affected by the exposure, current models are incapable of correctly decomposing the total effect[139]. Common causes of both adiposity and increased levels of biological risk factors which theoretically could be influenced by

physical activity include; 1) diet quality (e.g. diets with a high proportion of total energy intake from (processed) carbohydrate causing raised insulin, triglyceride and lowered HDL-cholesterol and may facilitate weight-gain[32]), 2) a genetic variation predisposing to elevated BMI and inferior glycaemic control[28, 228], or 3) poor mental health/stress causing weight-gain and elevated blood-pressure[229]. While these potential biological mechanisms are not documented to severely violate the assumption, the examples serve to highlight the complicated nature of effect-decomposition and the fallacies which may be committed when claiming causality from a statistical model. Measurement error is a substantial threat to correct identification of total effects and to the assessment of the relative contribution of direct/indirect effects. The consequence of non-differential measurement error in exposure assessment has been discussed above. While non-differential measurement error in the mediator will most likely result in downwards (towards the null) biased indirect effects (and simultaneous upwards-biased direct effects because the mediator is included in estimation of the direct effect[230]), the role of non-differential exposure measurement error is less intuitive. The indirect effect can be biased in either direction because measurement error of the exposure will tend to weaken the exposure-mediator association but will strengthen the mediator-outcome association (the mediator-outcome association is estimated in a model including the exposure variable). Which of these 2 consequences is more substantial will determine whether the indirect effect is biased towards or away from the null[230]. Regression calibration may be used to correct for non-differential measurement error but this was not pursued as available data[190, 193] was deemed insufficient. Sensitivity analysis estimating a range of effect-sizes under different measurement error assumptions could have been informative.

Early approaches for regression-based effect-decomposition relied on dichotomized decisions about statistical significance known as the "causal steps method"[231]. This approach is suboptimal as conclusions of statistical significance may be sample-size dependent and opposing direct/indirect

effects may cancel out total effects. Several methods for assessing mediation are now available as relatively easily implementable statistical software[136]. Widely used models for deducing direct/indirect effects are based on comparing 2 sequential regression models (as in the counter-factual approach) and are known as the "difference-method" or the "product-of-coefficients" method[139]. This approach is heavily inspired by the work of Baron and Kennedy[231]. The difference-method and the product-of-coefficients methods are identical for a continuous model on a difference scale. They produce valid estimates of the composition of effects only if there is no statistical interaction between the exposure and the mediator (in addition to all other assumptions being satisfied)[139], which may not hold for physical activity and adiposity[105]. The result is inability of the direct and indirect effects to sum to the total effect, which is necessary for evaluating their relative importance. In study 2, effects were decomposed while allowing for exposure-mediator interaction, but no strong influence was detected. This suggests application of the difference method in study 3 (and elsewhere in the literature) do not violate this part of the correct model-specification assumption. Other models which could have been applied were those based on structural equation modelling which offer substantial flexibility. The counterfactual approach was chosen because of its rigorous mathematical properties which were preferred over model flexibility (which comes at the cost of a proportionally larger sum of assumptions) and because the focus of the study was on estimating the indirect effect. Uncertainty around the indirect effect was calculated using a nominal 2.5^{th} to 97.5^{th} CI. This approach appropriately accounts for potential substantial non-normal distribution of indirect effects[232] which e.g. the Sobel-test does not. However, potential exposure-confounder or mediator-confounder interactions were not allowed for which may not adequately reflect the biology of insulin-resistance in growing children[168]. Using the counterfactual approach also necessitated dichotomization of the exposure variable as models including a continuous exposure, a continuous mediator, allowing for exposure-mediator and still providing correct effect-

decomposition are not currently available[136, 137]. Dichotomizing physical activity at any absolute cut-point does not mirror biology but when comparing variance explained by MVPA in its continuous form in a regression model including the composite-score, MVPA/day (continuous), age, gender and study, the r-squared was 0.071. The same model including the dichotomized PA exposure presented an r-squared of 0.061. That may be interpreted as these models explain roughly 7 and 6 % of the variation in the cluster-score, respectively. The same models with waist-circumference as dependent variable produced r-squared values of 0.496 and 0.495 for continuous and dichotomized MVPA, respectively, suggesting the decision to dichotomize did not materially distort model fit as compared to a linear model.

Representativeness of study samples

The samples included originated from populations of healthy children and adolescents suggesting wide generalizability of findings. Data from ICAD is heavily centred at the ages 9 to 15 years weakening inference to particularly younger children. The CHAMPS-study DK is based in the municipality of Svendborg which has a fairly homogenous White-European population of urban and rural residency. The main city has approximately 27.000 inhabitants. Based on summary statistics, parent at schools participating in the project had approximately 15% higher household income as compared with non-participating schools, while there was no difference in the educational level[216]. It is thus unclear how a similar intervention would fare in settings covering e.g. a more diverse population or in a population of predominantly lower or higher socioeconomic-status.

A composite risk score of standardized values of the risk factors will be unique to the sample. In study 2 and 3 the risk score was created by standardizing variables from 6 different samples of

individuals aged 6-18 years from the U.S. and 3 different European countries which increase generalizability. However, direct comparison of clinical characteristics from an internally derived composite score will be lowered in comparison with summing the number of risk-factors exceeding clinical cut-points as in the adult MetS definition. Strong evidence supporting such dichotomizations are still lacking in young people[122] and the stronger applicability of a continuous score in investigating aetiology such as gains in power speaks to its use.

Comparison with other literature

Prospective studies identified in study 1 generally suggested higher physical activity levels were associated with lower levels of adiposity indices over time. The results were not without exceptions and opposite associations. Higher physical activity levels were associated with more favourable biological risk marker levels. These observations were supported by an inverse, graded association between larger physical activity contrasts and metabolic homeostasis in study 2 and the graded association with intensity in study 3.

A systematic review published in 2016 only considered observational studies if they used objective physical activity methodology (primarily accelerometry was located) and included at least 1000 individuals for adiposity outcomes. Still, the review identified 72 studies on adiposity indices[66]. Cross-sectional studies consistently reported associations between higher levels of physical activity and lower levels of adiposity indices with MVPA associations being significant in 26 of 30 studies[66]. Results for 14 prospective studies were more equivocal and only 4 of 7 studies reported any significant association between MVPA and a measure of adiposity. Studies included in the 2016 review overlap considerably with those included in study 1. They differ by not imposing the minimum 2-year follow-up restriction and by considering studies published before October 2009.

These differences in inclusion criteria lead to the inclusion of 4 cohorts not considered in study 1. An earlier review[68] also restricted to objective methodology but only considering prospective studies published between 2004 and 2008 reported more consistent results. Seven of 8 studies reported any association between physical activity assessed by heart-rate monitoring, pedometry and accelerometry and the authors concluded there was "strong evidence" for a protective effect of physical activity on subsequent excessive weight-gain. Finally, a third review considering prospective studies published from 2000 to 2009 included 10 studies assessing physical activity by doubly labelled water, 24-hour calorimetry, and accelerometry[132]. The authors reported that only 4 of the 10 studies provided any evidence for a beneficial effect of physical activity on excess adipose tissue accumulation over time. Meta-analysis of randomized controlled trials conducted in overweight or obese young people support the concept that aerobic and/or resistance training may favourably impact adiposity indices[233, 234] and that greater exercise volumes produce larger benefits[234]. However, these meta-analyses were not restricted to studies without (planned) concomitant dietary or other life-style interventions limiting inference on causal effect of exercise per se. An association was more consistently observed with MVPA than with lower intensity activity in study 1 and larger effect-sizes for BMI and waist-circumference with higher intensities were observed in study 3. Together, these findings lend some support to the importance of total volume as a product of intensity and duration for the prevention of excessive adipose tissue accumulation in youth. Considering the totality of evidence, the role of physical activity in controlling excess weight-gain in young people over time is unclear and of unknown clinical relevance. The harmonized data-analysis of 6 small studies (total n=586) employed by Wilks and colleagues in 2011 suggested a substantial daily amount of physical activity energy expenditure of 1000 KJ was needed to induce a lowering of body-fat by 0.05%[79]. No updated pooled estimate is available but effect-sizes included in study 1 were generally small. Exceptions are observed and a

3.5% lower body fat per 10 minutes MVPA was reported in a study including pre-adolescent children at high risk of obesity[165]. Further, if the association between physical activity and adiposity is bi-directional even small population level improvements may meaningfully influence weight-trajectories.

A beneficial role of physical activity in modulating the biological risk markers is echoed by a similar systematic review[66]. Associations in study 1, 2, and 3 were particularly strong for indices of insulin-sensitivity, but were less convincing for fasting glucose. This observation is consistent with young people with functioning beta-cells being able to maintain normal blood glucose by compensation for deteriorating sensitivity by increased insulin secretion[235]. A meta-analysis of 24 experimental studies reported a pooled improvement in hepatic insulin sensitivity of 0.48 standard deviations[63]. Collectively, study 1, 2 and 3 lend support to the notion that total volume and intensity are the main determinants of favourable metabolic control. The duration of accumulation patterns did not appear of importance. Study 2 supports[76] and extends[78] previous observations of a continuous inverse association with metabolic control suggesting benefits continue to accrue above the accumulation of 90 minutes of MVPA/day. An inverse linear trend was also supported by the EYHS[172] but was less apparent in other studies[166, 171]. Thereby, a continuous improvement in metabolic control covering up to 15 minutes of vigorous activity or 90 minutes of MVPA appears evident from the very limited data.

The beneficial effects of larger physical activity intensity and volume are appreciated from randomized controlled trials[70, 236] and consistent with current activity recommendations[59]. The relative importance of intensity and volume in improving risk factors remains unclear and may be phenotype specific[70, 236]. From observational studies the "independent" association with either may be difficult to appreciate as higher intensity and greater volume are correlated in young people[76].

The effects of intensity-threshold within what is usually considered the vigorous activity domain on cardiometabolic risk markers was recently explored cross-sectionally in 11 588 young people from ICAD (cut-points ranging from >3365 counts/min to ≥ 6000 counts/min). The results were inconsistent with only few of a substantial number of analyses showing a statistically significant association for replacement of light with vigorous activity[90]. This concurs somewhat with results for vigorous activity in isolation in study 1. On the contrary, study 3 showed that physical activity within the spectrum usually defined as light to at least moderate or above was consistently associated with favourable cardiometabolic risk factors in a graded fashion. This apparent u-shaped association pattern may be an artefact of modest variation in vigorous activity within the samples and many individuals achieving zero vigorous activity with waist-worn accelerometry. This is supported by "vigorous" activity as typically defined by >6 metabolic equivalents (METs) in epidemiological studies (which may be achieved by slow running or playing basket/soccer) is substantially less intensive than exercise modalities employed in exercise programs. By including frequently used MVPA cut-points (2000 counts/min and 3000 counts/min) in study 2 these data may facilitate comparisons of absolute effect-size differences between studies applying different intensity cut-points.

Isotemporal substitution of short to medium and long bouts of activity in addition to the lack of statistical significance of the intensity-by-bout-duration interaction terms in study 3 suggested no additive or multiplicative effects of longer bout-durations on metabolic control, when total volume was accounted for. This is in agreement with a systematic review considering 7 observational studies[66]. While short-bouted activity does appear beneficial[84, 85] few studies have directly compared short- versus long-bouted activity with simultaneous control for differences in total activity volume. Holman and colleagues compared effect-sizes from 1-9 minute bouts and ≥10 minute bouts using c-statistics and found no difference in the odds of having a high composite risk

score[84]. Willis and colleagues compared 3 groups derived based on MVPA composition patterns and found lower BMI and waist-circumference in the group accumulating the highest proportion of activity in bouts[87]. The former analysis did not control for total activity while the later did adequately include total activity as a covariable. Some of the between-study variation is likely to be partly explained by different analytical approaches for handling total volume of activity and/or to applying different bout-definitions. It may be that a 60-second epoch is inadequate for capturing bouts of activity in children[48]. A study applying both 4-second and ≥5 minute bouts in boys did not find strong indication that correlations with waist-circumference and cardiorespiratory fitness differed markedly between the bout-definitions[86]. Some experimental evidence is available from a randomized cross-over trial including 70 healthy young adults in 3 experimental conditions; 1) uninterrupted, prolonged sitting, 2) prolonged sitting with 30 minutes of continuous walking, and 3) prolonged sitting interspaced with 18 100-seconds walking sessions[237]. Prolonged sitting interspaced with short-bouted walking resulted in improved glycaemic control in comparison with the continuous exercise bout. This would suggest greater metabolic control is obtained by short-bouted physical activity. Importantly, total exercise duration and intensity was matched between the exercise conditions but the inclusion of a sitting condition to the contrast (which included 8 hours of uninterrupted sitting in the continuous condition) makes the comparison suboptimal. To the best of my knowledge, other trials comparing short versus long bouts includes contrasts which are discordant in intensity or total volume. Additional benefits of any moderate activity bout duration on cardiometabolic risk markers are not evident in adults[238].

Study 1 did not convincing support detrimental associations between more time spent being sedentary and the levels of cardiometabolic risk markers. These findings are similar to conclusions reached in other reviews[73, 239, 240]. Available (limited) evidence from experimental settings in young people suggest prolonged, uninterrupted sitting is not detrimental to glycaemic regulation in healthy

adolescents in comparison with prolonged sitting but including breaks of walking at 30 % and 60 % peak oxygen uptake[241]. These conclusions are in contrast to experimental[241] and observational data in adults. In middle-aged adults objectively measured (accelerometry) sedentary time and sedentary patterns have been associated with higher mortality risk[242], possibly mediated by deteriorations in glycaemic control[243], although these findings have also been disputed[244]. The emergence of new modelling approaches to account for the co-dependent nature of physical activity and sedentary time may provide insights into the role of sedentary time independent of physical activity.

Study 2 suggested 78 % of the association between achieving 60 minutes of daily MVPA and metabolic control in healthy young people was attributed to the direct effect, with statistical significance of both direct and indirect effects. This relative contribution of direct and indirect effects was consistent at lower and higher physical activity levels, but the indirect effect varied from 31 % for HOMA-IR to 12 % for triacylglycerol. In study 3, including adiposity to regression-models attenuated effect-sizes for insulin by 6 % and 42 % at the ≥500 and ≥3000 counts/min cut-point, respectively. Effect-sizes for triglyceride were unaffected by BMI control at ≥500 counts/min but attenuated by 19 % at ≥3000 counts/min. Attenuation of effect-sizes appeared greater at higher intensities for insulin, glucose, triglyceride, and HDL-cholesterol. A meaningful direct association between physical activity and metabolic regulation is generally consistent with consensus of an "independent" direct effect as suggested by applications of the difference-method and from clinical studies[70, 100, 245]. In other studies applying formal effect-decomposition the physical activity-adiposity association have explained a larger proportion of the total association than observed in study 2. The indirect effect of MVPA through waist-circumference explained a statistically significant 44 % of the total effect on arterial stiffness in a cross-sectional study including 30 year old Brazilians[246]. Differences in indirect effects may owe to arterial stiffness representing years of accumulated exposure, while the biological risk factors investigated in study 2 respond more rapidly

to recent changes in behaviour. In a 2-year prospective study, DXA assessed body fat explained a statistically significant 62 % of the association between MVPA and insulin-sensitivity in 10 year old children with high risk of obesity[165]. The direct effect of physical activity on insulin-sensitivity was not statistically significant in the models which also included control for cardiorespiratory, fitness however this estimate may be overly conservative. Concerning clinical studies, three days of bed-rest have shown to reduce insulin-sensitivity in young adults[247]. Further, a pre-post study in overweight and obese 13 year old girls demonstrated 12 weeks of aerobic exercise 3 times a week for 40 minutes improved insulin mechanics following an oral glucose tolerance test without (detectable) concomitant changes in DXA assessed total or relative body fat[248]. The improvement in metabolic regulation may be related to the lack of control condition as a randomized controlled trial in 13-year old obese children showed no superior improvements in insulin sensitivity compared to control following 8 weeks of 35 minutes aerobic exercise 3 times per week. In that study changes in cardiorespiratory fitness were associated with changes in HOMA-IR indicating a dose-response relation. However, in a randomized controlled trial in healthy 12-year old children spanning 8 weeks, an increase in daily physical activity by cycling to school resulted in a -0.58 standard deviation lower composite risk score without detectably improvements in cardiorespiratory fitness[249]. School-based interventional approaches, of which most include some physical activity modifications, also suggest improvements in systolic blood pressure may be achieved without detectable improvements in BMI[44]. This is noteworthy given the higher reliability of BMI measurement in comparison with blood pressure. Further, some[250] but not all[23] studies reports higher cardiorespiratory fitness (which may be modulated by physical activity) is associated with lower diabetes risk irrespective of adiposity status. Finally, in a study including 334 161 individuals followed for 12 years, higher physical activity levels were associated with lower mortality in all strata of BMI or waist-circumference[251].

The most consistent association in study 4 was with lower waist-circumference gains. The association was significant across muscle-fitness indices but after control for cardiorespiratory fitness, muscular agility was not associated with waist-circumference gains. The association with muscular strength was stronger than that of power and agility and evident in both boys and girls. Controlling for waist-circumference in addition to cardiorespiratory fitness in study 4 eliminated nearly all associations between muscle-fitness and the non-adiposity composite risk score except for HDL-cholesterol in girls. Other prospective studies have shown higher muscle-fitness associated with a more favourable composite risk score including HOMA-IR, blood pressure, waist-circumference, triacylglycerol, and HDL-cholesterol in pre-adolescent children[91] but higher muscle-fitness has also been associated with higher blood pressure in adolescents[252]. Muscle-fitness associations with the composite score were not controlled for cardiorespiratory fitness in a comparable sample of 1635 6 - 11 year olds from the IDEFICS study. They were instead controlled for MVPA which may be counterintuitive as variation in muscle-fitness owing to variation in physical activity (assuming perfect measurement) would represent the effect of genetic disposition to higher muscle-fitness. In the IDEFICS study only lower body muscular power (standing long jump) was associated with lower waist-circumference gains over 2 years and only significantly so in girls[91]. The IDEFICS study also observed that control for BMI removed the association between lower body muscular power and the composite risk score in boys, while only resulting in slight attenuation of effect-sizes in girls. Sexual dimorphic associations were also reported in the study including adolescents[252].

Collectively, there is some support for the role of muscle-fitness in modulating biological risk factors in pre-adolescent children which is potentially explained by association with favourable energy balance. In contrast, muscular strength in adolescents presents a strong protective

association with metabolic control in young adulthood independent of waist-circumference[95, 253]. This discrepancy may relate to the source of variation in muscle-fitness between the age-groups. In agreement with data included in this thesis, the muscle-fitness association appears phenotype-specific in all studies[91, 252, 253]. An additional source of variation between studies is the normalization to body-size which appears without consensus. As shown in study 4 and described elsewhere[254] the impact of normalization may be substantial. In the IIDEFICS study muscular strength as assessed by handgrip was not normalised to body weight which is likely to induce negative confounding by adiposity. Control for BMI in statistical models is unlikely to remedy this artefact. Randomized controlled trials show that reductions in total body fat (DXA) with resistance training may be achieved in boys and girls[200, 255]. Insulin-sensitivity was improved only in boys. A systematic review of randomized controlled trials support beneficial risk marker adaptations may accrue with resistance training but also reports mixed findings with only 5 of 13 studies succeeding in improving the biological risk factors. Finally, 2 Mendelian Randomization studies using muscle-fitness associated alleles (from genome-wide association studies in adults) in a genetic risk score, found no genetic association with favourable risk factors levels[256]. There was also no association with type 2 diabetes risk[256] and conflicting results for CVD risk[256, 257].

Two school-based physical activity interventions provide comparable data to the long-term follow-up of the CHAMPS-study DK. A non-randomized controlled trial based on doubled physical education for 3 years followed 439 children for a total of 7 years. At the final follow-up, boys at intervention schools had a significantly lower blood pressure than boys at control schools, but no other outcomes (11 investigated) were affected[258]. Similarly, a 1-year randomized controlled trial (KISS) based on additional and improved physical education, active breaks during academic lessons, and physical activity home-work in 293 children, found only cardiorespiratory fitness was

still higher in intervention school children at the 4-year follow-up[259]. A total of 14 outcomes were included in that study. Attrition over time was substantial in both studies raising issues with potential selection bias. The most promising results of school-based physical activity interventions (evaluated immediately post-intervention) on composite risk scores have been found in the 2-year Sogndal school-intervention[260] and the 1-year evaluation of the KISS[261] study. These studies both included daily physical education led or planned by physical education specialists (60 minutes/day in Sogndal and 45 minutes/day in KISS). Reported differences in the composite risk scores which in both studies included body composition, lipids, blood pressure, cardiorespiratory fitness and glycaemic control were 0.37 and 0.18 standard deviations in favour of intervention schools. The statistically insignificant difference in the composite risk score between intervention and control schools in study 5 was 0.09 standard deviations in favour of intervention schools.

Considering previous efforts to increase overall physical activity levels by school-based approaches (physical education based or other behavioural interventions), meta-analysis of 12 studies suggests the pooled effect is minor and not statistically significant[262]. The large Active for Life Year 5 trial including more than 2000 U.K children reached the same conclusion[263]. Broadening the scope of interventions beyond school-based approaches suggests a statistically significant, albeit modest, increase of 4 minutes of MVPA/day may be achieved immediately post-intervntion[264]. However, even in studies with initial success in increasing physical activity levels the effects appear lost over time when interventional support is ceased[265]. This has caused some to speculate into the existence of an activitystat[221] which, in the presence of exposure to a physical activity initiative would ensure activity-homeostasis by reducing activity to the intrinsic set-point once external perturbations has ceased. Our data neither supports nor refutes the existence of an intrinsic activity set-point. Rather, it suggests physical activity interventions based on provision of additional physical education

should be coupled with behavioural models facilitating translation of physical activity into leisure time to achieve public health relevance. Given the physical activity data, it may not be surprising that current school-based physical activity intervention models do not appear to induce long-term benefits on cardiometabolic risk factors. Systematic work to synthesize effective implementation models is being conducted[266], but without data to answer the important question; what works in the long-term after cessation of intervention contact, application for public health improvements will remain limited.

Biological mechanisms

Physical activity effects on improved insulin sensitivity are well-described, albeit the mechanisms are not fully understood. Insulin-resistance, together with particularly abdominal ectopic fat deposition, is considered instrumental in the cascade of metabolic dysregulation leading to high risk of NCD morbidity and mortality[103]. Physical activity causes non-insulin dependent glucose uptake into skeletal- and heart-muscle and adipose tissue by primarily allocation of GLUT4 proteins to cell membranes[267]. GLUT4 proteins facilitate transport of glucose across the membrane, with skeletal muscle cells representing the main storage-point of glucose within the human body. Translocation of GLUT4 to muscle-membranes is facilitated by elevations in intracellular adenosine-mono-phosphate as a consequence of adenosine-tri-phosphate turnover and the effect is thus independent of decreased sensitivity to insulin. In addition to facilitating insulin-circumventing glucose uptake, physical activity is believed to increases de novo synthesis of GLUT4 proteins[267]. The effects of physical activity on insulin-sensitivity are efficiently demonstrated by site-specific adaptations in one-legged training studies[268] and a single exercise session have shown to improve sensitivity for as much as 48-72 hours compared to rest-conditions[269, 270]. As substrate utilization is partly determined by availability, this provides a mechanism linking insulin-resistance with dyslipidaemia as beta-

oxidation is reduced with inactivity[271]. Increased non-insulin dependent glucose-uptake would be considered part of the direct effect in study 2. However, several others consequences of activity which are not adiposity dependent may explain improved metabolic regulation. Physical activity could increase cardiorespiratory- or muscle-fitness and some have suggested higher fitness explains a substantial portion of the effect of physical activity on metabolic markers[272, 273]. This is however not supported by experimental studies[70, 249].

Improvements in muscle-fitness by physical activity require sustained and intensive muscle-contractions suggesting relatively large volumes of activity are needed. A large volume of energy expenditure over a long period of time may prevent excess weight-gain as suggested by study 4. Further, larger lean mass and corresponding higher resting energy expenditure and/or capacity for glucose uptake, changes in muscle-fibre type composition[70], and/or increased mitochondrial/enzymatic capacity and function[274] are potential mechanism which could explain metabolic adaptations with higher muscle-fitness. These mechanisms would be expected to be largely independent of weight-loss but little support for this notion was found in study 4. Further, the apparent lack of genetic association between handgrip strength and metabolic regulation and morbidity[256, 257] suggests is may be regular engagement in physical activity and not the phenotypic adaptations which explains most of the positive metabolic benefit seen with resistance training. In type 2 diabetics, muscle-fitness benefits appear additive to cardiorespiratory fitness[275] which has also been reported in adolescents[276]. More work on potential additive effects of high muscle-and cardiorespiratory fitness in high-risk as well as healthy young people is however needed. In contrast to adolescents and adults, children respond to resistance-type exercise with an increase in synapse firing frequency, synchronization, and antagonist coordination, and less so by an increase in muscle-fibre cross-sectional area[99]. These unique adaptations could explain the apparent

discrepancy in health-benefits between muscle-fitness in pre-adolescent and adolescent children. The biological rationale explaining why there would be sexual dimorphic mechanisms in play in pre-adolescent children but not in adolescents[95] is unclear.

Conclusion

This thesis supports and extends the substantial body of evidence suggesting higher levels of physical activity are associated with favourable metabolic regulation and potentially with lower levels of adiposity in young people. Concerning adiposity, the appearance of conflicting directions of associations in conjunction with unquantified and unknown clinical relevance of the totality of the evidence, it still appears premature to conclude physical activity is a major source of variation in adiposity-status in young people. The collective inference from prospective studies in study 1 and the direct comparison of intensity thresholds in study 3 is that favourable adaptations are particularly evident with activities performed at an intensity corresponding to minimum moderate intensity. Thereby, this part of current physical activity guidelines is supported. Beneficial effects of physical activity are likely to accrue irrespective of the duration of accumulation patterns as long as the total volume is identical. The collective inference from currently available prospective data on the role of sedentary time does not suggests population policy directed towards young people should be weighed more against decreasing sedentary time if this is done at the expence of efforts towards increasing MVPA.

Engagement in activities of sufficient intensity and frequency to elicit muscle-fitness adaptations provided cardiometabolic benefits in pre-adolescent children independent of cardiorespiratory fitness chiefly through associations with adiposity. Muscle-fitness promoting activities should thus

be promoted in addition to aerobic activities to obtain maximal cardiometabolic benefit. Overall, the data concerning muscle-fitness associations with biological risk factors independent of adiposity appears ambiguous from sources other than cross-sectional studies. Roughly four-fifths of the association between physical activity and metabolic control was not adiposity dependent suggesting the major constituent of the association is attributable to physical activity *per se*. It also suggests related models controlling for adiposity in youth are only slightly conservative. When indices of insulin-resistance are considered, models may however be substantially more conservative. Whether this conservative estimate is appropriate or not will ultimately depend on the research question of interest.

From studies 1 to 4 and previous evidence it is reasonable to suggest a right-shift of the population-distribution of physical activity will improve the metabolic health of young people, potentially leading to improved long-term health gains. Provision of an augmented physical education program from Kindergarten to grade 6 did not appear an effective strategy to achieve this aim. This likely owes to the physical education program failing to result in immediate or sustainable differences in overall physical activity levels of the children.

The results of this thesis should be interpreted in the light of lack of appropriate temporal exposure-outcome sequence in some studies, the possibility of residual and unmeasured confounding, and the high attrition in the evaluation of the school-based intervention.

Policy and clinical perspectives

From available data is appears unsubstantiated to suggest school-based approaches will increase physical activity levels and improve the population-levels of cardiometabolic risk markers. A multi-factorial approach including radical and innovative policy decisions in multiple segments of society such as increased school physical activity, facilitating daily active transportation while limiting car-based passive transportation, efforts to maintain youth sports-participation in adolescence, tailored family-based physical activity counselling and/or legislation supporting workplace physical activity is probably needed. A substantial shift in population distribution of risk markers need not be reached but if slight sustainable changes are made the strategy is likely to represent meaningful primordial prevention.

There was no evidence of multiplicative interaction between physical activity and adiposity suggesting physical activity should be recommended for maintaining metabolic homeostasis irrespective of weight status or achievement of weight-loss in high-risk youth. Muscle-fitness associations appeared additive to cardiorespiratory fitness particularly in controlling excessive adipose tissue accumulation. Thereby, support is found for engagement in resistance-type activities in addition to aerobically challenging activity as is now advocated by many guidelines. As per current guidelines for youth but contrary to adult recommendations, it does not appear justified to include specifications on bout-duration into guidelines or exercise prescriptions. Physical activity may be accumulated in a single or interspaced pattern which maximizes feasibility.

Future research directions

A more detailed understanding of the dose-response relationship between physical activity in youth and subsequent risk markers is needed. Particularly, studies applying objective methodology should attempt to follow larger cohorts of young people for longer periods of time and pursue linking with established clinical or behavioural databases. Estimation of absolute and relative risks at a range of physical activity levels will additionally serve to facilitate strong public health messages and may be used for cost-effectiveness calculations of different interventional strategies. Until these data become available, identification of retrospective data-sources containing physical activity surrogates such as muscle- and cardiovascular fitness[23] linked with updated morbidity/mortality-registries will provide valuable information and should be pursued were available. Potential bout-by-intensity interactions at higher intensities than included here (>3000 counts/min) may be interesting to explore as bouts of high intensity may result in fitness adaptations which is unlikely to occur at lower intensities.

The sources of the substantial between-study heterogeneity in the physical activity-adiposity association should be carefully examined in future studies. Identification and quantification of characteristics explaining heterogeneity at the study-design, sample, measurement methodology, and analytical level will serve to improve understanding of the complexities of physical activity in relation to energy balance. Due to variation in methods, reporting and analysis in the published literature this aim will be difficult to achieve in meta-analysis of published estimates. More likely, meta-analysis of individual participant data is needed. Finally, future studies should attempt to include direct assessments of adiposity in addition to anthropometrical indices. Decomposition of the physical activity association with metabolic homeostasis should be pursued in studies of different ages and demographic settings. These pursuits could include other potential mediators than

adiposity. These should preferably be performed in data including the appropriate temporal lag and an evaluation of estimate sensitivity to a range of assumptions. Estimation of the relative importance of direct/indirect effects for clinical end-points will be particularly informative. Because of the potential for reverse causation bias, an observational approach may need to control for pre-baseline physical activity[139] which will reduce applicability of many datasets. Clinical trials exploring the relative contribution of direct and indirect effects would also be valuable.

The role of high muscle-fitness and resistance training independent of aerobic activity or cardiorespiratory fitness deserves further scrutiny. New studies should consider observational and experimental research in different age-groups and from the general population. Experimental research would benefit from identifying optimal training regiments for exercise prescription. The role of adaptations in specific muscle-fitness phenotypes would be valuable to explore in observational as well as experimental studies. In observational studies in particular there is a need for wider acknowledgement of the potential for adiposity-related confounding under different body-weight normalization schemes. Preferably, more than one index is presented to facilitate evidence synthesis. Additional exploration of muscle-fitness health benefits as additive to cardiorespiratory fitness benefits and independent of body-composition should be pursued.

Adaptation to current physical activity intervention models appears needed. Additional involvement of family or community components may facilitate translation of higher physical activity during school to leisure time. Creation of lasting habits is needed for the formation of sustainable public health gains and previous intervention models do not appear successful in achieving this aim. Current work to ensure short-term efficacy is valuable[266] but without future interventions pre-planning for long-term follow-up and including a careful consideration of procedures to minimize attrition information to guide public health policy will remain limited.

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Study 1

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Review

Physical activity, sedentary behavior, and long-term cardiovascular risk in young people: A review and discussion of methodology in prospective studies

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Abstract

The long-term effects of physical activity (PA) or sedentary behavior on cardiovascular health in young people are not well understood. In this study, we use a narrative format to review the evidence for a prospective association with adiposity and other well-established biological cardiovascular risk factors in healthy young people, considering only studies with at least 2 years of follow-up. PA appears to elicit a long-term beneficial effect on adiposity and particularly markers of cardiovascular health. With adiposity, however, a few studies also reported that higher levels of PA were associated with higher levels of adiposity. Time spent sedentary does not appear to be related to adiposity or markers of cardiovascular health independent of PA. We then discuss the uncertainties in the underlying causal chain and consider a number of alternative modeling strategies, which could improve our understanding of the relationship in future studies. Finally, we consider the current methodology for assessing PA and sedentary time.

Keywords: Adiposity; Adolescents; Cardiovascular health; Children; Long-term; Metabolic syndrome score

1. Introduction

Cardiovascular disease (CVD) continues to be the number one cause of death and disability in the world.[1,2] While CVDs such as coronary heart disease and stroke manifest clinically in middle age or older adulthood, their origins begin much earlier.[3] Exposures acting during growth and maturation may exert long-term effects on cardiovascular physiology and have a major impact on the development of CVD. For example, evidence shows that alterations in fetal environment such as exposure to maternal smoking and nutritional status, childhood socioeconomic position and overweight independently predispose to development of CVD in adulthood.[4–6] Similarly, lack of physical activity (PA) during childhood and youth may lead to cardiovascular disturbances and progression of atherosclerosis that could contribute to development of CVD in adulthood. Indeed, organizations and governments have identified a population-wide promotion of healthy lifestyle including PA in young people as a key priority for primordial prevention of CVD.[7] A major advance in population-based studies of the influence of childhood or youth PA on long-term cardiovascular health outcomes has been the application of objective methods to assess PA that prevents bias related to recall and social desirability.[8] This has also facilitated the possibility to obtain detailed information on frequency, duration, and intensity of PA.

Two earlier reviews, published in 2010 and 2011, respectively, systematically addressed studies on the observational associations between objectively assessed PA and adiposity outcomes.[9,10] These reviews highlighted the importance of distinguishing between cross-sectional and prospective studies as Jimenez-Pavon and colleagues[9] found a negative association

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in 32 of 41 cross-sectional studies (78%), while Wilks and colleagues,[10] who only considered prospective studies, found a negative association in 4 of 10 studies (40%). Because cross-sectional studies have a number of limitations, including inability to infer causality, this inconsistency is important to address by considering recently published experimental and prospective studies. In 2014, Tanaka and colleagues[11] published a review on the prospective association between changes in objectively measured sedentary behavior (SED) and measures of adiposity. However, by imposing this restriction the authors were only able to include 3 studies. The review found evidence for a positive association in 1 of the 3 studies. In summary, the previous evidence synthesis did not clearly support a prospective association between PA/SED and adiposity outcomes. In contrast to these reviews on adiposity outcomes, we are aware of no published reviews on the prospective association between PA and biological risk factors in young people.

This narrative review aims to give an overview of the evidence from population-based studies relating objectively assessed PA and SED in childhood or youth with long-term (≥2 years) cardiovascular risk factor progression. In addition, we will discuss the current methodological challenges and future direction in the objective assessment of PA in large-scale studies following young people over time.

2. Scope of the review

In our discussion we will consider PA as a behavior separately from SED. This is because a body of literature has identified behaviors such as TV-viewing[12,13] and total sitting time[13] to predict all-cause and cardiovascular mortality in adults, independent of other domains of PA. Our discussion will consider observational studies which

- Used an objective measure to quantify whole-day PA (i.e., total activity, light PA (LPA), moderate PA (MPA), moderate-to-vigorous PA (MVPA), or vigorous PA (VPA)) or SED at baseline.
- Included healthy, population-based samples of children and adolescents aged ≤18 years at baseline and followed the same individuals for a period of ≥2 years.
- Related an exposure to any form of the conventional biological risk factors (recommended for use in CVD risk stratification among asymptomatic adults[14]) with the addition of indices of insulin-resistance. We considered all forms of adiposity outcomes.
- Were published after October 29, 2009, as this was the final search date in the review by Wilks and colleagues[10] (applies for PA–adiposity investigations only).

We identified relevant studies from our records by conducting a search on PubMed using combinations of the relevant exposures and outcomes and by going through the reference lists of the identified studies. As there are a greater number of studies on adiposity outcomes than studies on biological risk factors, we will address these in separate paragraphs. Furthermore, we will briefly discuss findings from controlled or randomized controlled intervention studies conducted in a general (young) population.

3. How do PA and SED associate with adiposity and biological risk factors?

3.1. PA and adiposity

We identified a total of 13 published studies which fulfilled our criteria.[15–27] The duration of follow-up ranged from 2 to 7 years with 9 studies covering ≤3 years.[15–18,22–25,27] The studies included samples aged 4–18 years at baseline. The median (25th–75th quartiles) study size was 554 (315–984). Twelve studies used accelerometry as their exposure variable, while 1 used a combination of accelerometry and heart rate.[16] The outcome variables were more heterogeneous. Nine studies (69%)[17–20,22–24,26,28] reported a significant association between PA and a measure of adiposity such that a higher level of PA was associated with a lower level of adiposity (negative association). Surprisingly, 3 studies reported significant associations in the opposite direction.[16,20,23] When examining the relative importance of specific PA intensities, significant negative associations were apparent with LPA in 0 of 3 studies (0%), with MPA in 1 of 4 studies (25%)[17] and with VPA in 2 of 4 studies (50%).[17,24] Finally, significant negative associations with adiposity were found for MVPA in 7 of 8 studies (87%).[18–20,23–26] Four studies included SED as a covariate, which generally had a small effect on the estimates and only affected the conclusion in 1 study.[25] The choice of outcome measure in adiposity studies appeared of importance as all studies (4 in total) that considered MVPA and used a reference method for assessing adiposity such as DXA or densitometry[29] reported a negative association with adiposity.[18,20,23,26] In contrast, 6 of the MVPA studies used a body mass index (BMI)-based measure with significant negative associations to adiposity found in 3 studies, while 2 studies found significant positive associations. The latter appears to be explained by an increase in muscle or bone tissue as both of these studies also reported significant associations between PA and fat free mass. If PA associates with fat free mass, which is suggested in the literature,[30,31] future studies should use methods which are accurate enough to separate fat mass from lean mass.

3.2. SED and adiposity

We identified a total of 9 published studies which fulfilled our criteria.[15,20,21,24–27,32,33] The duration of follow-up ranged from 2 to 7 years with 5 studies having ≤2.5 years of follow-up.[15,24,25,27,32] The studies included youth 4–18 years at baseline. The median (25th–75th quartiles) study size was 554 (403–984). Eight studies used accelerometry, while 1 used a combination of accelerometry and heart rate.[32] The measures used for outcomes were more heterogeneous. Two studies (22%)[25,33] reported a significant association between SED and adiposity; more time spent on SED was associated with a higher level of adiposity. One study found an association in the opposite direction, i.e., higher SED associated with lower adiposity.[20] Of the 2 studies reporting a positive association, 1 used a relatively high cut-point to define SED (<1100 counts/min (CPM)),[25] which is very likely also to include time spent on LPA.[34] This finding is surprising as none of the 3 studies examining LPA separately from SED found an association and 1 of these even found a negative association.[16,17,27] Six studies used a

cut-point of <100 CPM to define SED,[15,21,24,26,27,33] which is comparable to the cut-point of 1.5 METs[34] used in the combination study. In order to determine the independent association between SED and adiposity, it is important to adjust for other dimensions of PA. This was performed in 7 studies by adjusting for MVPA. The isolated effect of this adjustment was, however, not possible to infer, as either the studies did not report the estimates that were not adjusted for MVPA or the studies adjusted for other covariates when including the adjustment for activity.

3.3. PA, SED, and biological risk factors

We identified a total of 8 published studies which fulfilled our criteria for PA,[17,20,35–40] while 1 study used SED.[20] The study using SED found no significant associations with any of the biological risk factors before or after adjustment for MVPA.[20] The duration of follow-up in the PA studies ranged from 3 to 12 years with 4 studies having ≤3 years of follow-up. Included participants were 5–16 years at baseline. The median (25th–75th quartiles) study size was 293 (209–466). Seven studies used accelerometry and 1 used pedometers.[40] Five studies used a composite risk score[20,35,37–39] and 6 studies provided results for individual risk factors. Four of 5 studies (80%)[20,37–39] reported a significant negative association between PA and the composite score, meaning that a higher level of PA was associated with a more favorable cardiovascular profile. Results for the individual risk factors were generally coherent with 4 of 5 studies reporting a significant negative association with insulin resistance,[20,36,38,40] 0 of 3 with glucose, 2 of 3 with triglyceride[37,38] and 3 of 4 with blood-pressure.[17,37,38] Two of 3 studies reported significant positive associations with high-density lipoprotein cholesterol (HDL-C).[20,38] In contrast to studies on adiposity outcomes, studies on biological risk factors included little reporting of specific PA intensities. Only 3 studies gave results for different intensities.[17,36,39] Two of these studies demonstrated that VPA, but not lower intensities, was significantly associated with the risk factors[17,39] while 1 study showed similar associations for total activity and MVPA.[36] MVPA was used in 5 studies with 4 reporting significant associations.[20,36–38]

3.4. Intervention studies

Dobbins et al.[41] published a Cochrane-review in 2013 which included a total of 44 randomized-controlled trials with interventions ranging from 12 weeks to 6 years. They found little evidence for an effect on blood-pressure or mean cholesterol. However, another review found that studies in general showed a significant, but small, effect of PA on HDL-C and triglycerides, but no effect on total cholesterol or low-density lipoprotein cholesterol (LDL-C).[42] A review synthesizing effects on PA, fitness, and motor skills[43] concluded that strong interventions carried out by educated physical education (PE) teachers and including around 5 PE lessons per week are needed to improve these parameters. In the Sogndal school-intervention study, the implementation of the intervention was carefully controlled and included 60 min PA on all school days, and part of the activity was of high intensity.[44] They found an improvement in cardiovascular fitness of 15% in the least fit quartile compared with the similar quartile in controls. This suggests that the intervention was intensive and in that study, nearly all the biological risk factors also improved.[44] The intervention in Sogndal was designed based on findings from the Copenhagen School Child Intervention Study wherein an increase in PE lessons from 2 to 4 lessons a week revealed minimal or no changes in risk factors.[45] Later, the Childhood Health, Activity, and Motor Performance School Study Denmark (the CHAMPS-study DK) tested a PE dose of 6 h per week and found improvements in a composite cardiovascular risk score.[46] In Iceland, Hrafnkelsson et al.[47] conducted an intervention wherein PA was gradually increased from 30 to 60 min per school day over 2 years. They did not find improvements in any risk factors, but the study was quite small with only 3 intervention schools included in the cluster randomization. Moreover, accelerometer measurements showed only 4 min more MVPA in the intervention group in the first year and no difference in the second year when compared to the control group.

4. Perspectives

An important question when addressing the relationship between PA and biological risk factors is whether the associations are independent or mediated by adiposity.[48,49] This issue is important for understanding the etiology of cardiometabolic risk and is crucial for public health efforts as an independent effect would mean that an increase in PA would reduce CVD risk, even though no change in adiposity was achieved. Cross-sectional literature suggests that adjusting for adiposity attenuates the association slightly, indicating that the effect of PA on biological risk factors is not entirely explained by adiposity.[50–53] Our review included 3 studies which adjusted their models for changes in adiposity,[36,54] with the effect of this adjustment only reported in 1.[37] Metcalf and colleagues[37] reported that adjusting for change in BMI or waist-circumference had virtually no influence on their estimates. Further, 3 studies included an adiposity measure in their composite score,[35,38,39] which highlights the complexity of this issue. By doing so, adiposity is treated equal to the biological risk factors, but if adiposity is actually the cause of these,[55] this could drive the associations. A promising methodological advancement is the emergence of mediation analysis and structured equation models.[56] These use 2-stage regression to decompose associations into, e.g., total, direct, and indirect effects.[57] With these more formal quantifications of mediation, it may be possible to assess the relative importance of a putative indirect effect in relation to the direct effect. If the indirect effect is small, from a public health perspective, it is of little importance whether adiposity is a mediator or a confounder. However, more work is needed to improve the methods and address issues such as varying degrees of measurement error in exposure/mediator variables and reverse causation, which are not resolved by using these methods.[58] Hence, these limitations should be considered when interpreting the models.

The reviewed studies considered numerous different models to investigate the prospective associations. A frequently used model was the "determinant"-model. Here, a follow-up outcome or a change in outcome (the two are equivalent when adjusted for baseline outcome) was regressed on a baseline exposure. However, not all studies adjusted for baseline outcome, which is important as the size and direction of change from baseline to follow-up are expected to be associated with the baseline level of the outcome because of regression to the mean and flooring/ceiling effects. The potential impact of not adjusting for baseline outcome is demonstrated in 1 study where the direction of the association was inverted following this adjustment.[20] Another frequent model was the "change"-model in which the absolute change in outcome over time was modeled on the absolute change in exposure. In this model, bias may arise by not adjusting for baseline values of both exposure and outcome. In the reviewed studies, only one considered both in their models of absolute change.[40] As some studies adjusted for baseline exposure, some adjusted for baseline outcome and some adjusted for neither, this may be the cause of some of the heterogeneity in the study results. The change-model, however, has been criticized for being a "masked" cross-sectional study as it could be the outcome which changed first,[10] again making inference of the causal pathway difficult. To better establish the temporal relationship between PA and cardiovascular risk factors, future studies could attempt to collect (and analyze) repeated measurements of exposures and outcome during follow-up, which enables the possibility to specify a time lag between PA and the change in risk factor. Further, with repeated measurements, a structural equation modeling approach could be used to assess the importance of early life exposure versus change in that exposure over time, while taking into account the correlations between time-points. Interpretation of results was further challenged in several studies as an isotemporal substitution model (ISM)[59] was used, but only one mentioned this interpretation in their reporting.[32] An ISM may arise when multiple intensity domains and total wear time is included in the same model. The interpretation of an ISM would be the effect of changing a unit of time of, e.g., LPA with the same unit of time of MVPA. This is not the same as that of the more frequently used partition model.[59] We will not favor one model over the other but will recommend that future studies clearly state their models and their interpretation of these. Finally, even though 4 of our reviewed studies considered some form of adjustment for dietary factors, finer control of measures of diet quality and quantity would also help improve future observational studies.

The use of accelerometry in the assessment of PA and SED in prospective studies is encouraged by its feasibility and higher validity than self-report.[8] Nevertheless several shortcomings with accelerometers have been identified and include both technical and reactivity issues and controversies regarding how best to define non-wear and various intensity cutoff-points.[60] The technical shortcoming of the accelerometer is the misrepresentation of important health-related activities like cycling,[61] strength training, static exercise, aquatic activities and the intensity leveling-off with increased running speed.[62,63] Misrepresentation of beneficial PAs with moderate and vigorous intensity could explain some of the diversity of the prospective PA and adiposity associations reported. The assessment of SED using the <100 CPM cutoff point has been shown to have an acceptable sensitivity (71.7%) and specificity (67.8%) to estimate sitting time, with no optimal cutoff points for sitting plus standing.[64] Thus, the SED estimation in the study by Basterfield and colleagues[25] included activities with higher intensity than standing and suggests a measure that is far from the concept of SED defined by the posture allocation of sitting. Most studies investigating the association of SED with adiposity did not show any significant association, and this could partly be explained by the sensitivity and specificity of the cutoff point. The definition of the non-wear is also problematic. The definition used across the selected studies is quite different, and it has been shown that various non-wear definitions generate substantially different results with a significant impact on the assessment of SED.[65] The comparison across different studies seems to require a consensus and harmonization on what definitions of cutoff points should be used and non-wear handled. Additionally, more research is needed to investigate the shortcomings of the accelerometer and how new solutions can be feasibly applied in large-scale studies.

In summary, a beneficial effect of PA on long-term change in adiposity in young people was generally observed, but some studies found the opposite effect. This may be explained by the marker of adiposity used. In observational as well as intervention studies among children or adolescents, PA had a protective association with established biological risk factors. These findings suggest that providing the right opportunities for children and adolescents to engage in PA is important for primordial prevention of CVD. Sedentary behavior did not appear to increase long-term adiposity independent of other activity domains and almost no evidence was available with biological risk factors for this exposure. Future studies should attempt to better decompose the causal chain between PA and CVD risk factors, and there is a need for improved reporting and interpretation of statistical analyses, harmonization of data-reduction and development of methods to distinguish between different SED behaviors.

Authors' contributions

JT identified relevant studies, extracted the information, and drafted the manuscript; JCB, LBA, NCM, KF, and AG identified relevant studies and drafted the manuscript. All authors have read and approved the final version of the manuscript and agree with the order of presentation of the authors.

Competing interests

None of the authors declare competing financial interests.

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Study 2

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ORIGINAL ARTICLE

Does adiposity mediate the relationship between physical activity and biological risk factors in youth?: a cross-sectional study from the International Children's Accelerometry Database (ICAD)

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BACKGROUND/OBJECTIVES: To model the association between accumulating 60 daily minutes of moderate-to-vigorous physical activity and a composite score of biological risk factors into a direct and an indirect effect, using abdominal obesity as the mediator.
SUBJECTS/METHODS: Cross-sectional data from the International Children's Accelerometry Database (ICAD) including 6–18-year-old children and adolescents ($N = 3412$) from 4 countries providing at least 3 days of accelerometry-assessed physical activity. A standardized composite risk score was calculated from systolic blood pressure and fasting blood samples of insulin, glucose, triacylglycerol and inverse HDL-cholesterol. Abdominal obesity was assessed by the waist-circumference:height ratio. Two-stage regression analysis, allowing for exposure–mediator interaction, was used for the effect decomposition.
RESULTS: Participants achieving 60 daily minutes of moderate-to-vigorous physical activity had a 0.31 (95% CI: − 0.39, − 0.23) standard deviations lower composite risk score than those achieving less than 60 min. Modelling the associations suggested that 0.24 standard deviations (95% CI: − 0.32, − 0.16) was attributed to the direct effect and − 0.07 (95% CI: − 0.11, − 0.02) to the indirect effect indicating that 22% of the total effect was mediated by central adiposity. Modelling 30 and 90 min of moderate-to-vigorous physical activity per day resulted in changes in the direct but not the indirect effect.
CONCLUSIONS: One hour of daily moderate-to-vigorous physical activity was associated with clinically relevant differences in metabolic control compared to engagement in less than this minimally recommended amount. The majority of the difference was explained by the direct effect of physical activity.

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INTRODUCTION

Available evidence from prospective cohort studies suggests that higher levels of physical activity are, in a dose-response manner, protective against all-cause mortality[1] and incident cardiovascular disease and type 2 diabetes.[2] Similarly, in children and adolescents, physical activity is inversely associated with the biological risk factors (blood pressure, triacylglycerol, HDL-cholesterol and glycaemic control) comprised in the metabolic syndrome[3] which strongly predicts cardiovascular disease, type 2 diabetes and mortality in adults.[4]

Excess adipose tissue accumulation is one of the cornerstones in the aetiology of metabolic dysfunction[4] and elevated body mass index (BMI) in youth has been shown to predict incident type 2 diabetes, the metabolic syndrome and advanced atherosclerosis in young adulthood similarly to elevated levels of three or more of the biological risk factors.[5] However, the risk of type 2 diabetes and cardiovascular disease in young adulthood also appears to increase with advanced 'clustering' of the risk factors in youth irrespective of the combination of its constituent parts.[5,6] Further, a recent meta-analysis suggested that 46% and 76% of the effect of BMI on coronary heart disease and stroke, respectively, was mediated through blood pressure, cholesterol and glucose levels highlighting the importance of maintaining metabolic homeostasis.[7] Thus, identifying modifiable factors which can target biological risk factors in youth is relevant for primordial prevention of disease and for tailoring interventions for high-risk individuals.

Physical activity influences 24 h blood pressure[8] and modest amounts of activity may improve glucose and lipid metabolism compared to an uninterrupted prolonged sedentary state.[9] Since physical activity is the largest modifiable component of total energy expenditure, it plays an important role in energy balance and hence weight gain which occurs naturally during growth; indeed some, but not all, studies have suggested objectively measured moderate-to-vigorous physical activity (MVPA) is prospectively associated with accumulation of less adipose tissue in youth.[10–13] Thus, benefits from physical activity may originate from at least two sources and previous studies in youth supports an independent protective effect on the risk factors.[14–17] However, the relative contributions of these consequences of physical

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activity are not fully understood and previous studies have not described the associations in detail. We have previously reported a direct effect of MVPA on individual risk factors[16] and now extend this work by (1) formally quantifying the indirect component, that is, the effect on the risk factors attributed to the association between physical activity and adiposity, (2) allowing for potential exposure-mediator interaction, and (3) using a composite score of biological risk factors to maximize information on metabolic dysfunction.[14]

METHODS

Study design and participants

This study is based on secondary data from the International Children's Accelerometer Database (ICAD), an international pooling of data from 20 studies in which accelerometry was used to assess physical activity in children 3–18 years of age. The project has been detailed elsewhere.[18] All accelerometry data included in ICAD were centrally cleaned, reprocessed and harmonized. For these analyses, eligible studies (Copenhagen School Child Intervention Study (CoSCIS), National Health and Nutrition Examination Survey (NHANES) 03/04 and 05/06, and European Youth Heart Study (EYHS) Portugal, Estonia and Denmark) all provided data on fasting insulin, glucose, triacylglycerol, HDL-cholesterol and systolic blood pressure.[19–21] From these studies, 8799 youth aged 6–18 years provided a reliable accelerometer file (146 files were invalid). Of these, 4231 individuals accumulated at least 3 days of valid data, a fasting blood sample (no blood was drawn from children under the age of 12 in NHANES, excluding 1897) and a measure of waist-circumference. A further 819 participants were excluded due to missing data on covariates leaving 3412 participants (38% of participants available from these studies in ICAD) for the present analysis. The analysed sample was older, accumulated two more minutes of daily MVPA, and had more favourable biological risk factor profiles as compared to non-analysed individuals. A comparison of characteristics is available in Supplementary Table 1. Descriptive characteristics of included participants stratified by study are shown in Table 1.

Exposure

Raw accelerometry files were reprocessed using commercially available software (KineSoft v3.3.20, Loughborough, UK). All files were analysed using a 60-s epoch and non-wear was defined as 60 min of consecutive zeroes, allowing for up to 2 min of non-zero interruptions.[18] Participants were included if they had a minimum of 3 days of $\geqslant$ 500 min of wear time in the 0700 hours to 2400 hours. In those studies in which physical activity was measured more than once, we used the first measurement only. We defined MVPA as $\geqslant$ 2296 counts per min as this cut-point has shown a classification specificity and sensitivity for four metabolic equivalents of 88% and 92%, respectively, against indirect calorimetry in a validation study of 5–15-year-old youth.[22] This cut-point is equivalent to the acceleration of the hip/waist during brisk walking, so would not include slow walking. MVPA was averaged across valid days.

Outcome

The main outcome was a standardized continuous risk score[14] comprised of the aforementioned biological risk factors (using HOMA-IR as measure of glycaemic control calculated as; (insulin × glucose)/22.5)) and created by standardizing each risk factor for age and sex in a linear regression analysis with the standardized residuals representing z-scores with a mean of 0 and a standard deviation (s.d.) of 1. HDL-cholesterol was multiplied by − 1 prior to standardization. The residuals were subsequently averaged and the score standardized. In this composite score, a lower score represents a more favourable risk profile. Body height was added to the standardization of systolic blood pressure. Standardization was done for the complete analytical sample. Individual risk factors are presented as secondary outcomes.

Mediator

Waist-circumference:height ratio was used as a marker of abdominal obesity. Waist-circumference was measured midway between the lower rib margin and the iliac crest in the EYHS and CoSCIS studies and just above the iliac crest at the midaxillary line in the NHANES. A previous study found the mean differences between these measurement sites to be: 0.8 cm in 6–

Table 1. Study characteristics

Study		*CoSCIS*	*EYHS DENMARK*	*EYHS ESTONIA*	*EYHS PORTUGAL*	*NHANES 2003/4*	*NHANES 2005/6*
Country and year of data acquisition		Denmark, 01–05	Denmark, 97-98 and 03/04	Estonia, 98–99	Portugal, 99–00	US, 03–04	US, 05–06
Age (years)	Range	6–11	8–17	8–17	9–16	12–18	12–18
Participants	*N* (% girls)	358 (47)	1022 (55)	535 (55)	499 (50)	523 (45)	475 (50)
Height (cm)	Mean (SD)	124 (7)	149 (15)	152 (17)	147 (15)	165 (10)	164 (10)
Weight (kg)	Median(IQR)	24.0 (22.1–26.7)	36.7 (31.0–52.7)	42.6 (29.9–56.0)	39.0 (31.1–53.9)	60.8 (50.6–72.6)	58.9 (49.3–71.3)
Waist circumference (cm)	Median(IQR)	55.0 (52.3–58.0)	61.5 (57.3–68.4)	61.4 (56.2–67.6)	63.9 (58.5–69.5)	76.8 (69.4–87.0)	75.7 (69.7–86.6)
WC/H-ratio	Median(IQR)	0.45 (0.43–0.46)	0.42 (0.40–0.45)	0.41 (0.39–0.43)	0.43 (0.41–0.46)	0. 46 (0.42–0.53)	0.46 (0.42–52)
composite-score	Mean (SD)	−0.13 (0.85)	0.17 (0.92)	0.05 (0.88)	−0.44 (0.92)	0.01 (1.14)	0.12 (1.12)
MVPA/day (min)	Median(IQR)	55 (41–82)	45 (25–68)	55 (35–83)	46 (27–72)	34 (20–54)	28 (17–44)
Physical activity-categories	%	13/43/26/18	31/38/21/11	19/38/24/19	28/37/22/13	43/36/15/6	53/33/9/4
Ethnicity (white)	%	NA	96	98	99	21	25
Birthweight (g)	Mean (SD)	NA	3406 (586)	3543 (603)	3418 (505)	NA	NA
Mothers' BMI (kg m^{-2})	Median(IQR)	NA	22.8 (20.9–25.6)	23.1 (20 9–26.5)	25.3 (22.8–28.2)	NA	NA
Sexual maturity	%	NA	60/10/2/13/16	44/9/9/19/19	18/47/2/8/24	NA	NA
Household income quartiles	%	NA	NA	NA	NA	22/17/32/28	19/22/28/32
Mothers Education	%	NA	31/36/33	29/33/38	89/6/5	NA	NA

Abbreviations: CoSCIS, Copenhagen School Child Intervention Study; EYHS, European Youth Heart Study; NHANES, National Health and Nutrition Examination Survey; MVPA, moderate-to-vigorous physical activity; BMI, body mass index; IQR, inter-quartile range; SD, standard deviation. Waist-circumference was measured midway between the lower rib margin and the iliac crest in the EYHS and CoSCIS studies and just above the iliac crest at the midaxillary line in the NHANES studies. Mothers' BMI is based on self-reported height and weight. Household income quartiles in ascending order is based on distribution in complete NHANES data sets. WC:H-ratio, waist-circumference:height ratio; NA, not applicable; SD, standard deviation; IQR, 25th–75th percentile.

11-year-old boys, 1.3 cm in 6–11-year-old girls, 1.5 cm in 12–19-year-old boys and 12–19-year-old girls.[23] *A priori* selected putative confounders of the exposure-outcome, exposure-mediator or mediator-outcome relations, which were available from ICAD, were used as covariates; these included age, sex, ethnicity (White or not), birth weight (continuous), mother's BMI (continuous), sexual maturity (Tanner stages) and mother's education (high/medium/low) from EYHS studies. Available from NHANES was age, sex, ethnicity (White, Black, Asian and Hispanic) and household income (quartiles). Age and sex were available from CoSCIS.

Mediation analysis (effect decomposition)

We used a two-stage regression approach to decompose the total effect on the respective outcome into a direct effect and an indirect effect thereby providing a quantitative estimate of the relative importance of these. An important aspect when conducting mediation analysis is to consider possible interactions between the exposure and the putative mediator.[24,25] Allowing for potential interaction, if such is present, provides not only correct effect decomposition, but determining the presence of exposure–mediator interaction is of importance in understanding aetiology with potential practical relevance for tailoring interventions. Mediation analysis allowing for exposure–mediator interaction has been defined within the counterfactual framework.[26–28] For these effects to be identified in the presence of exposure–mediator interaction, it is necessary to estimate the change from one fixed level of exposure to another (exact definitions given below). We therefore dichotomized mean MVPA per day for adherence to the WHO physical activity recommendation for 5–17-year-old youth of at least 60 min of MVPA per day (yes/no). Using the two-stage regression approach we first fit (Model 1) a linear regression model for the outcome (*Y*) on achieving the respective activity exposure (*a*) controlling for waist-circumference:height ratio (mediator (*m*)), including a physical activity × waist-circumference:height ratio interaction term and controlling for confounding variables (*c*). Secondly, a linear regression model (Model 2) for the waist-circumference:height ratio on the respective activity exposure controlling for confounding variables is fitted.

$$\mathrm{E}\left[Y\middle|a,m,c\right] = \beta_0 + \beta_1 a + \beta_2 m + \beta_3 am + \beta_i c_i \quad (1)$$

$$\mathrm{E}\left[M\middle|a,c\right] = \theta_0 + \theta_1 a + \theta_i c_i \quad (2)$$

The coefficients from these regressions are then used to estimate the average direct and indirect effects in the population.[28] As the focus of this analysis is to decompose the putative sources of the total association we estimate the natural direct and indirect effects.[27,28] Using the counterfactual framework, the direct effect can be interpreted as the contrast between achieving the activity target and not achieving the activity target, while for each individual fixing the mediator to the level it would have assumed if the activity target had not been achieved. In other words, the direct effect estimates the effect of the activity target on the composite risk score not acting through abdominal obesity. Similarly, the indirect effect can be interpreted as the contrast between fixing the mediator to the level it would have assumed had the activity target been achieved versus the level it would have assumed had the activity target not been achieved, while setting the activity target to not achieved. That is, the indirect effect is the effect of the activity target acting on the composite risk score by the activity target influencing abdominal obesity which, in turns, affects the composite risk score.

Statistical analysis

Descriptive data are summarized as means with s.d. or medians with 25th and 75th percentiles dependent on distributional properties. All models were checked for normality and homoscedasticity of residuals and inspected for a linear association between continuous dependent and independent variables. HOMA-IR, triacylglycerol and waist-circumference: height ratio were transformed by the natural logarithm prior to estimations. When using logarithmically transformed variables as outcomes these were multiplied by 100 so they can be interpreted as a difference in percentage.[29] Results are presented as difference (standardized betas or percentage) in composite risk score, or individual risk factors, with 95% CI. The primary activity contrast was above or below 60 min of MPVA per day. To assess a possible graded association we further compared the following MVPA contrasts: (1) <30 versus $\geqslant 30$ min, (2) <30 versus $\geqslant 60$ min, (3) <30 versus $\geqslant 90$ min, and (4) <90 versus $\geqslant 90$ min. Total, direct and indirect effects were estimated separately for each study and were subsequently pooled by weighting the estimates (meta-analysis). A bootstrap procedure using 1000 repetitions, with replacement, was used to derive the study-specific 95% bias-corrected confidence intervals for the indirect effect as the 2.5th and the 97.5th percentiles. In meta-analysis the study weights are usually defined as 1/(standard error^2). Because the standard errors in this application are derived from a bootstrap procedure (in contrast to s.d./$\sqrt{N}$), the standard errors of the total, direct and indirect effects will not necessarily be identical if using 1/(standard error^2) as weights. This would result in failure of the decomposition to sum to the total effect in the meta-analysis, which is counterintuitive. Therefore, study weights were given as ($\sqrt{}$study sample size)/($\sqrt{}$total sample size). Specifying the weights as such precluded using a random-effects meta-analysis (see supplementary Table 2 for study weights). The consequence of using a fixed rather than a random effects approach is that relatively more weight will be given to larger studies. The Q-statistic and I^2 were used to assess between-study heterogeneity. The proportion of the total effect which can be attributed to the indirect effect was calculated as (indirect effect/total effect) × 100. To assess the robustness of results for meeting the 60-min MVPA target we performed the analysis stratified by sex and conducted the following sensitivity analyses: (1) omitting, in turn, one study from the meta-analysis, (2) reanalyse all data only controlling for age and sex, (3) treating the two age groups in EYHS studies as individual studies, (4) converting CoSCIS and EYHS waist-circumference measurements to NHANES equivalents using previously published equations,[23] (5) using BMI as mediator, (6) using an empirically derived waist-circumference to height exponent (allometric scaling), (7) including accelerometer wear-time as covariate, (8) including all participants with at least one valid day ($n = 3792$), (9) defining a valid day as $\geqslant 600$ min, (10) including season of physical activity assessment as a covariate to account for seasonal variation in physical activity, (11) defining MVPA as counts per min >1999 and, (12) estimated the size of a dichotomous vector of unmeasured or residual confounding (U) necessary to completely explain away the difference between the activity contrast. The latter analysis was performed assuming a 20% and 50% difference in prevalence of U simulating moderate and substantial confounding, respectively. Analyses were conducted using the PARAMED and METAN modules in Stata IC v.14.1 (StataCorp, College Station, TX, USA).

RESULTS

The median number of valid participant days was four with 79% of the sample achieving more than 3 days. Seven per cent of the

Table 2. Daily min of moderate-to-vigorous physical activity and clinical characteristics for the physical activity contrasts

	0–30 min of MVPA per day (n = *1076*)	*30 − <60 min of MVPA per day* (n = *1274*)	*60 − <90 min of MVPA per day* (n = *668*)	*⩾90 min of MVPA per day* (n = *394*)
MVPA (min per day)	17.8 (10.3–24.2)	43.6 (36.8–51.3)	72.8 (66.0–81.0)	107.8 (97.6–128.0)
HOMA-IR	1.9 (1.2–2.8)	1.5 (1.0–2.3)	1.3 (0.8–1.9)	1.1 (0.7–1.7)
Systolic BP (mm Hg)	106.0 (100.0–113.3)	104.7 (97.7–111.0)	102.0 (96.3–108.5)	100.7 (94.3 –08.0)
Triacylglycerol (mmol l^{-1})	0.8 (0.6–1.1)	0.8 (0.6–1.1)	0.6 (0.5–0.9)	0.6 (0.5–0.8)
HDL-c (mmol l^{-1})	1.4 (1.2–1.6)	1.5 (1.3–1.7)	1.4 (1.3–1.7)	1.5 (1.3–1.7)

Abbreviations: MVPA, moderate-to-vigorous physical activity; BP, blood pressure; HDL-c, high density lipoprotein-cholesterol. Data is median (25th–75th percentile).

Table 3. Total, direct and indirect effects of physical activity on the composite risk score

Physical activity contrast	*Sample* (n)	*Total effect (95% CI)*	*Direct effect (95% CI)*	*Indirect effect (95% CI)*	*Percent of total effect attributable to indirect effect*
< versus ⩾ 60 min MVPA per day	3412	− 0.307 (−0.389, − 0.225)	− 0.239 (−0.317, − 0.161)	− 0.068 (−0.112, − 0.023)	22%
< versus ⩾ 30 min MVPA per day	3412	− 0.240 (−0.320, − 0.160)	− 0.174 (−0.248, − 0.100)	− 0.066 (−0.102, − 0.030)	28%
< versus ⩾ 90 min MVPA per day	3412	− 0.391 (−0.508, − 0.273	− 0.331 (−0.438, − 0.224)	− 0.060 (−0.132, 0.013)	15%
< 30 versus ⩾ 60 min MVPA per day	2138	− 0.400 (−0.506, − 0.293)	− 0.304 (−0.403, − 0.205)	− 0.096 (−0.154, − 0.037)	24%
< 30 versus ⩾ 90 min MVPA per day	1470	− 0.578 (−0.733, − 0.423)	− 0.455 (−0.601, − 0.031)	− 0.123 (−0.233, − 0.013)	21%

Abbreviations: CI, confidence interval; MVPA, moderate-to-vigorous physical activity; HDL-c, high density lipoprotein-cholesterol. The composite risk score is comprised of standardized values of HOMA-IR, systolic blood pressure, triacylglycerol and inverse HDL-c. HOMA-IR and triacylglycerol were transformed by the natural logarithm before standardization. Exposures are the various physical activity contrasts coded as 0 for below the speCified threshold and 1 for above. Estimates are weighted means with 95% CI from fixed effects meta-analysis with study weights given as ($\sqrt{}$study sample size)/($\sqrt{}$total sample size).

sample did not have data from a weekend day, while 73% had data from two. Clinical characteristics and daily min of MVPA for the activity contrasts are shown in Table 2. Meeting the activity target of 60 min of MVPA per day, versus not meeting it, was associated with a − 0.31 (−0.39, − 0.23) s.d. lower composite risk score. This effect was composed of a direct effect of − 0.24 (−0.32, − 0.16) s.d. and an indirect effect of − 0.07 (−0.11, − 0.02) s.d. suggesting that 22% of the effect was attributable to the mediator (Table 3). Tests of between-study heterogeneity were highly significant for total, direct and indirect effect (all p's < 0.01). The I^2 indicated that 78–91% of the total variation was due to heterogeneity. Meeting the activity target of 30 min of MVPA per day, versus not meeting it, had a total effect on the risk score of − 0.24 (−0.32, − 0.16) s.d., a direct effect of − 0.17 (−0.25, − 0.10) s.d. and an indirect effect of − 0.07 (−0.10, − 0.03) s.d., that is, 28% of the total effect was attributable to the mediator. Similarly, meeting the 90 min of MVPA per day target versus not meeting the target resulted in a total effect on the risk score of − 0.39 (−0.51, − 0.27) s.d., a direct effect of − 0.33 (−0.44, − 0.24) s.d. and an indirect effect of − 0.06 (−0.13, 0.01) s.d.; hence, 15% of the total effect was attributable to the mediator. The confidence interval for the indirect effect included zero. When exploring the larger MVPA contrasts, results were similar with larger total effects explained primarily by larger direct effects.

We thereafter repeated the analyses using the individual risk factors as outcomes (Table 4). Meeting the activity target of 60 min of MVPA per day, versus not meeting it, resulted in a 15% lower HOMA-IR score (−0.20, − 0.10), a 1.9 mm Hg lower systolic blood pressure (−2.64, − 1.25), a 9% lower triacylglycerol level (−0.12, − 0.05), and a 0.05 mmol l^{-1} higher HDL-c level (0.02, 0.08). The percentage explained by the mediator varied from 12 to 31%.

When stratifying by sex, results for boys were largely similar to the sex-adjusted estimates with a total effect of meeting the PA target of 60 min of MVPA per day, versus not meeting it, on the risk score of − 0.31 (−0.42, − 0.21) s.d., a direct effect of − 0.22 (−0.32, − 0.12) s.d. and an indirect effect of − 0.09 (−0.15, − 0.04) s. d. In girls, effects were smaller with a total effect of − 0.19 (−0.37, − 0.00) s.d., a direct effect of − 0.18 (−0.47, 0.11) s.d. and an indirect effect of 0.03 (−0.25, 0.32) s.d. All effects in girls had confidence intervals overlapping zero. However, in the NHANES studies very few girls (24 in total) achieved 60 min of MVPA per day resulting in imprecise estimates for these studies.

Removing the exposure–mediator interaction had little impact on estimates. The total effect with no interaction was − 0.31 (−0.39, − 0.23) s.d., a direct effect of − 0.24 (−0.31, − 0.17) s.d., and an indirect effect of − 0.07 (0.11, − 0.03) s.d. None of our sensitivity analyses substantially changed results (Supplementary Figures 1–3). Excluding one study at a time resulted in little variation of the total effect (Supplementary Figures 1–3), but the composition changed substantially with estimates explained by the mediator varying from 15 to 35%. To reduce our estimates of the direct effect to zero, the influence of U on the risk score would have to be − 0.62 and − 1.55 s.d. under the moderate and substantial confounding scenarios, respectively. For the indirect effect, the required influence of U was estimated as 0.34 and 0.14 s.d. under moderate and substantial confounding scenarios, respectively.

DISCUSSION

The result of this effect decomposition model indicates that the majority of the association between meeting the 60 min MVPA per day target and metabolic control can be attributed to the direct effect of MVPA, rather than to an indirect effect by abdominal adiposity. The data demonstrated an inverse, graded relationship for the direct, although not the indirect effect.

A recent publication from the Cardiovascular Risk in Young Finns Study reported that a one s.d. increase in four different composite risk scores in youth predicted incident type 2 diabetes

Table 4. Total, direct and indirect effects of physical activity on individual metabolic risk factors using below versus above 60 min of MVPA per day as activity contrast

Risk factor	*Sample* (n)	*Total effect (95% CI)*		*Direct effect (95% CI)*	*Indirect effect (95% CI)*	*Percent of total effect attributable to indirect effect*
HOMA-IR (%)	3412	−0.150 (−0.203, −0.098) [−0.243]		−0.104 (−0.153, −0.055) [−0.169]	−0.046 (−0.076, −0.016) [−0.074]	31%
Systolic blood pressure (mm Hg)	3412	−1.895 (−2.644, −1.146) [−0.205]		−1.620 (−2.345, −0.895) [−0.176]	−0.275 (−0.548, −0.002) [−0.029]	15%
Triacylglycerol (%)	3412	−0.085 (−0.119, −0.051) [−0.157]		−0.075 (−0.110, −0.040) [−0.131]	−0.010 (−0.022, 0.001) [−0.026]	12%
HDL-c (mmol l^{-1})	3412	0.048 (0.021, 0.075) [0.118]		0.034 (0.007, 0.061) [0.072]	0.014 (0.004, 0.025) [0.046]	29%

Abbreviations: CI, confidence interval; MVPA, moderate-to-vigorous physical activity; HDL-c, high density lipoprotein-cholesterol. HOMA-IR and triacylglycerol are logarithmically transformed and multiplied by 100 so can be interpreted as a difference in percentage. The contrast is an exposure below (coded as 0) vs above (coded as 1) 60 min of MVPA per day. Estimates are unstandardized weighted means with 95% CI from fixed effects meta-analysis with study weights given as ($\sqrt{}$study sample size)/($\sqrt{}$total sample size). Values in brackets are standardized betas.

and advanced atherosclerosis between 15 and 25 years later. The magnitude was substantial with an increased risk between 30 to 78% for type 2 diabetes and 12 to 61% for advanced atherosclerosis, depending on the definition of the composite score.[30] Thus, our estimate of a 0.31 s.d. lower risk score for meeting, compared with not meeting, the 60 min MVPA target is clinically relevant. Further, meeting the 60 min MVPA target was associated with a 1.9 mm Hg lower systolic blood pressure of which less than one fifth of the effect was through abdominal adiposity. Assuming such a difference is maintained over time, estimated reductions in stroke and ischaemic heart disease in middle age are likely not trivial.[31]

The observed direct association between MVPA and the composite risk score not through abdominal adiposity was inverse and graded. In opposite, we found no indication of a graded relationship with the composite score for the association through abdominal adiposity when contrasting meeting versus not meeting the 30, 60 and 90 min MVPA targets and in absolute terms the contribution of abdominal adiposity to the association was modest. This is not surprising given the unclear role of physical activity for the prevention of excess adipose tissue accumulation in youth.[12,13] Importantly, with increasing activity contrasts (below 30 min vs above 60 and above 90 min) the absolute magnitude of the indirect effect increased, although not in relation to the total effect as indicated by the percent attributable to the indirect effect. This suggests that large activity contrasts are needed to reduce adipose tissue to a degree where this reduction may improve metabolic control whereas, in comparison, even meeting the 30 min MVPA target conferred a three times higher reduction in the composite score compared with those not meeting this amount of MVPA. Therefore, indices of adiposity may not be the most sensitive marker of improved metabolic control following a physical activity intervention. Further, increases in physical activity at the population level are likely to be important even if adiposity is not affected. We observed very little effect of allowing for the interaction between physical activity and abdominal adiposity and the interaction terms were not statistically significant (results not shown) indicating that the relationship between meeting these physical activity contrasts and abdominal adiposity is additive only. This observation may be interpreted as the effect of, for example, accumulating 60 min of daily MPVA on metabolic control is similar across levels of abdominal adiposity, potentially relevant for intervention studies in high-risk populations.

An effect of physical activity on metabolic control irrespective of weight loss is biologically plausible. Physical activity improves insulin sensitivity up to 72 h in adults[32] and stimulates glucose uptake in the muscle through a non-insulin-dependent impact on the GLUT4 protein allocation. Insulin resistance, together with abdominal obesity, may be the common factor behind hypertension, dyslipidaemia and chronic low grade inflammation.[4] An independent effect of physical activity on metabolic risk factors in youth is in concert with previous publications.[14–17,33] We extend these observations by formal effect decomposition modelling and allowing for interaction between physical activity and abdominal adiposity to describe the associations in more detail. Our results corroborate previous studies suggesting a direct effect of MVPA on metabolic control since these associations are usually only slightly attenuated following adjustment for adiposity indices in the model.[17] Plausibility of a direct effect is further supported by the following observations: (1) An improvement in metabolic control without concurrent loss of adipose tissue was reported in an uncontrolled study of 21 overweight girls after 12 weeks of aerobic training,[34] (2) one-legged training studies demonstrate site-specific adaptations in glucose metabolism[35] which, by design, are independent of abdominal fat mass, and, (3) in adults, physical activity is associated with a reduced risk of mortality in all strata of adiposity.[36] In a cross-sectional study in young Brazilian adults, Horta *et al.*[37] found that the indirect effect of MVPA

through waist-circumference explained 44% of the total effect of MVPA on arterial stiffness. Such a high indirect effect may be due to differences in age in comparison with the present study or that arterial stiffness represents years of accumulated exposure, while the other biological risk factors respond more rapidly to recent changes in behaviour.

The present study should be interpreted in the light of several limitations. (1) The total effect and the effect decomposition is based on the assumptions of no residual or unmeasured exposure–outcome, exposure–mediator or mediator–outcome confounding as well as no mediator–outcome confounder affected by the exposure. In our analysis, we controlled for important biological variables such as birth weight and maturation as well as mother's BMI and markers of socioeconomic status and ethnicity in all but the CoSCIS study. Importantly, in models only adjusted for age and sex results were virtually unchanged. Further, when we, under simplistic assumptions, simulated an unmeasured confounder (for example diet, a genetic factor or smoking) the impact of such would have to be substantial to completely explain the total effect under the moderate confounding scenario, but only minor to explain the indirect effect under the substantial confounding scenario. However, the decomposition should be interpreted in the light of the lack of control of diet quality and quantity which could potentially confound all paths in the analysis. (2) The study is cross-sectional so we cannot infer direction of causality or even claim that adiposity is in fact a mediator as it could also be conceptualized as a confounder. Our position is that the fundamental laws of thermodynamics speak to a not purely confounding role, irrespective of the intrinsically complicated nature of weight management. Under the assumptions of our decomposition, the major constituent of the association was direct; suggesting physical activity positively influences metabolic control irrespective of either mediating or confounding from adiposity. However, it is conceivable that the association between physical activity and adiposity is bidirectional.[38] To investigate this issue, we re-arranged the exposure–mediator order and modelled associations with being overweight/obese (according to IOTF age-and gender-specific BMI cut-points as no international waist-circumference cut-points exists) versus not being so, using physical activity in its continuous form and allowing for exposure–mediator interaction. While being overweight/obese, as compared to not being so, was associated with a nearly one standard deviation higher composite risk score (total effect with 95% CI: 0.81 (0.72, 0.86)), the data suggest only 2% of this association (indirect effect with 95% CI: 0.02 (0.002, 0.03)) is attributable to the association between being overweight/obese and lower physical activity levels. This lends support to our hypothesized pathway where it is physical activity which prevents excess body weight, but non-identical levels of measurement error in physical activity and adiposity assessment questions a direct comparison of the indirect effects. (3) We were unable to account for the sampling procedure in the NHANES studies or the composite data structure in EYHS and CoSCIS. The latter tend to artificially deflate variance estimates. (4) We were unable to weight our meta-analytic estimates based on precision (standard error) and maintain meaningful effect decomposition. When we omitted one study at a time, the total effect was robust while the decomposition was more sensitive. However, the between study heterogeneity was substantial, the cause of which should be an area of further study. (5) Waist-circumference:height ratio is not a direct measure of abdominal adiposity, which may introduce measurement error in our mediator biasing our indirect effect towards the null.[39] However, the correlation between waist-circumference and fat mass assessed by dual-energy X-ray absorptiometry in children is high ($r > 0.88$) and associations with health outcomes are nearly identical between the two measures[40] giving us confidence in the use of waist-circumference as indicator of abdominal adiposity. (6) Accelerometry is an imperfect measure of physical activity which will have resulted in an underestimation of the total effect and may also have influenced the relative contributions of direct and indirect effects. Further, lack of consensus concerning measurement protocols and data-reduction decisions limits the ability to make direct comparisons between independent manuscripts. (7) Excess adipose tissue accumulation represents only one of many mediators of health benefits from physical activity. For example, physical activity may also improve cardiorespiratory fitness which is highly associated with metabolic risk factors.[17] Our direct effects would include associations owing to physically active youth having a higher cardiorespiratory fitness, and future studies could consider attempts to further decompose physical activity associations with health outcomes. In summary, habitual levels of MVPA was associated, in an inverse, graded relationship, with a strong direct effect on a composite risk score of established cardiovascular disease risk factors in youth, while the indirect effect through adiposity was smaller in comparison. Thus our results extend previous reports of an independent effect of physical activity by doing a formal decomposition and allowing for exposure–mediator interaction.

Availability of data and materials

Data from ICAD are available per request as a supported access resource. Analysed data are de-identified from the main ICAD database, available only for the approved analyses, and cannot be shared by the authors. Analyses included in this manuscript can be reproduced by requesting a new data release.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

JT conceived the study, performed the analysis, led the analysis and writing of the manuscript. AB conceived the study, analyzed the data and critically revised the manuscript. NCM conceived the study, analyzed the data, critically revised the manuscript and is an ICAD Collaborator. LBA conceived the study, analyzed the data, critically revised the manuscript and is an ICAD Collaborator. LBS analyzed the data, critically revised the manuscript and is an ICAD Collaborator. UE analyzed the data, critically revised the manuscript and is an ICAD Collaborator. SB analyzed the data and critically revised the manuscript. All authors approved the final version of the manuscript. JT is the guarantor of this work.

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Supplementary Information

Exact study weights, characteristics of non-analysed sample, and impact of sensitivity analyses on point estimates and 95 % confidence intervals for total, direct and indirect effects on the clustered risk score contrasting above versus below sixty minutes of MVPA/day. Analyses detailed in the main manuscript.

Table of contents

The following sensitivity analyses were applied.

- Converting CoSCIS and EYHS waist-circumference measurement site to NHANES site using correction formulas given by Patry-Parisien et al. (2012).
- Allometric scaling of WC to height
- Using BMI as mediator
- Including all participants with at least one valid day (n=3792)
- Defining a valid day as at least 600 minutes of wear-time (n=3095)
- Treating the two age groups (9- and 15 years old) in EYHS studies as individual studies
- Removing the exposure-mediator interaction
- Including accelerometer wear-time as covariable
- Omitting all confounding variables except age and sex
- Including season of physical activity measurement as covariate
- Setting MVPA cut-point to >1999 counts/min
- Omitting, in turn, each study from the meta-analysis

Supplementary Table 1. Comparison of characteristics for analysed (n=3412) versus non-analysed (n=5533) samples from the six studies with relevant data.

		Analysed sample (n=3412)	N (non-analysed sample)	Non-analysed sample	Adjusted[a] difference (beta-coefficient with 95% CI)	P-value[b]
Age[c]	Median(IQR)	12.1 (9.6 – 15.4)	5533	10.6 (8.8 – 14.6)	NA	<0.001
Gender (girls)[d]	%	51	5533	51	NA	0.52
Height (cm)	Mean (SD)	150.4 (136.7 – 166)	5500	145.7 (132 – 162)	0.42 (0.25 to 0.59)	<0.001
Weight (kg)	Median(IQR)	43.9 (30.9 - 59.1)	5507	42.4 (29.2 - 58.7)	-1.2 (-1.5 to -1.0)	<0.001
Waist-circumference (cm)	Median(IQR)	65.0 (58.0 – 73.3)	5428	67.5 (58.3 - 77.5)	-2.1 (-2.4 to -1.89)	<0.001
MVPA/day (min)[e]	Median(IQR)	43.4 (25.0 - 67.9)	4154	41.5 (25.5 - 62.6)	2.3 (1.6 to 3.0)	<0.001
Above 60 minutes MVPA/day[d,e]	(%)	31	4154	28	NA	0.001
Above 90 minutes MVPA/day[d,e]	(%)	12	4154	8	NA	<0.001
logHOMA-IR	Mean (SD)	0.40 (-0.04 - 0.86)	1583	0.16 (-0.44 - 0.67)	0.08 (0.06 to 0.10)	<0.001
Systolic BP (mmHg)	Mean (SD)	104.0 (97.3 – 111.0)	4752	104 (96.7 - 111.3)	0.1 (-0.1 to 0.3)	0.37
logTriacylglycerol (mmol/l)	Mean (SD)	-0.36 (-0.66 - -0.04)	1952	-.34 (-0.61 - 0.01)	-0.05 (-0.06 to - 0.03	<0.001
HDL-c (mmol/l)	Mean (SD)	1.42 (1.24 - 1.66)	4657	1.4 (1.2 - 1.66)	0.02 (0.01 to 0.02)	<0.001

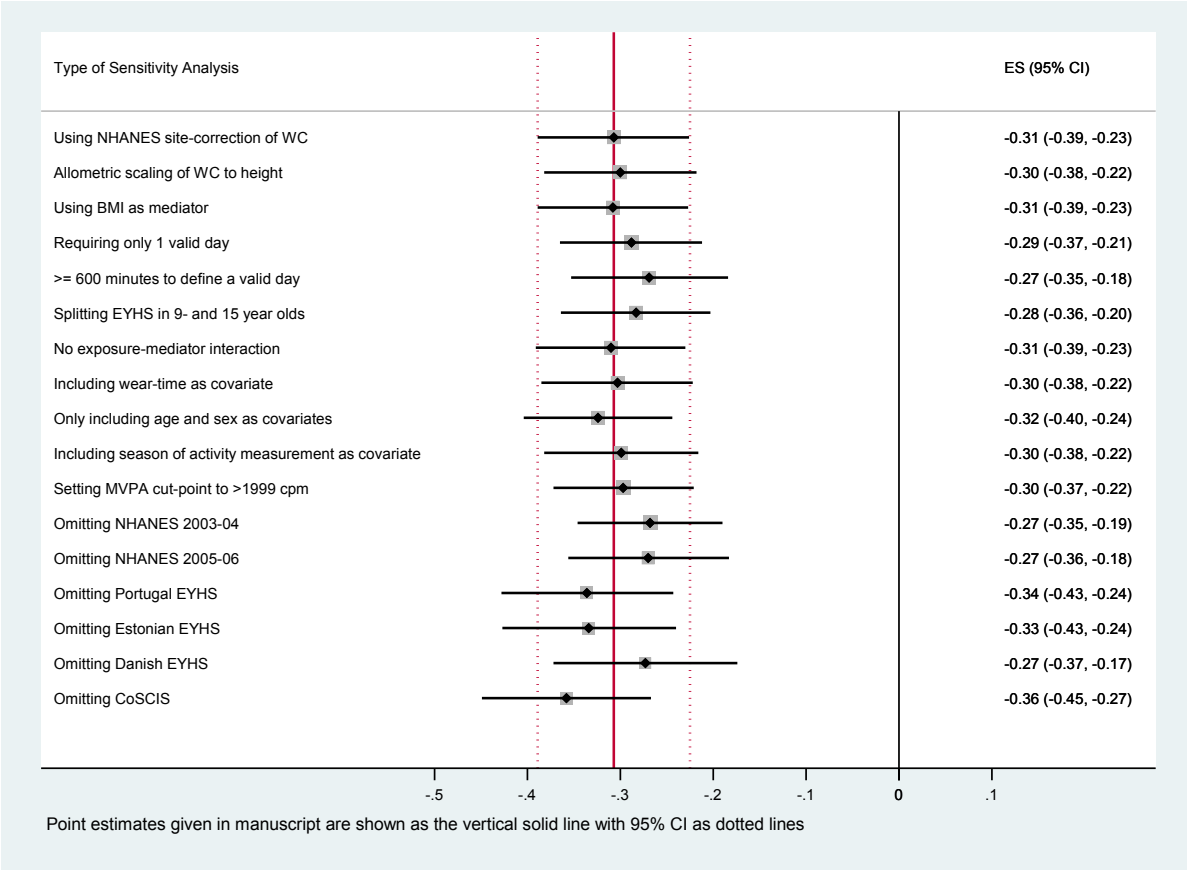
NA: not applicable. IQR: 25th – 75th percentile. Descriptive characteristics are crude values. [a]Adjusted difference from a linear regression model including age as covariate (non-analysed sample as reference). [b]P-value is for between-sample comparison from the linear regression model unless otherwise indicated. [c]Unpaired t-test. [d]chi-squared test. [e]Only participants with >3 days with 500 minutes of wear-time included.

Supplementary Table 2. Study weights

Study	Sample size	Square-root (sample-size)	Weight (%)
CoSCIS	358	18.9	13.4
EYHS DK	1022	31.9	22.7
EYHS ESTONIA	535	23.1	16.4
NHANES 05/06	475	21.8	15.5
NHANES 03/04	523	22.9	16.2
EYHS Portugal	499	22.3	15.8
Sum	3412	141	100

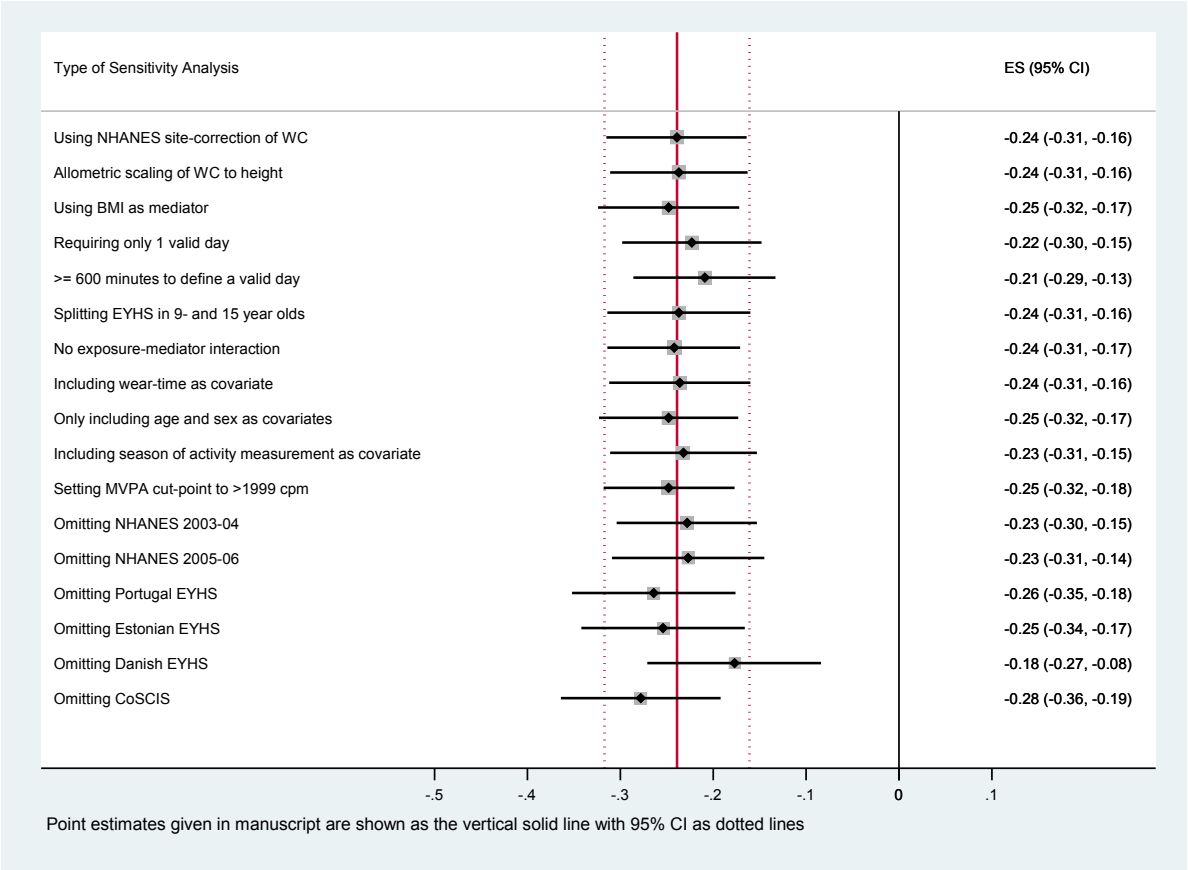
Discrepancies due to rounding.

Supplementary Figure 1. Sensitivity analyses for total effect



The following sensitivity analyses were applied in order of appearance on the figure: 1) Converting CoSCIS and EYHS waist-circumference measurement site to NHANES site using correction formulas given by Patry-Parisien et al. (2012). 2) Allometric scaling of WC to height. 3) Using BMI as mediator. 4) Including all participants with at least one valid day (n=3792). 5) Defining a valid day as at least 600 minutes of wear-time (n=3095). 6) Treating the two age groups (9- and 15 years old) in EYHS studies as individual studies. 7) Removing the exposure-mediator interaction. 8) Including accelerometer wear-time as covariable. 9) Omitting all confounding variables except age and sex. 10) Including season of physical activity measurement as covariate (coded as 0: January-March, 1: April-September, 2: October-December). 11) Setting MVPA cut-point to >1999 counts/min. 12) Omitting, in turn, each study from the meta-analysis.

Supplementary Figure 2. Sensitivity analyses for direct effect



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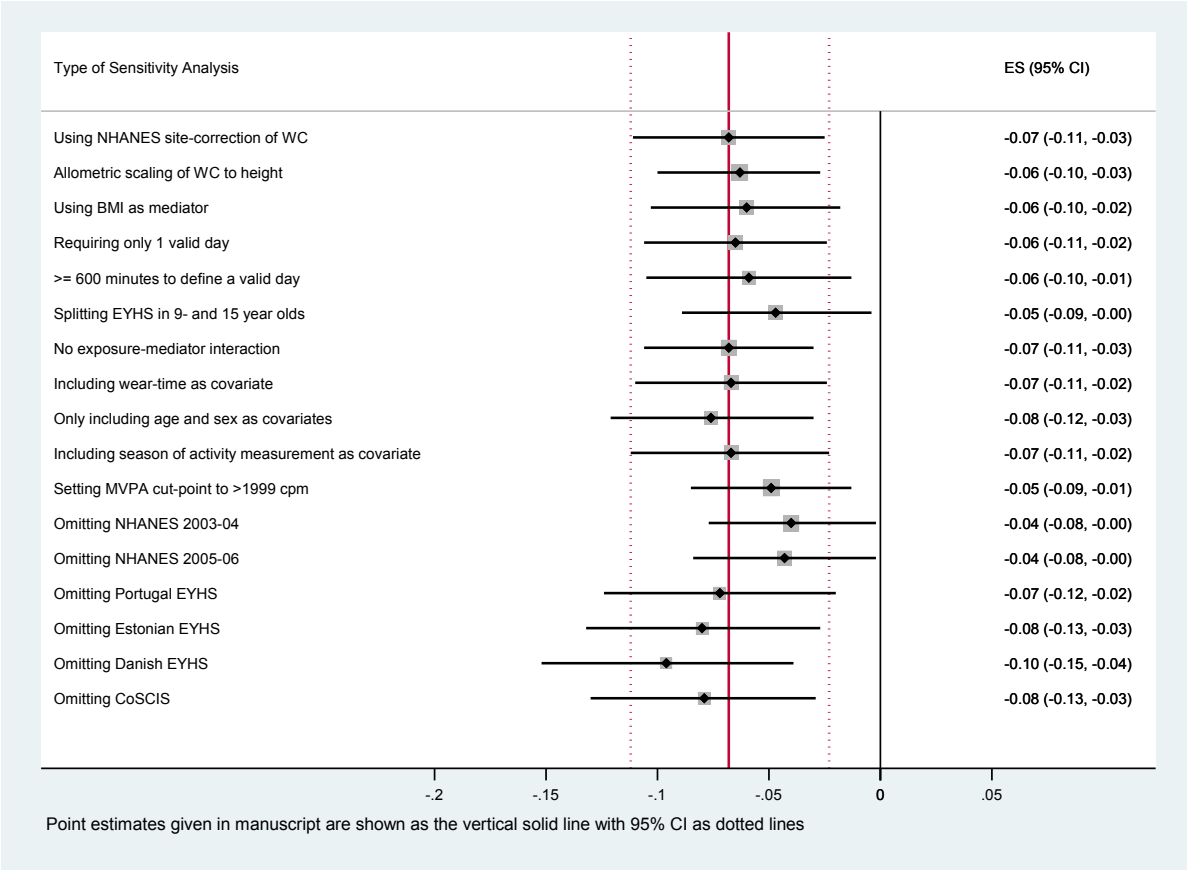
Supplementary Figure 3. Sensitivity analyses for indirect effect



The following sensitivity analyses were applied in order of appearance on the figure: 1) Converting CoSCIS and EYHS waist-circumference measurement site to NHANES site using correction formulas given by Patry-Parisien et al. (2012). 2) Allometric scaling of WC to height. 3) Using BMI as mediator. 4) Including all participants with at least one valid day (n=3792). 5) Defining a valid day as at least 600 minutes of wear-time (n=3095). 6) Treating the two age groups (9- and 15 years old) in EYHS studies as individual studies. 7) Removing the exposure-mediator interaction. 8) Including accelerometer wear-time as covariable. 9) Omitting all confounding variables except age and sex. 10) Including season of physical activity measurement as covariate (coded as 0: January-March, 1: April-September, 2: October-December). 11) Setting MVPA cut-point to >1999 counts/min. 12) Omitting, in turn, each study from the meta-analysis.

Study group members

Does adiposity mediate the relationship between physical activity and biological risk factors in youth? – A cross sectional study from the International Children's Accelerometry Database (ICAD)

Jakob Tarp, Anna Bugge, Lars Bo Andersen, Luis B. Sardinha, Ulf Ekelund, Soren Brage, Niels Christian Møller. On behalf of the International Children's Accelerometry Database (ICAD) Collaborators.

Study 3

Physical activity intensity, bout-duration and cardiometabolic risk markers in young people

Short title: Physical activity and cardiometabolic risk in youth

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Conflict of interest

The authors declare no conflict of interest.

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Abstract

Objectives: Determine the role of intensity and bout-duration in modulating associations between physical activity and cardiometabolic risk markers in young people.

Methods: A cross-sectional study from the International Children’s Accelerometry Database including 38 306 observations. Accelerometry data was summarized as time accumulated in sixteen combinations of intensity thresholds (≥500 to ≥3000 counts/min) and bout-durations (≥1 to ≥10 minutes). Outcomes were body-mass-index, waist-circumference and biochemical markers. A composite risk score was created. Associations were analysed as 1) linear mixed models controlling for age, sex, wear-time and random effects for participant and study, 2) meta-regression, and 3) isotemporal substitution of short to longer bouts.

Results: Physical activity was significantly and inversely associated with the risk factors irrespective of cut-point, with increasing intensity associated with larger effect-sizes for most outcomes. Each 1000 counts/min increase in intensity threshold was associated with a -0.027 (95% CI: -0.039 to -0.014) standard deviations lower composite risk score, and a -0.064 (95% CI: -0.09 to -0.038) kg/m^2 lower body mass index. Despite a pattern of increasing effects-sizes with longer bout-durations (e.g. composite risk score -0.071 (95% CI: -0.085 to -0.058) standard deviation for ≥1 minute bouts and -0.104 (95% CI: -0.143 to -0.064 for ≥10 minutes bouts), little evidence to support additional benefits of longer bouts of physical activity was found, when total volume was accounted for.

Conclusions: Physical activity intensity modified the association between physical activity and cardiometabolic risk factors. The data did not support additional benefits of physical activity when accumulated in long compared to shorter bouts.

Introduction

Cardiovascular disease accounted for 17.6 million deaths worldwide in 2016, making it the leading cause of non-communicable disease mortality (1). While the disease is generally a concern in adulthood, cardiometabolic risk factors are present from a much earlier age, for example endothelial damage leading to atherosclerosis may first develop in adolescence (2). In addition, previous evidence suggests cardiometabolic risk factors may track from childhood and adolescence into adulthood (3; 4). Taken together, this makes it paramount to understand the modifiable determinants of cardiometabolic risk factors in young people. In adults, participation in physical activity improves levels of biological risk factors such as glycaemic control, lipids, and blood pressure (5; 6), and this association is also present in children and adolescents (7-9).

Current national and international physical activity guidelines recommends adults should accumulate moderate-to-vigorous physical activity (MVPA) or vigorous physical activity in bouts of at least 10 minute duration (10; 11). For children and adolescents, at least 60 minutes per day of MVPA is recommended (10; 11). In some (10; 12), but not all (13), national guidelines no minimum bout duration for MVPA is specified for youth and studies investigating this issue remain scarce in this age group (14-17).

Further, although it is well-established that choice of movement intensity cut-offs affects physical activity summary measures from accelerometers such as time spent in MVPA (18), it has not been sufficiently explored how associations with cardiometabolic risk factors may be modulated by intensity thresholds, let alone combined with an evaluation of bout duration. Therefore, the purpose of this study is to assess how physical activity of different intensities and accumulated in bouts of varying duration relates to cardiometabolic health in young people.

Methods

Study design and Participants

This study is based on secondary data from the ICAD (http://www.mrc-epid.cam.ac.uk/Research /Studies/) which contains harmonized objectively measured physical activity data from studies in youth across the world (19). All studies were based on participant/parental written informed consent and consulted with their respective research boards to ensure appropriate ethical approval of data-sharing. Included studies were conducted between 1997 and 2009 in eleven countries: five in the U.S. (20-24), four in the U.K. (25-28), two each in Denmark (29; 30), Australia (31; 32), and Switzerland (33; 34), and one each in Estonia (29), Norway (29), Portugal (29), Belgium (35),

Scotland (36), and Brazil (37). A total of 44 869 physical activity files were available from the ICAD database. After exclusion of participants due to; insufficient or unreliable physical activity data, missing outcome data, or age outside the 4 – 18 year range, a final sample size of 38 306 observations from 29 800 unique individuals was included.

Physical activity data reduction

A detailed description of the protocol for harmonization of physical activity data is provided elsewhere (19). In short, available raw data files were reanalysed to create directly comparable variables across all contributing ICAD studies. Epoch length was harmonized to 60 seconds due to the lack of availability of shorter epochs in older studies (KineSoft version 3.3.20, KineSoft, Saskatchewan, Canada). For this analysis, all epochs producing counts ≥ 30 000 were deemed incompatible with human movement behaviour and considered non-wear. Non-wear was further defined as strings of identical count values for >60 consecutive minutes in the data time-series (Python, Python Software Foundation, Delaware, United U.S.). These strings were removed before summation of activity and wear time. As strings of identical count values are unlikely to represent true movement behaviour, this approach will both remove continuous zero counts and reduce data with technical malfunction (i.e. count plateau). To avoid extreme outliers, days with recorded mean counts/min above the 99.9th percentile (2125 counts/min (cpm)) or below the 0.1th percentile (36 cpm) were discarded. Three or more days of ≥500 minutes of wear-time between 7 am and midnight were required for a participant to be included in this analysis. To investigate the effect of higher intensity of physical activity we defined four increasing, but arbitrarily chosen, cut-points; ≥500cpm, ≥1000cpm, ≥2000cpm, and ≥3000cpm. Further, we summarized time above these cut-points as uninterrupted bouts of ≥1 (includes all activity), ≥2, ≥5 (medium), and ≥10 (long) minutes. A bout was terminated when counts dropped below the respective activity cut-point. Variables were

derived for each day and averaged across valid days for analysis. These data reductions lead to sixteen combinations of intensity and bout-duration.

Assessment of cardiometabolic risk factors

Outcome variables consisted of two anthropometric (waist-circumference, body mass index (BMI) and five biological (insulin, glucose, triglyceride, diastolic blood pressure, HDL-cholesterol) markers reflecting established cardiometabolic risk factors. Standardised methods were used to measure height and weight across all studies with BMI calculated as weight (in kilograms) divided by height (in metres) squared. BMI was the only outcome available from all studies. Waist-circumference was measured by the same procedure (WHO) in all contributing studies except the U.S. National Health and Nutrition Examination Survey (NHANES). The latter used a metal anthropometric tape placed at the midaxillary line (just above the iliac crest) (20; 21), as opposed to the midpoint between the lowest rib and iliac crest (25-27; 29; 30; 33; 34; 36; 37). We converted NHANES data to WHO measurement methodology by applying a correction formula (38). Blood pressure was measured in ten studies, all using repeated measurements with automated (25; 29; 30; 36; 37) or manual (20; 21) methods after at least five min of rest. Only diastolic blood pressure is presented in this study. Eight and seven studies obtained fasting measures of lipid (triglyceride and HDL-cholesterol) and glycaemic (glucose and insulin) metabolism, respectively. All using standardized procedures (20; 21; 29; 30; 34). To maximize information on the latent cardiometabolic risk profile, we additionally calculated two composite risk scores using standardized values (z-scores) of the risk factors (39). Composite score one included BMI, the homeostasis assessment model of insulin resistance (HOMA-IR) (40), triglyceride, mean arterial pressure (calculated as 1/3*systolic blood pressure + 2/3*diastolic blood pressure), and inverse

HDL-cholesterol. Composite score two was identical but excluded BMI. All variables were standardized for age and sex with mean arterial pressure additionally standardized for height and BMI, HOMA-IR, and triglyceride as log-transformed before standardization. The composite score was standardized to a mean of zero and standard deviation of one before analysis.

Statistical Analysis

Central tendencies of continuous variables are presented as mean (standard deviation) or median (25^{th}-75^{th} percentiles) based on distributional properties. Bout/intensity intercorrelations were explored using Spearman's partial correlation controlling for age, sex, wear-time and study. Data from studies was pooled into one dataset, and separate multivariable linear mixed regression models were used to analyse associations between the nine outcomes and sixteen combinations of intensity and bout durations while including the co-variates age, sex, and wear-time. Diastolic blood pressure and waist-circumference were additionally adjusted for stature. The non-adiposity composite score, insulin, glucose, triglycerides, HDL-cholesterol, and diastolic blood pressure were additionally controlled for BMI in secondary models. Participants and studies were modelled as "random-effects" in all models. Additional adjustment for number of included days produced minimal changes in coefficients. Regression models were visually inspected for normal-distribution of residuals, variance homoscedacity, and linearity between independent and dependent variables as well as for influential observations (Cook's d). All model assumptions were verified and no transformation of variables necessary. Regression coefficients and 95 % confidence intervals are presented graphically in the form of forest plots, and represent the difference in outcome per 10 min/day positive difference in physical activity. To directly model whether physical activity spent in medium or long bouts confers an additional health benefit over an identical amount of shorter

bouts of physical activity, we used an isotemporal substitution approach (41). These models took the form (omitting error term):

$$Y = \beta_0 + \beta_1 \text{Physical Activity}_{\geq 5\text{-}9 \text{ minute bouts at intensity}} + \beta_2 \text{Physical Activity}_{\geq 10 \text{ minute bouts at intensity}} + \beta_3 \text{Total Physical Activity}_{\text{at intensity}} + \beta_4 \text{Wear-time} + \beta_5 \text{Age} + \beta_6 \text{Sex} + \zeta_1 \text{Study} + \zeta_2 \text{Participant}$$

This model constraints total physical activity above the intensity threshold, thereby allowing for investigation of its composition (42). The coefficients β_1 and β_2 thus represents the effect of substituting time spent in physical activity of 1-4 minutes duration (short bout-duration) with an equal amount of time spent in medium or long bout-durations of MVPA (41). Meta-regression was used to explore trends in the influence of intensity and bout duration on the outcomes. An intensity-by-bout duration interaction term was added in a separate meta-regression model to explore potential heterogeneity in effects across bout duration/intensity combinations. Estimates for bout-durations of ≥3 and ≥7 minutes were added to the meta-regression to increase information. Confidence intervals in meta-regression were adapted to account for non-independence of coefficients (43). Analyses were conducted using Stata/IC version 15.0. Significance tests were two-sided, and *p* values less than 0.05 were considered statistically significant. We did not include adjustment for multiple testing and provide an interpretation of data based on the pattern of results.

Results

Characteristics of the study sample

Participant and study characteristics, including number of available studies and participants, are presented in Table 1, Table 2 and in Supplementary File Table S1. The median age of participants was 11.7 (11.1 – 13.6) and 26 % of the sample was overweight or obese. Each observation contributed a median of 6 (4 - 6) days with a mean of 13.2 (1.2) hours of wear-time/day. Boys spent

a higher percentage of their time above each intensity/bout combination threshold (all p-values <0.001, Figure 1). Correlations between bout durations were high but decreased with higher intensity thresholds (correlation matrix shown in Supplementary File Table S2).

Associations between combinations of intensity and bout durations with cardiometabolic risk factors

Forest plots of bout/intensity combinations and their associations with the composite risk score, non-adiposity composite risk score, and BMI are shown in figure 2 and 3. As the overall pattern of association was similar for the remaining outcomes, we show results (with and without BMI adjustment) for insulin, glucose, triglyceride, HDL-cholesterol, diastolic blood pressure and waist-circumference in Supplementary File figure S1 – S6. Intensity/bout combinations were negatively associated with the cardiometabolic risk factors suggesting participants with higher activity levels had more favourable risk profiles irrespective of intensity threshold and bout-duration in the range examined. Additional control for BMI attenuated effect-sizes, with attenuation appearing greater (absolute and relative) at higher intensities (figure 1 and Supplementary file figure S1 – S5. Overall, the data suggested a pattern of increasing effect-sizes with activity accumulated at higher cut-points e.g. a ten minute difference in total physical activity ≥500cpm was associated with a -0.014 standard deviations (95% CI: -0.018 to -0.01) lower composite risk score and a -0.016 (95% CI: -0.022 to -0.011) kg/m^2 lower BMI. In comparison, additional ten minutes of activity ≥3000cpm was associated with a -0.069 standard deviations (95%CI: -0.081 to -0.056) lower composite risk score and a -0.141 (95% CI: -0.157 to -0.125) kg/m^2 lower BMI. A pattern of increasing effect-sizes with increasing bout-durations was observed within all intensity thresholds. E.g. a ten minutes difference in total physical activity ≥2000cpm was associated with a -0.043 standard deviations (95% CI: -

0.051 to -0.035) lower composite risk score, while ten minutes of the same intensity obtained in medium, and long bouts were associated with a -0.065 (95% CI: -0.078 to -0.052) and -0.081 (95% CI: -0.101 to -0.061) standard deviations lower composite score, respectively. Effect-sizes for glucose and triglycerides followed an irregular pattern at ≥2000cpm and ≥3000cpm with weaker associations observed with medium and long bout-durations (Supplementary File figure S2 and S3).

Meta-regression suggested independent contributions of intensity for all outcomes except for glucose and triglyceride were confidence intervals straddled zero (coefficients shown in Supplementary File table S3). No statistical support for independent effects of bout-duration on outcomes was found. Each 1000 cpm increase in the activity threshold was associated with a -0.026 (-0.039 to -0.014) standard deviations and a -0.064 (-0.09 to -0.038) kg/m^2 difference in the beta-coefficient for the composite score and BMI, respectively. When adding the intensity-by-bout duration interaction term, this did not reach statistical significance for any outcome (coefficients shown in Supplementary File table S3).

Isotemporal substitution of short for medium and long bouts of physical activity

Supplementary file Table S4 includes quintiles of residual variation in bouted physical activity after controlling for total activity volume (≥1 minute bouts), sex, age and wear-time. Associations with isotemporal activity substitution and the composite risk score, non-adiposity risk score and BMI are shown in table 3 (other outcomes shown in Supplementary File table S5 and S6). Replacing ten minutes/day of activity accumulated in short bouts with an identical amount of same intensity time accumulated in medium or long bouts, produced mixed associations with the cardiometabolic risk

factors. E.g. substituting short bout activity above 500cpm with physical activity accumulated in long bouts was associated with a -0.032 (95% CI: -0.047 to -0.018) standard deviation lower composite score, but substituting ten minutes of short bout activity above 3000cpm with the same amount of activity accumulated in long bouts was associated with a 0.066 (95% CI: 0.013 to 0.118) standard deviation *higher* composite score.

Discussion

These data suggests physical activity intensity is associated with cardiometabolic risk markers in youth irrespective of the pattern of accumulation. Activity accumulated at higher intensities produced progressively stronger benefits as indicated by lower levels of risk markers and a favourable body composition within the range examined. Results for bout-duration were equivocal with meta-regression as well as isotemporal substitution models providing little evidence for an additional benefit of bouted activity above that of a strong correlation with total physical activity. Consistent with previous data, controlling for adiposity did not fully explain associations (44).

Physical activity intensity

It is well-established that physical activity improves cardiometabolic risk factors in children and adolescents (7; 9; 45). In epidemiological studies, physical activity is often operationalized by MVPA which is frequently defined in the range of 2000 to 3200 cpm in youth (46). We show that favourable associations with the risk factors are already present at the lower end of the intensity spectrum, in the range of what is often considered light physical activity, but increase in magnitude

as intensity increases. Intensity-dependent associations are consistent with the results of randomized-controlled trials comparing high-intensity interval training with continuous lower intensity exercise (47). In children, 3000cpm corresponds to walking at approximately 4-5 km/h (48; 49) and should thus be readily attained by healthy individuals. However, considering the sample spent on average ≈ 3 % of their time above 3000cpm and less than 50 % of boys and girls accumulated any 10-minute bout above this cut-point, accrual of beneficial associations at lower intensities is a useful observation for clinicians. It is possible that any locomotion is beneficial and limiting sedentary behaviour is paramount, a notion partly reflected in the Canadian 24-hour movement guidelines (50). However, isotemporal substitution of sedentary behaviour with light physical activity in young people does not, at least for adiposity, support this notion (51). An additional argument for higher intensity activity is potential fitness adaptations. Higher fitness-levels in adolescence are strongly linked with future risk of cardiovascular disease (52).

Physical activity bouts

Consistent with literature, a strong correlation between bouted and total physical activity was observed (14; 17). This suggests a direct inference from associations with longer bout-durations as compared with short bout-duration would be confounded by total activity. Therefore, an isotemporal substitution approach was used to model the impact of replacing short bouted for medium and long bouted activity while holding total activity constant. These models did not suggest physical activity accumulated in longer bout-durations will produce more pronounced benefits than shorter bouts (at least down to a one minute bout) when the total volumes are identical. Meta-regression models supported this notion as neither the coefficient for bout-duration nor the interaction between bout-duration and intensity was statistically significant. Other studies on

the impact of bout-duration have produced mixed findings. In 2498 children aged eight to seventeen years participating in the NHANES, MVPA accumulated in short (1-4 minutes) bouts was equally associated with adiposity status than MVPA accumulated in bouts of longer duration (15). Both short and longer bout-durations were associated with lower odds of overweight and obesity after controlling for the total volume of activity (15). Also using NHANES data, Holman and colleagues presented similar associations between MVPA accumulated in short (<5 minutes), medium (≥5 minutes), and long (≥10 minutes) bouts and cardiometabolic risk factors (14). Likewise, Stone and colleagues did not find bout duration to modify the association between physical activity and waist-circumference or cardiorespiratory fitness (17). Finally, in another U.S. based study, BMI and waist-circumference but not cardiometabolic risk markers were associated with bout patterns in 396 8-year old children (16). A collective inference on any additional health benefit from longer-bouted activity above that of short-bouted activity from the literature is difficult to extract as studies are discordant in their analytical approach for examining this issue. Any specific biological mechanism favouring longer bout-durations under identical total volumes of activity also remain unidentified.

Limitations

Our operationalization of the cut-points included all activity above the respective threshold and did not consider an isolated intensity range (e.g. light physical activity, moderate-physical activity). As such, our estimates of e.g. ≥500cpm would include more than one of the "conventional" intensity domains without distinguishing their relative contributions to the estimate. The pattern of results for substitution models was counterintuitive with conflicting directions of associations. This could suggest issues with collinearity which was indeed large. However, residual-analysis indicated meaningful (i.e. reasonable intervention target) variation in bouted activity remained after

controlling for total activity and correlations did not differ substantially in magnitude from what is reported from e.g. substitution of distinct fatty acids (53). Additionally, a minute-for-minute comparison of short and long bouts above a certain threshold may in fact be confounded by intensity of the underlying behaviour. This bias would most likely favour longer bout-durations (54). The applied definition of bouts did not allow for interruptions in the time-series. Whether bouted behaviour is better captured by allowing for e.g. an 80 % interruption is unclear. Data was cross-sectional so we are unable to infer on the direction of association. Finally, we controlled for age, sex and study, hence the possibility of confounding cannot be rejected.

Conclusion

In this cross-sectional study including nearly 40 000 observations physical activity intensity, but not bout-duration, appeared the major determinant of variation in cardiometabolic risk factors within the ranges examined. These data does not endorse including specific bout-durations in activity recommendations but suggests physical activity of any accumulation pattern may be promoted by authorities, clinicians, and parents to improve cardiovascular health in young people.

Table 1. Participant characteristics

	Girls (n=18 810)*	Boys (n=10 990)*
Age (years)	11.8 (10.6 - 13.8)	11.4 (9.6 - 12.0)
Body Height (cm)	152.4 (141.9 - 159.8)	145.7 (135.5 - 155.6)
Body Weight (kg)	45.6 (35 - 55.7)	38.0 (30.2 - 49.8)
Wear-time (hours/day)**	13.2 (1.3)	13.1 (1.3)
Counts/min	453 (348 - 586)	620 (491 – 768)

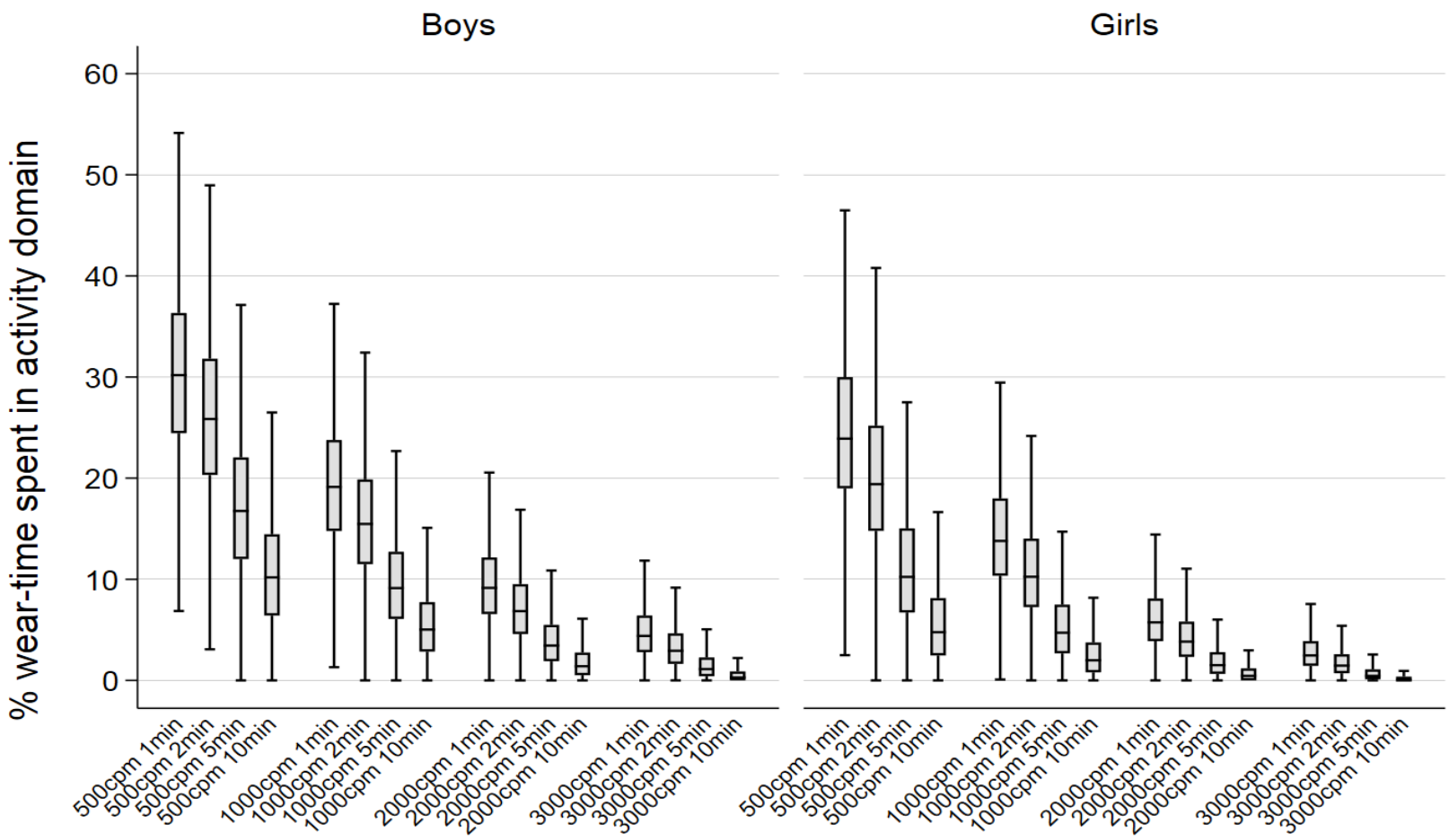
Values are median with 25th - 75th percentile unless noted otherwise. *Unique participants.**Mean (SD)

Table 2. Outcome characteristics

	No. of Studies	Girls (%)	n*	Median**	25 - 75th percentile**	ICC-study	ICC-participant	Contributing studies
Composite risk score	6	52	4338	-0.07	-0.65 to 0.58	0.08	0.51	4, 5, 6, 10, 12, 15
Non-adiposity composite score	6	52	4338	-0.01	-0.66 to 0.66	0.08	0.35	4, 5, 6, 10, 12, 15
Insulin (pmol/l)	7	52	4728	42.78	27.28 to 64.80	0.07	0.65	4, 5, 6, 10, 12, 15, 20
Glucose (mmol/l)	7	52	4756	5.00	4.70 to 5.30	0.17	0.13	4, 5, 6, 10, 12, 15, 20
Triglyceride (mmol/l)	8	52	5104	0.69	0.51 to 0.95	0.07	0.51	4, 5, 6, 10, 11, 12, 15, 20
Diastolic BP (mmHg)	10	52	13 598	58.7	54.0 to 64.0	0.18	0.37	1, 4, 5, 6, 9, 10, 11, 12, 14, 15
HDL-c (mmol/l)	8	51	7456	1.43	1.22 to 1.68	0.03	0.55	4, 5, 6, 10, 11, 12, 15, 20
BMI	21	62	29 751	18.69	16.55 to 21.77	0.05	0.86	all
Waist-circumference	14	52	19 015	64.50	58.8 to 72.0	0.07	0.80	1, 4, 5, 6, 9, 10, 11, 12, 13, 14, 15, 16, 19, 20
Overweight / obese (%)	21	62	29 734	18 / 8				all

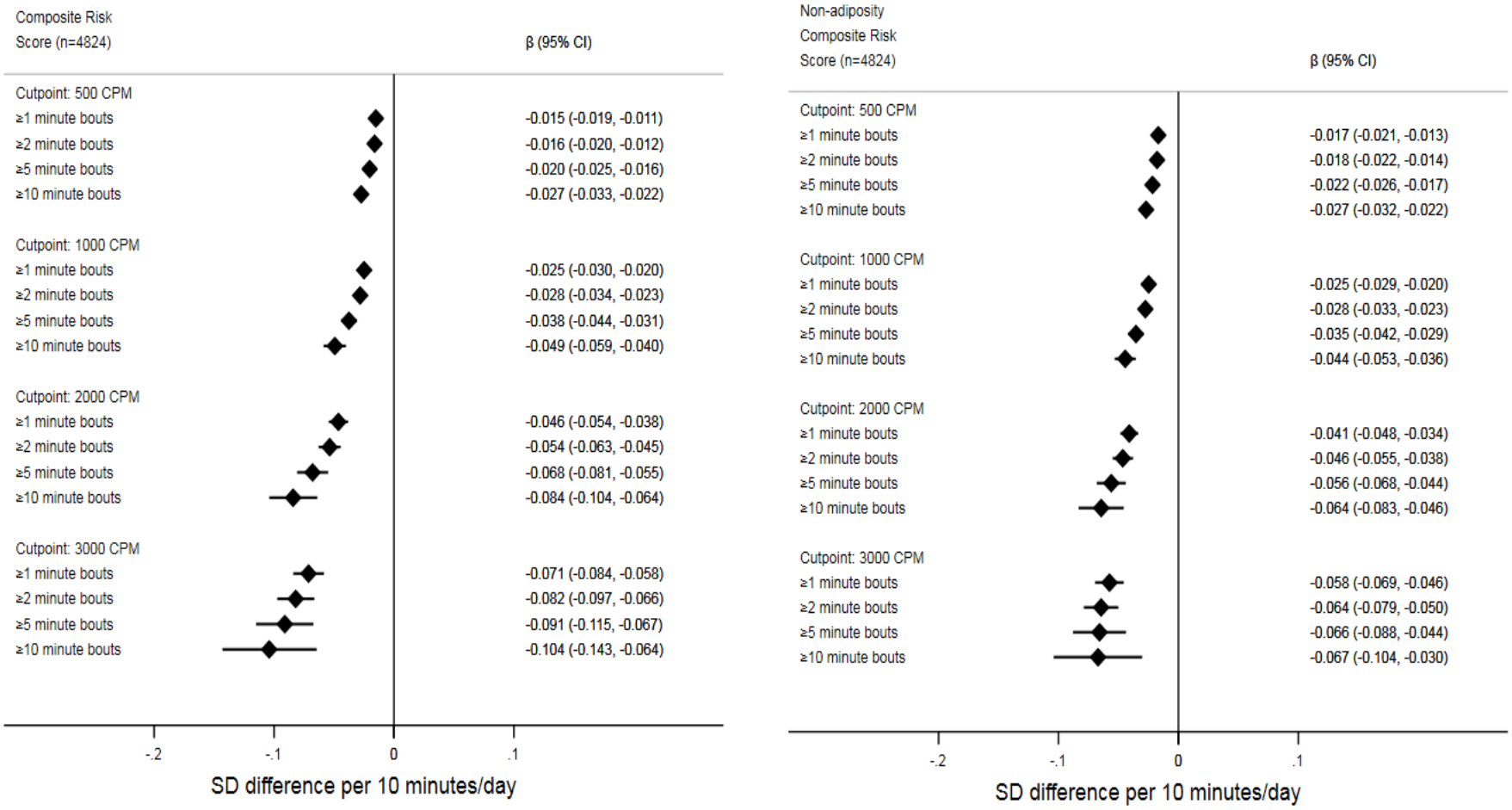
*Unique participants. **For prospective studies, only study "baseline" data is included in the table. Overweight and obesity defined according to IOTF cut-offs. Study indicators (NHANES waves counted separately): 1: ALSPAC, 2: Belgium Pre-School Study, 3: CLAN, 4: CoSCIS, 5: Danish EYHS, 6: Estonian EYHS, 7: HEAPS, 8: IBDS, 9: MAGIC, 10: NHANES 2005-06, 11: Norways EYHS, 12: NHANES 2003-04, 13: PEACH, 14: Pelotas, 15: Portugal EYHS, 16: SPEEDY, 17: TAAG, 18: CHAMPS UK, 19: Ballabeina Study, 20: KISS, 21: CHAMPS US.

Figure 1. Activity patterns in 10 990 boys and 18 810 girls



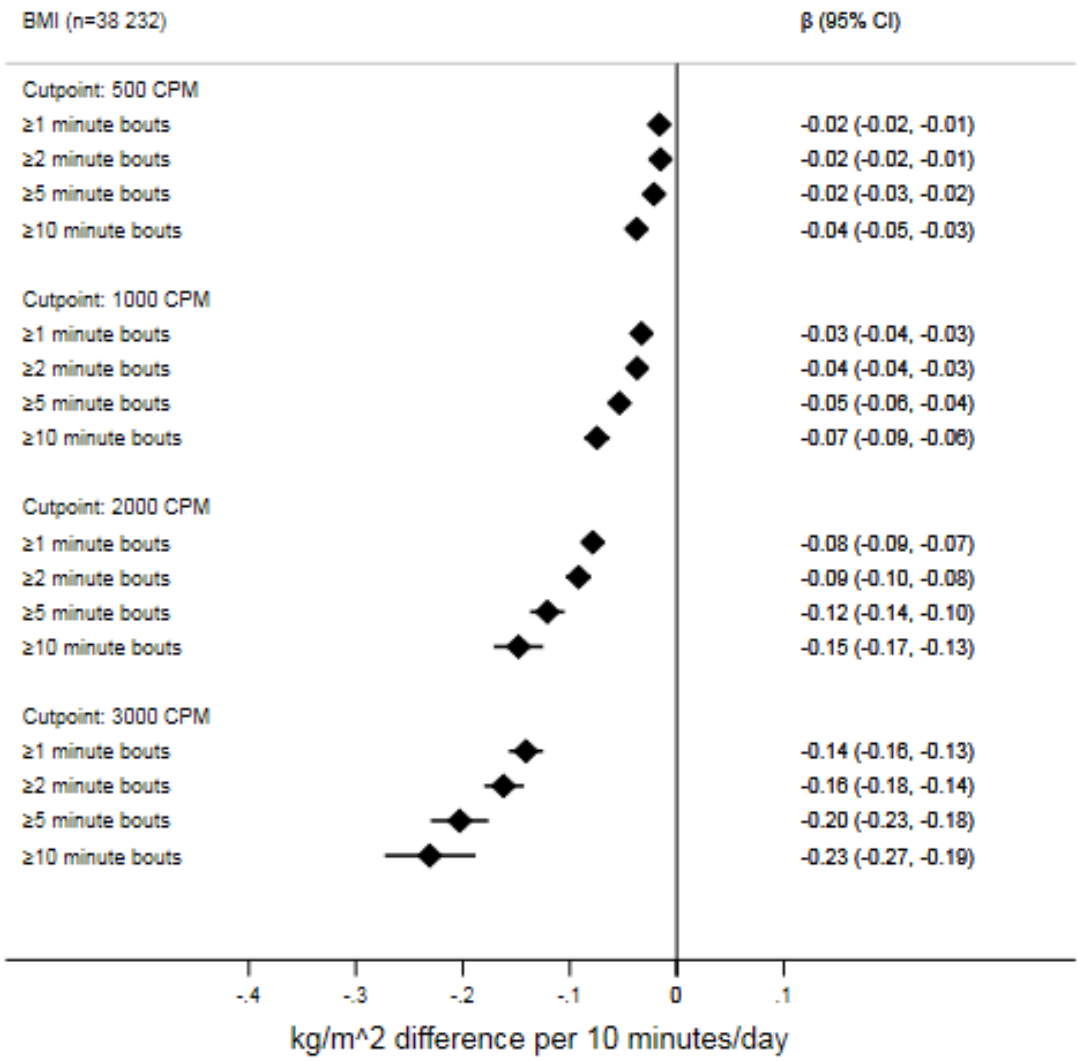
Box-plot displays percentage of wear-time spent in intensity/bout combinations in boys and girls. Outside values not shown.

Figure 2. Forest plot of associations between intensity/bout combinations and composite risk scores



Beta-coefficients and 95 % CI from linear mixed regression models controlled for age, sex, wear-time including study and participant as "random-effects". Non-adiposity composite risk score additionally controlled for BMI.

Figure 3. Forest plot of associations between intensity/bout combinations and BMI



Beta-coefficients and 95 % CI from linear mixed regression models controlled for age, sex, wear-time including study and participant as "random-effects".

Table 3. Associations for composite risk score, non-adiposity risk score, and BMI from isotemporal substitution of short to medium and long bouts of physical activity

	Composite risk score (n= 4338)			Non-adiposity composite risk score (n= 4338)*			BMI (n= 38 232)		
	Beta	95% CI	p-value	Beta	95% CI	p-value	Beta	95% CI	p-value
Medium bouts$_{500cpm}$	-0.060	-0.035 to 0.025	0.68	-0.016	-0.043 to 0.011	0.25	0.103	0.071 to 0.135	<0.001
Long bouts$_{500cpm}$	-0.032	-0.047 to -0.018	<0.001	-0.026	-0.040 to -0.013	<0.001	-0.017	-0.034 to 0.0002	0.052
Medium bouts$_{1000cpm}$	-0.039	-0.075 to -0.003	0.03	-0.036	-0.070 to -0.002	0.04	-0.022	-0.061 to 0.019	0.29
Long bouts$_{1000cpm}$	-0.044	-0.062 to -0.026	<0.001	-0.029	-0.046 to -0.012	0.001	-0.079	-0.100 to -0.058	<0.001
Medium bouts$_{2000cpm}$	-0.038	-0.091 to 0.015	0.17	0.057	-0.222 to 0.336	0.69	-0.079	-0.136 to -0.022	0.006
Long bouts$_{2000cpm}$	-0.006	-0.037 to 0.026	0.72	0.172	0.012 to 0.333	0.04	-0.070	-0.104 to -0.036	<0.001
Medium bouts$_{3000cpm}$	0.081	0.003 to 0.158	0.04	0.105	0.032 to 0.177	0.005	-0.004	-0.089 to 0.081	0.92
Long bouts$_{3000cpm}$	0.066	0.013 – 0.118	0.01	0.099	0.050 to 0.149	<0.001	0.022	-0.034 to 0.079	0.44

Beta-coefficients with 95% confidence intervals from linear mixed regression models. Coefficients are interpreted as replacing 10 minutes of physical activity accumulated in shorter bouts (1-4 minutes) with 10 minutes of same intensity physical activity but accumulated in the respective bout-duration (≥5-9 or ≥10 minute bouts). Cpm: counts/min, CI: confidence interval, BMI: body mass index. *Non-adiposity composite risk score controlled for BMI.

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Supplementary File

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Table S1. Study characteristics

Study	Year	N*	Country	Age in years (median (range))**	Counts/min (median (IQR))**	Prospective data
ALSPAC	03-07	6418	England	12 (11 - 15)	549 (446 - 678)	Yes
Belgium Pre-School Study	06; 08-09	115	Belgium	5 (4 - 6)	565 (486 - 683)	Yes
CLAN	01; 04; 06	1104	Australia	11 (5 - 17)	662 (538 - 794)	Yes
CoSCIS	01-05	607	Denmark	7 (6 - 11)	683 (568 - 808)	Yes
Danish EYHS	97-98; 03-04	1193	Denmark	10 (8 - 17)	533 (375 - 699)	Yes
Estonian EYHS	98-99	647	Estonia	10 (8 - 17)	595 (432 - 762)	No
HEAPS	02-03; 06	1265	Australia	11 (4 - 15)	663 (539 - 810)	Yes
IBDS	98-07	576	US	6 (5 - 14)	684 (576 - 793)	Yes
MAGIC	02	278	Scotland	4 (4 - 5	712 (613 - 847)	No
NHANES 2005-06	05-06	2132	US	13 (6 - 18)	471 (348 - 630)	No
Norways EYHS	99-00	366	Norway	10 (9 - 10)	688 (546 - 864)	No
NHANES 2003-04	03-04	2046	US	13 (6 - 18)	491 (362 - 650)	No
PEACH	06-09	1197	England	11 (10 - 13)	515 (430 - 629)	Yes
Pelotas	06-07	270	Brazil	13 (13 - 14)	376 (288 - 477)	No
Portugal EYHS	99-00	1050	Portugal	10 (8 - 18)	524 (410 - 664)	Yes
SPEEDY	07	1890	England	10 (10 - 11)	563 (460 - 685)	No
Project TAGG	02-06	6884	US	14 (10 - 17)	367 (298 - 450)	Yes
CHAMPS UK	06-07	454	England	11 (4 - 16)	520 (407 - 644)	No
Ballabeina Study	08-09	546	Switzerland	5 (4 - 7)	678 (566 - 793)	Yes
KISS	05-06	488	Switzerland	10 (6 - 13)	639 (526 - 773)	Yes
CHAMPS-US	03-06	274	US	5 (4 - 6)	701 (592 - 795)	No

*Unique participants. **Only observations available at "baseline" included if prospective data available

Table S2. Correlation matrix of physical activity intensity/bout-duration combinations

	500 cpm 1-min	500 cpm 2-min	500 cpm 5-min	500 cpm 10-min	1000 cpm 1-min	1000 cpm 2-min	1000 cpm 5-min	1000 cpm 10-min	2000 cpm 1-min	2000 cpm 2-min	2000 cpm 5-min	2000 cpm 10-min	3000 cpm 1-min	3000 cpm 2-min	3000 cpm 5-min	3000 cpm 10-min
500cpm 1-min	-	0.99	0.93	0.80	0.95	0.90	0.77	0.61	0.77	0.69	0.54	0.39	0.62	0.53	0.39	0.25
500cpm 2-min	-	-	0.96	0.84	0.96	0.93	0.81	0.65	0.80	0.72	0.57	0.42	0.65	0.56	0.41	0.27
500cpm 5-min	-	-	-	0.93	0.96	0.96	0.91	0.76	0.85	0.80	0.67	0.50	0.71	0.64	0.49	0.32
500cpm 10-min	-	-	-	-	0.88	0.91	0.95	0.88	0.85	0.84	0.76	0.62	0.73	0.69	0.56	0.39
1000cpm 1-min	-	-	-	-	-	0.99	0.90	0.75	0.91	0.84	0.68	0.52	0.77	0.68	0.51	0.34
1000cpm 2-min	-	-	-	-	-	-	0.94	0.80	0.94	0.89	0.74	0.56	0.80	0.72	0.55	0.37
1000cpm 5-min	-	-	-	-	-	-	-	0.92	0.93	0.93	0.85	0.68	0.83	0.79	0.64	0.44
1000cpm 10-min	-	-	-	-	-	-	-	-	0.85	0.88	0.89	0.79	0.79	0.78	0.70	0.53
2000cpm 1-min	-	-	-	-	-	-	-	-	-	0.98	0.87	0.70	0.94	0.88	0.71	0.50
2000cpm 2-min	-	-	-	-	-	-	-	-	-	-	0.92	0.76	0.95	0.92	0.76	0.54
2000cpm 5-min	-	-	-	-	-	-	-	-	-	-	-	0.88	0.89	0.92	0.86	0.64
2000cpm 10-min	-	-	-	-	-	-	-	-	-	-	-	-	0.76	0.81	0.85	0.77
3000cpm 1-min	-	-	-	-	-	-	-	-	-	-	-	-	-	0.98	0.84	0.62
3000cpm 2-min	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.91	0.69
3000cpm 5-min	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.82
3000cpm 10-min																-

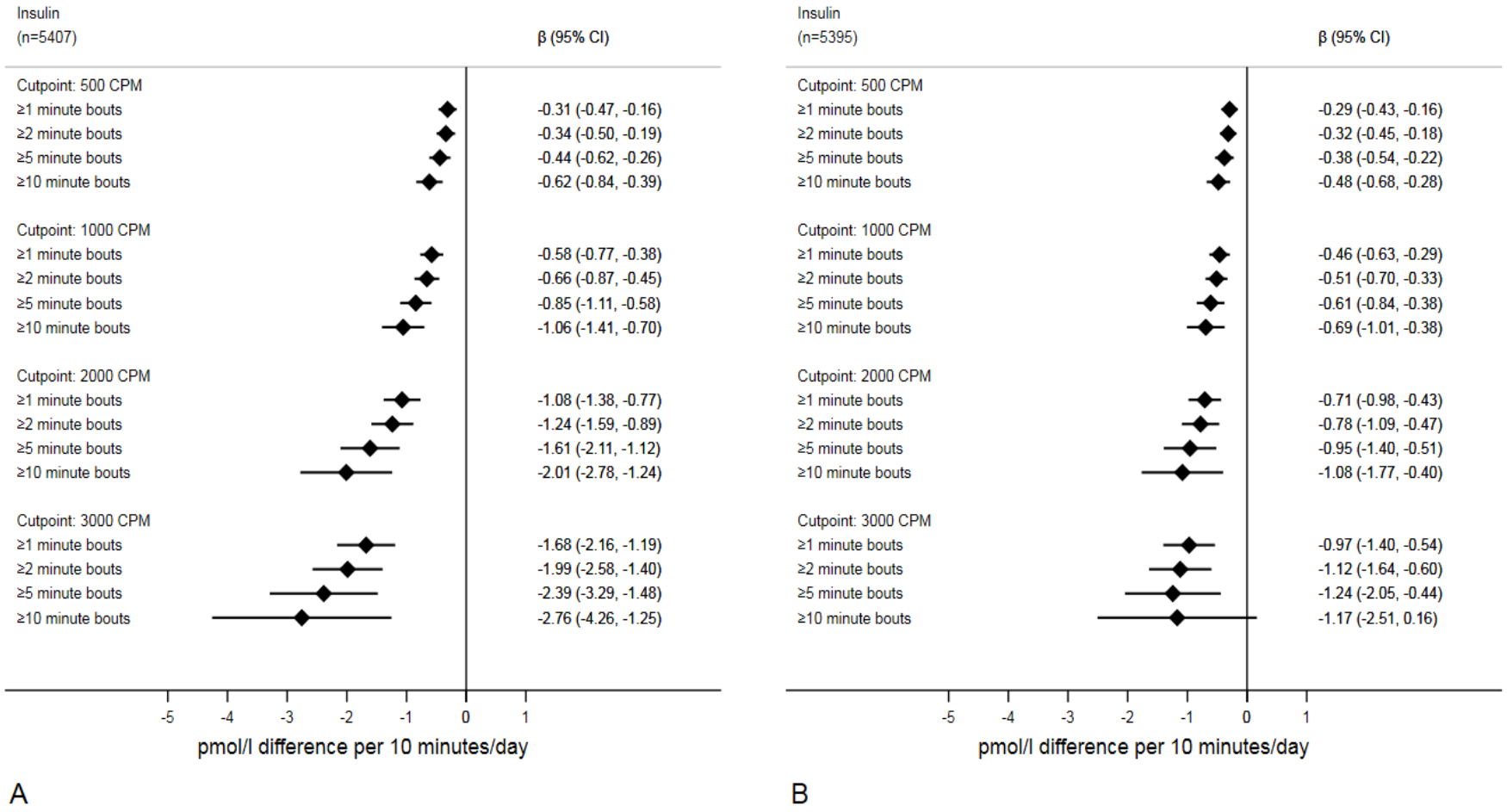
Correlations are Spearman’s rho controlling for age, sex, wear-time and study

Table S3. Meta-regression coefficients

	β Bout-duration (95% CI)	β Intensity (95% CI)	Bout-duration-by-intensity threshold interaction term (95% CI)*
Composite risk score (z-score)	-0.002 (-0.005 to 0.0005)	-0.027 (-0.039 to -0.014)	-0.002 (-0.006 to 0.003)
Non-adiposity composite risk score (z-score)	-0.002 (-0.004 to 0.0008)	-0.019 (-0.031 to -0.007)	-0.0009 (-0.005 to 0.003)
Insulin (pmol/l)	-0.048 (-0.138 to 0.042)	-0.667 (-1.075 to -0.259)	-0.048 (-0.222 to 0.126)
Glucose (mmol/l)	-0.0003 (-0.002 to 0.0009)	-0.003 (-0.009 to 0.003)	-0.0002 (-0.002 to 0.002)
Triglyceride (mmol/l)	-0.0003 (-0.001 to 0.0006)	-0.004 (-0.009 to 0.0005)	-0.00002 (-0.002 to 0.002)
HDL-cholesterol (mmol/l)	0.0005 (-0.0002 to 0.001)	0.006 (0.002 to 0.009)	0.0005 (-0.0008 to 0.002)
Diastolic blood pressure (mmHg)	-0.004 (-0.018 to 0.01)	-0.073 (-0.14 to -0.007)	-0.004 (-0.028 to 0.021)
Body mass index (kg/m^2)	-0.005 (-0.012 to 0.002)	-0.064 (-0.09 to -0.038)	-0.004 (-0.009 to 0.002)
Waist-circumference (cm)	-0.019 (-0.048 to 0.01)	-0.208 (-0.31 to -0.11)	-0.013 (-0.033 to 0.006)

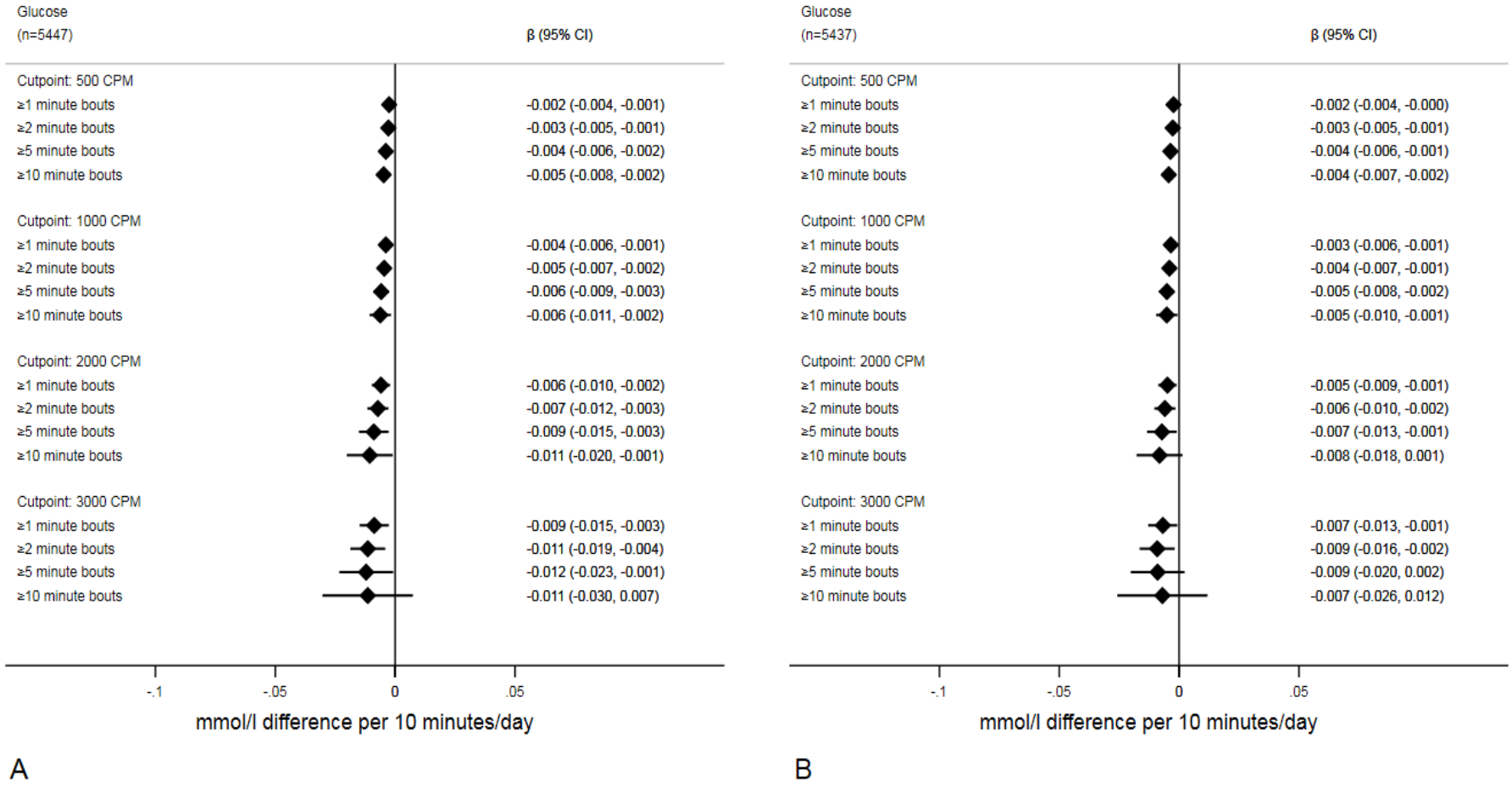
Coefficients per 1-minute increase in bout-duration and 1000 cpm increase in intensity threshold. Meta-regression includes estimates from: 1, 3, 5, 7, and 10 minutes bout duration and 500, 1000, 2000, and 3000 counts/min intensity thresholds. Confidence intervals adapted to non-independence of coefficients by recalculating the standard error (SE) as: (√(number of coefficients (20) – 1)) x SE obtained from the meta-regression model. *Interactionterm fitted in separate model.

Figure S1. Forest plot of associations between intensity/bout combinations and insulin levels



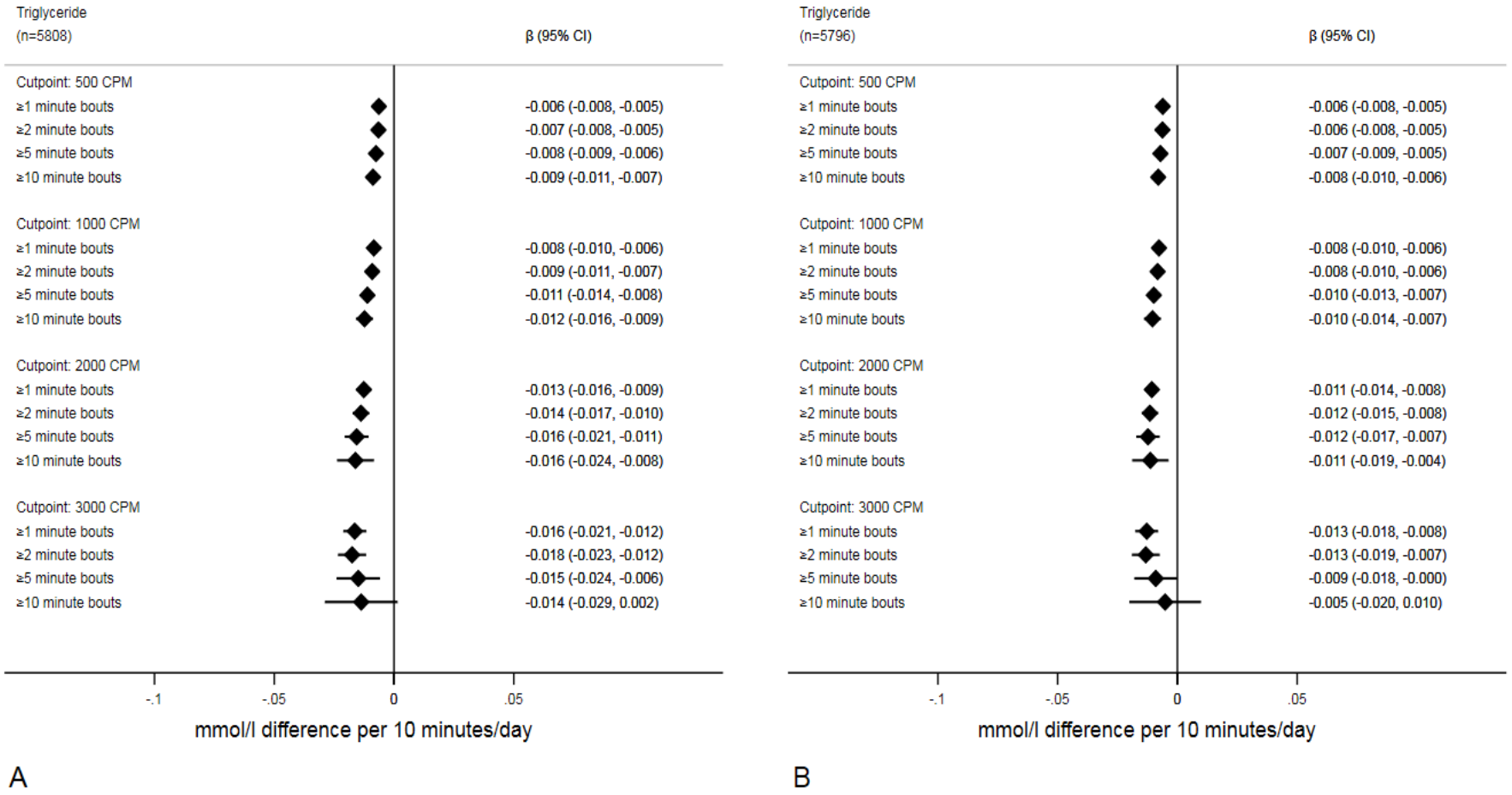
Beta-coefficients and 95 % CI from linear mixed regression models controlled for age, sex, wear-time including study and participant as "random-effects" (A). Panel (B) including control for BMI.

Figure S2. Forest plot of associations between intensity/bout combinations and glucose levels



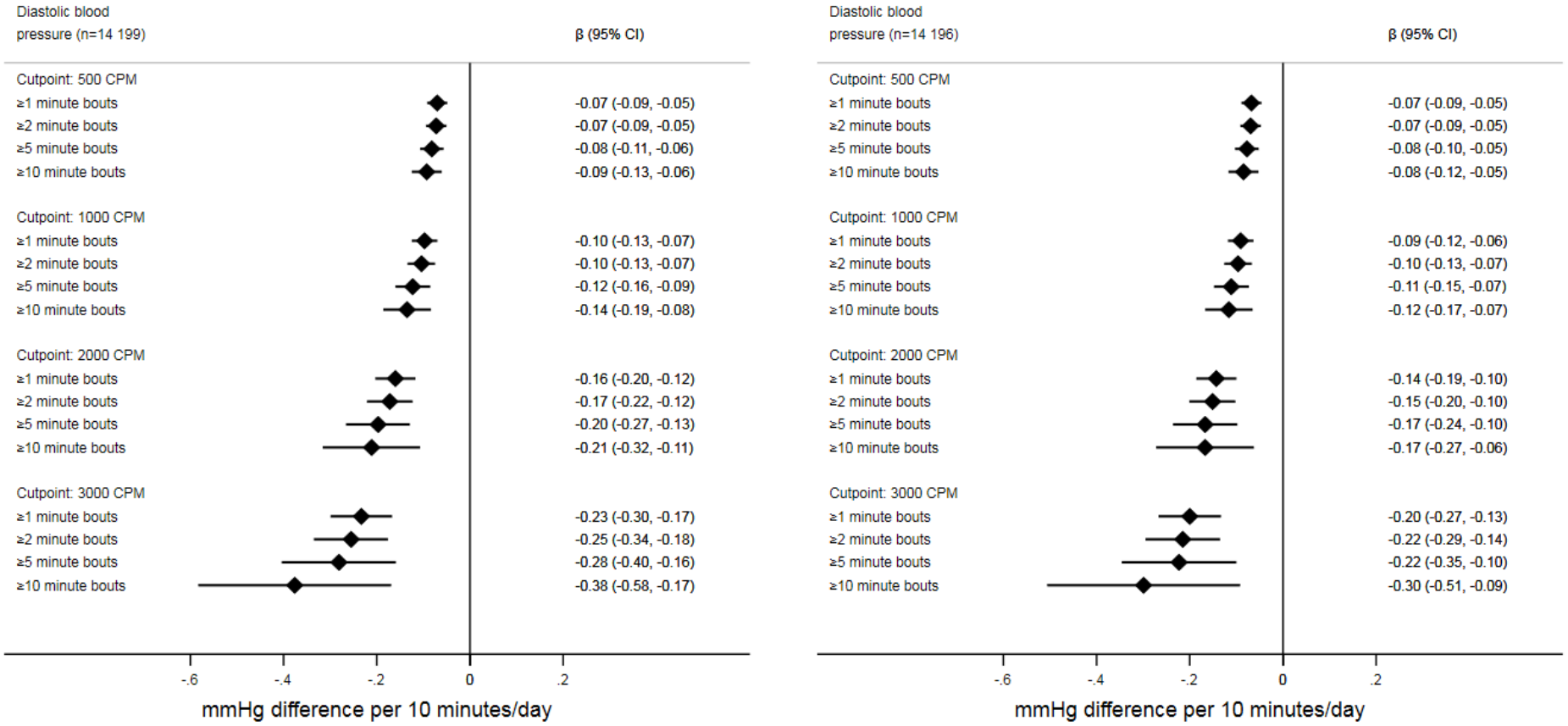
Beta-coefficients and 95 % CI from linear mixed regression models controlled for age, sex, wear-time including study and participant as "random-effects" (A). Panel (B) including control for BMI.

Figure S3. Forest plot of associations between intensity/bout combinations and triglyceride levels



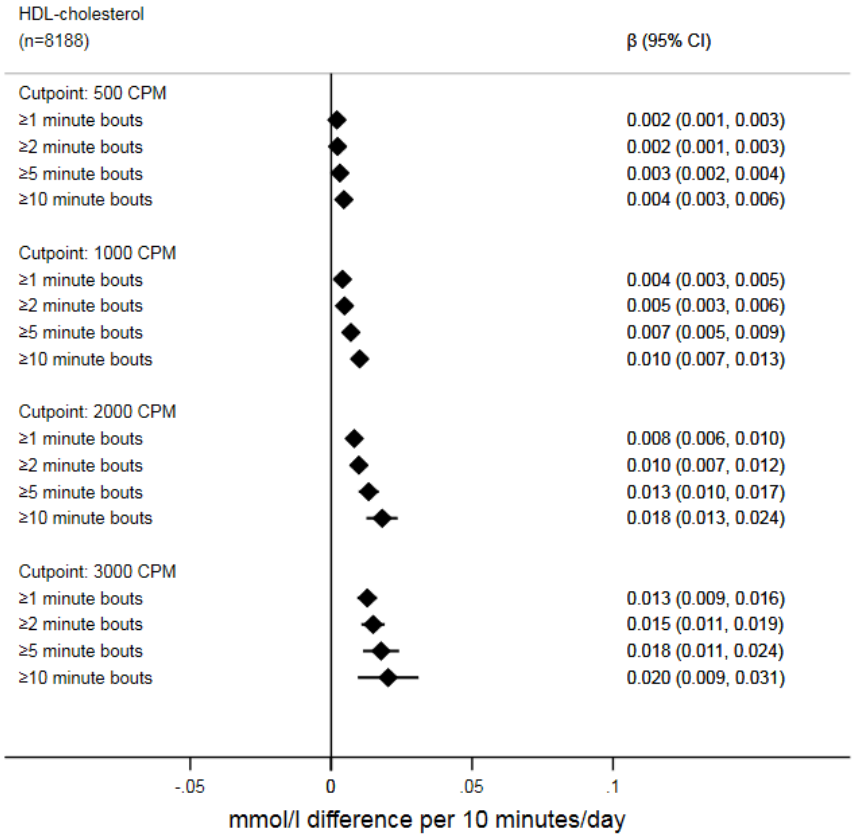
Beta-coefficients and 95 % CI from linear mixed regression models controlled for age, sex, wear-time including study and participant as "random-effects" (A). Panel (B) including control for BMI.

Figure S4. Forest plot of associations between intensity/bout combinations and diastolic blood pressure

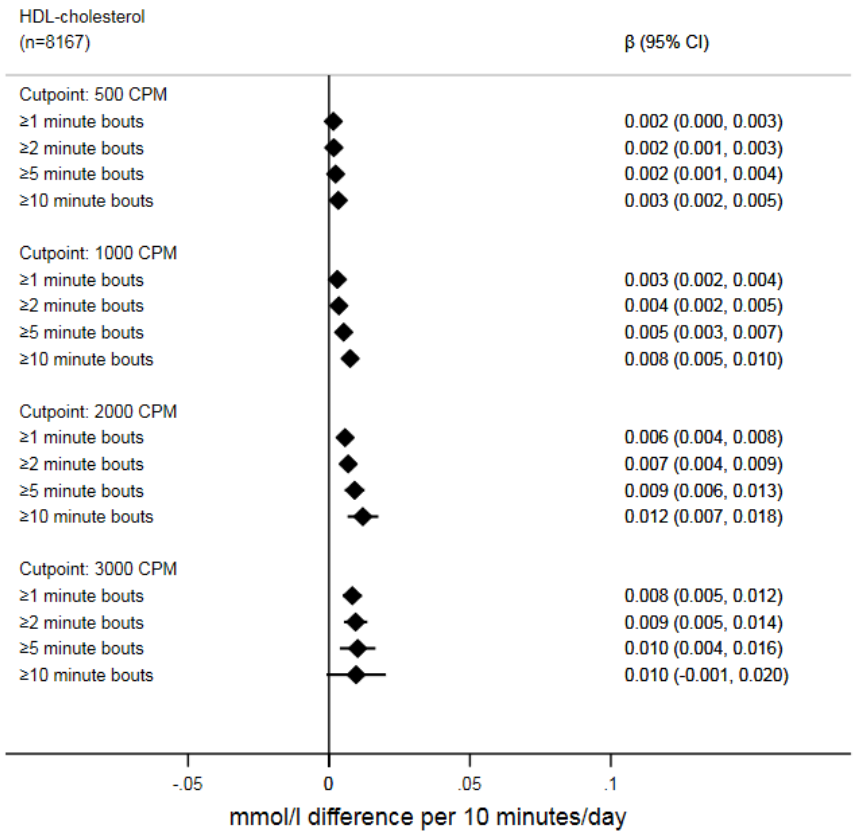


Beta-coefficients and 95 % CI from linear mixed regression models controlled for age, sex, body height, wear-time including study and participant as "random-effects" (A). Panel (B) including control for BMI.

Figure S5. Forest plot of associations between intensity/bout combinations and HDL-cholesterol



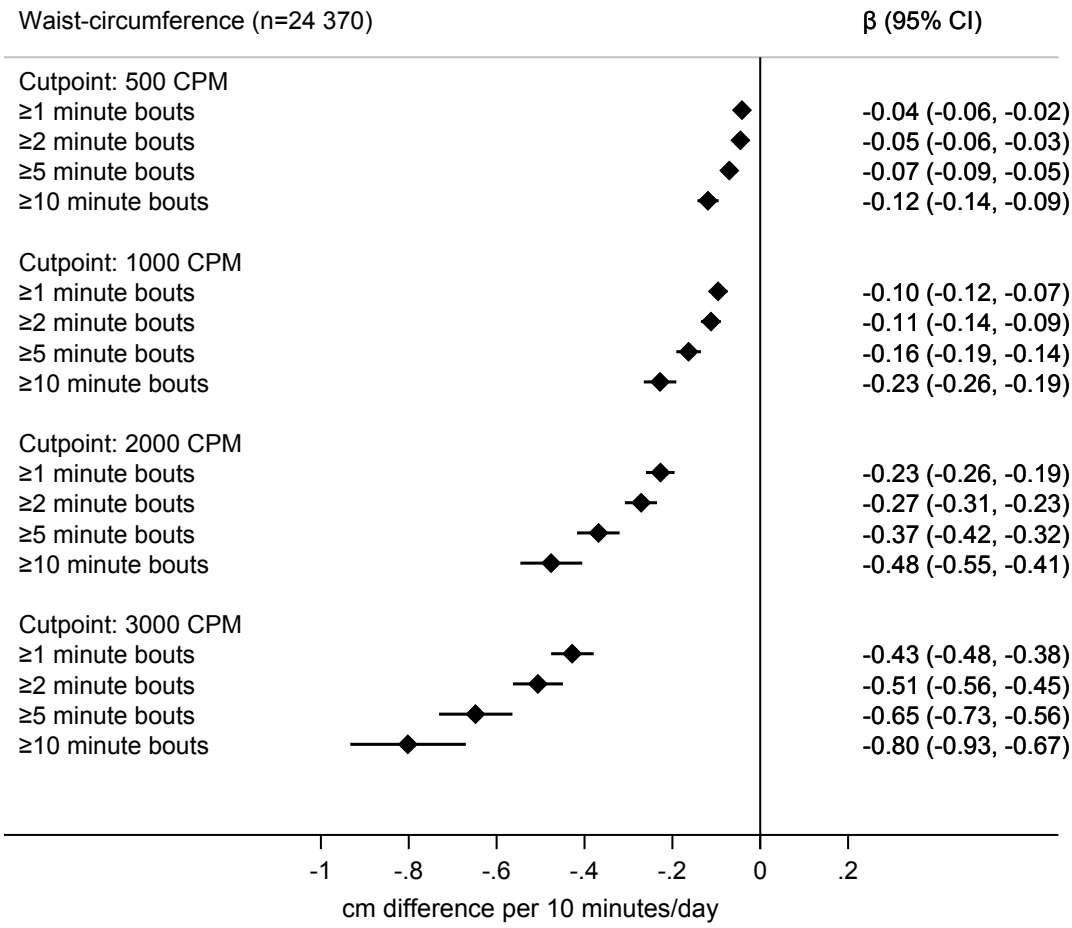
A



B

Beta-coefficients and 95 % CI from linear mixed regression models controlled for age, sex, wear-time including study and participant as "random-effects" (A). Panel (B) including control for BMI.

Figure S6. Forest plot of associations between intensity/bout combinations and waist-circumference



Beta-coefficients and 95 % CI from linear mixed regression models controlled for age, sex, body height, wear-time including study and participant as "random-effects". Waist-circumference data harmonized by correction formula.

Table S4. Residual variation in bout physical activity in n=38 306 observations from ICAD

	Quintile 1 (least time in bouts)	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Medium bouts$_{500cpm}$	-21.6 (7.6)	-8.2 (2.5)	-0.3 (2.2)	7.7 (2.6)	22.3 (9.1)
Long bouts$_{500cpm}$	-27.0 (9.1)	-10.6 (3.1)	-0.8 (2.7)	9.3 (3.3)	29.0 (13.4)
Medium bouts$_{1000cpm}$	-17.6 (6.5)	-6.5 (2.0)	-0.2 (1.7)	6.3 (2.1)	18.1 (7.6)
Long bouts$_{1000cpm}$	-18.7 (6.8)	-7.2 (2.1)	-0.7 (1.7)	5.9 (2.2)	20.7 (10.7)
Medium bouts$_{2000cpm}$	-10.8 (4. 3)	-3.8 (1.2)	-0.0 (1.0)	3.6 (1.2)	11.1 (5.4)
Long bouts$_{2000cpm}$	-9.6 (3.6)	-3.7 (1.0)	-0.5 (0.8)	2.7 (1.1)	11.1 (7.1)
Medium bouts$_{3000cpm}$	-6.4 (2.8)	-2.1 (0.7)	-0.1 (0.5)	1.9 (0.6)	6.7 (4.3)
Long bouts$_{3000cpm}$	-4.9 (2.0)	-1.8 (0.5)	-0.3 (0.4)	1.1 (0.5)	6.0 (5.2)

Quintiles of residual variation from regressing bout-duration on age, sex, wear-time and total physical activity at intensity cut-off. Data is mean (SD). Medium (≥5-9 minutes) and long (≥10 minutes).

Table S5. Associations for insulin, glucose, and triglyceride from isotemporal substitution of short to medium and long bouts of physical activity

	Insulin (n= 5407)			Glucose (n= 5447)			Triglyceride (n=5808)		
	Beta	95% CI	p-value	Beta	95% CI	p-value	Beta	95% CI	p-value
Medium bouts$_{500cpm}$	0.130	-0.969 to 1.230	0.82	-0.017	-0.031 to -0.003	0.02	-0.006	-0.017 to 0.006	0.322
Long bouts$_{500cpm}$	-0.765	-1.315 to -0.215	0.006	-0.011	-0.019 to -0.005	0.001	-0.005	-0.011 to 0.0002	0.061
Medium bouts$_{1000cpm}$	-0.958	-2.320 to 0.403	0.17	-0.021	-0.039 to -0.004	0.02	-0.016	-0.030 to -0.003	0.02
Long bouts$_{1000cpm}$	-0.820	-1.499 to -0.141	0.02	-0.006	-0.015 to 0.002	0.14	-0.004	-0.011 to 0.003	0.272
Medium bouts$_{2000cpm}$	-0.884	-2.856 to 1.087	0.38	-0.008	-0.034 to 0.017	0.53	0.001	-0.019 to 0.021	0.937
Long bouts$_{2000cpm}$	-0.156	-1.362 to 1.050	0.80	0.000	-0.015 to 0.015	0.98	0.017	0.005 to 0.029	0.006
Medium bouts$_{3000cpm}$	1.037	-1.895 to 3.969	0.49	-0.002	-0.039 to 0.036	0.92	0.054	0.025 to 0.084	<0.001
Long bouts$_{3000cpm}$	1.265	-0.757 to 3.286	0.22	0.012	-0.014 to 0.037	0.37	0.041	0.021 to 0.062	<0.001

Beta-coefficients with 95% confidence intervals from linear mixed regression models. Coefficients are interpreted as replacing 10 minutes of physical activity accumulated in shorter bouts (1-4 minutes) with 10 minutes of same intensity physical activity but accumulated in the respective bout-duration (≥5-9 or ≥10 minute bouts). Cpm: counts/min, CI: confidence interval.

Table S6. Associations for diastolic blood pressure, HDL-cholesterol, and waist-circumference from isotemporal substitution of short to medium and long bouts of physical activity

	Diastolic blood pressure (n= 14 199)			HDL-cholesterol (n= 8188)			Waist-circumference (n=24 370)*		
	Beta	95% CI	p-value	Beta	95% CI	p-value	Beta	95% CI	p-value
Medium bouts$_{500cpm}$	-0.084	-0.241 to 0.074	0.30	0.001	-0.007 to 0.009	0.720	0.136	0.029 to 0.244	0.013
Long bouts$_{500cpm}$	-0.046	-0.125 to 0.033	0.25	0.007	0.003 to 0.011	<0.001	-0.164	-0.220 to -0.108	<0.001
Medium bouts$_{1000cpm}$	-0.107	-0.300 to 0.087	0.28	0.007	-0.002 to 0.017	0.142	-0.146	-0.279 to -0.013	0.031
Long bouts$_{1000cpm}$	-0.006	-0.101 to 0.089	0.90	0.013	0.008 to 0.017	<0.001	-0.287	-0.355 to -0.219	<0.001
Medium bouts$_{2000cpm}$	0.057	-0.222 to 0.336	0.69	0.007	-0.008 to 0.021	0.358	-0.271	-0.456 to -0.085	0.004
Long bouts$_{2000cpm}$	0.172	0.012 to 0.333	0.04	0.007	-0.001 to 0.016	0.085	-0.302	-0.410 to -0.193	<0.001
Medium bouts$_{3000cpm}$	0.628	0.217 to 1.040	0.003	-0.008	-0.030 to 0.013	0.445	-0.020	-0.293 to 0.254	0.889
Long bouts$_{3000cpm}$	0.257	-0.024 to 0.538	0.07	-0.012	-0.026 to 0.003	0.117	-0.079	-0.258 to 0.099	0.384

Beta-coefficients with 95% confidence intervals from linear mixed regression models. Coefficients are interpreted as replacing 10 minutes of physical activity accumulated in shorter bouts (1-4 minutes) with 10 minutes of same intensity physical activity but accumulated in the respective bout-duration (≥5-9 or ≥10 minute bouts). Cpm: counts/min, CI: confidence interval. *Waist-circumference data harmonized by correction formula.

Study 4

TITLE

Muscle-fitness changes during childhood associates with improvements in cardiometabolic risk factors: A prospective study

SHORT TITLE

Muscle-fitness and cardiometabolic risk factors

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Conflict of Interest

The authors declare no conflict of interest exist

ABSTRACT

Objectives: To study associations between changes in composite and single muscle-fitness phenotypes and cardiometabolic risk factors during childhood.

Study design: A prospective observational study including 6-11 year old children (n=512) at baseline. Associations between two-year changes in muscular strength (handgrip strength), muscular power (standing vertical jump displacement), and muscular agility (short shuttle-run) with changes in the homeostasis model assessment of insulin resistance, systolic blood pressure, triglyceride, ratio of total cholesterol to high-density-lipoprotein-cholesterol, waist-circumference, and a composite risk score of these. We modelled sequential mixed linear regressions controlling for changes in cardiorespiratory fitness, waist-circumference and other putative confounding variables.

Results: Statistically significant associations, standardized beta (95% confidence intervals), were observed between changes in the composite risk score and changes in composite muscle-fitness -0.19 (-0.30 to -0.07), muscular strength -0.15 (-0.25 to -0.06), and agility 0.14 (0.04 to 0.23), but not muscular power -0.06 (-0.14 to 0.03). In sex-stratified analysis, changes in composite muscle-fitness were associated with the composite risk score when controlled for changes in cardiorespiratory fitness in girls -0.20 (-0.39 to -0.03), but not in boys -0.03 (-0.19 to 0.14). Subsequent control for changes in waist-circumference removed association with a non-adiposity

composite risk score in girls. Muscle-fitness was strongly associated with changes in waist-circumference in both girls -0.21 (-0.37 to -0.05) and boys -0.23 (-0.35 to -0.11) after controlling for cardiorespiratory fitness.

Conclusions: Our data support a unique role of muscle-strengthening activities in the promotion of metabolic health and prevention of excess adipose tissue accumulation in children.

Key words

Youth, biological risk factors, metabolic, muscle strength, physical activity, fitness

INTRODUCTION

The importance of high muscle-fitness for non-communicable diseases (NCDs) prevention is now recognized in national and international physical activity guidelines [1, 2]. Muscle-fitness may be divided into distinct sub-components such as strength, power, and endurance and high levels of these phenotypes may not provide identical protection against disease [3]. Similarly, more functionally based components of fitness, such as muscular agility, could have distinct physiological consequences. Young people constitute a first line of primordial prevention of NCDs as a physically active youth may improve likelihood of a physically active adult life [4]. Additionally, high levels of biological risk factors for NCDs (i.e. blood pressure, lipids and glucose metabolism) in youth is associated with type 2 diabetes and advanced atherosclerosis in young adulthood [5] and to premature mortality [6]. Experimental evidence in adolescents indicates that engagement in

resistance-based physical activity promotes muscle-fitness, reduces adiposity indexes and may improve insulin sensitivity compared to control conditions [7-10]. However, this data has limited generalizability as participants are, primarily, adolescents selected based on high risk (i.e. overweight or obese) and the provision of short-term, high-intensive and structured activities that are unlikely to resemble the behavior particularly children engage in. This makes observational data an important adjunct to the totality of evidence and studies in younger children particularly relevant. Previous non-interventional studies of changes in NCD risk factors following changes in muscle-fitness phenotypes [11-13] have been ambiguous with negative [12], positive [13] and no association [11] being reported.

Therefore, the purpose of the present study conducted in pre-adolescent children was to analyze; 1) the association between changes in muscle-fitness phenotypes and established cardiometabolic risk factors over two years, and 2) whether associations were independent of cardiorespiratory fitness and waist-circumference.

METHODS

Study design and population

The CHAMPS-study DK is a natural experiment [14] implemented as a controlled intervention study including children (kindergarten to fourth grade) from ten public schools in the municipality of Svendborg, Denmark. Briefly, the CHAMPS-study DK evaluated a trebling of curricular physical education in six intervention schools with four matched schools serving as controls [15-18]. A total of 1507 children were invited to participate in the study of which parents or legal guardians of 1209 children provided written informed consent. Outcome measurements were performed at baseline in

fall 2008 and repeated two years later, while fitness assessments from baseline and 18 months later were used. All measurements were performed at schools by trained research staff following standardized procedures. Eligible participants for these analyses (n=512) are those having available information on changes in cardiometabolic risk factors, changes in muscle and cardiorespiratory fitness, and on available putative confounding variables. The CHAMPS-study DK was approved by the ethics committee of the Region of Southern Denmark (S-20080047) and conducted in accordance with the Declaration of Helsinki.

Exposure

Upper body muscular force (strength) was measured as the highest maximum voluntary contraction (in kilograms) of the dominant hand following two attempts on an analogue handgrip dynamometer (Smedley´s dynamometer, Scandidact, Odder, DK) while standing. The result was divided by body weight for analysis because this index is frequently reported [19]. Muscular power was measured by a vertical jump test as the highest vertical displacement (in centimeters) in a minimum of three attempts, with additional attempts prompted if continuous improvement was observed. Participants were asked to perform a maximal jump with allowance of countermovement. Muscular agility was measured using the fifty meter short shuttle-run. It was performed as ten laps on a five-meter lane and measured in seconds. Less elapsed time reflects higher muscular agility. We derived a standardized composite muscle-fitness score by standardizing and averaging each muscle-fitness phenotype (using inverted shuttle-run) [18]

Outcomes

Fasting (overnight) blood samples were obtained and handled following standardized procedures by experienced biomedical laboratory scientists as detailed elsewhere [16]. All blood samples were analysed for insulin, glucose, triglyceride, high-density-lipoprotein-cholesterol (HDLc) and total cholesterol (TC) in a certified laboratory at the University of Vienna. Systolic blood pressure was measured with a suitable cuff size on the left arm using an automated oscillometric blood pressure monitor (Welch Allyn, New York, USA) vital signs monitor 300 series with FlexiPort™. The child was resting in the sitting position for five min before monitoring. Five subsequent values were recorded with one-min intervals or until the last three values had become stable. The mean of the last three recordings was used in the analysis. Waist-circumference was measured to the nearest 0.5 centimeter across the umbilical cord following a gentle expiration (Seca 201, Seca Corporation, Hamburg, Germany). At least two measurements were performed with a third undertaken if the two differed by more than one centimeter.

Other variables

Cardiorespiratory fitness was assessed using a field-test (Andersen-test) lasting ten minutes with fifteen seconds of intermittent running and pausing. Total distance covered was used as a proxy of cardiorespiratory fitness [20, 21]. Weight was measured to the nearest 0.1 kg on an electronic scale (Tanita BWB-800S, Tanita Corporation, Tokyo, Japan) wearing light clothes. Stature was measured to the nearest 0.5 cm using a portable stadiometer (Seca 214, Seca Corporation, Hamburg, Germany). Both measures were conducted barefoot. Sexual maturity was self-reported by indicating resemblance to five drawings (progressive rating 1 – 5) of secondary sex characteristics as described by Tanner[22]. Pubic hair was used in boys and breast development in girls. Because only 4

% of the sample defined themselves in category 3-5 we re-coded the sample as being either pre-pubertal (stage 1) or pubertal (stages 2-5). Sexual maturity at the time of risk factor ascertainment was used to classify follow-up status. Parents returned a mailed questionnaire which inquired on parent's height, weight, educational attainment and history of cardiovascular disease, hypertension or diabetes in parent, siblings or grandparents.

STATISTICS

Descriptive data are summarized as means with standard deviations (SD) or medians with 25th and 75th percentiles dependent on distributional properties. Standard visual model diagnostics were used to confirm model fit. No indication of problematic collinearity was detected (variance inflation factors did not exceed 1.92) and estimates were robust to removal of observations with standardized residuals above/below two SD. Relative stability (tracking) of single and composite muscle-fitness from baseline to follow-up was assessed by Pearson's product-moment correlation coefficient (rho) while controlling for age and sex. Fisher's transformation was used to derive a 95% confidence interval (CI).

As the primary outcome we created a standardized composite score [23] of the cardiometabolic risk factors; insulin resistance (defined by the homeostasis model assessment of insulin resistance (HOMA-IR) calculated as (insulin in IU/l x glucose in mmol/l)/22.5)[24], systolic blood pressure, triglyceride, TC:HDL-c ratio and waist-circumference. The score was created by standardizing each risk factor for age and sex in a linear regression analysis with the standardized residuals representing z-scores with a mean of 0 and a SD of 1. The residuals were subsequently averaged and the composite score standardized. Stature was added to the standardization of systolic blood pressure and waist-circumference. In this composite score, a lower score represents a more

favorable risk profile. Non-normal distributed variables were log-transformed prior to standardization. Associations are presented as standardized betas with 95% CI.

To elucidate the prospective associations between individual and composite muscle-fitness phenotypes with individual and composite cardiometabolic risk factors we defined for each muscle-fitness phenotype a series of multivariable mixed effects linear regressions. We used the following covariates from the questionnaire in all models; mother's body mass index (BMI) (continuous, calculated as body weight in kg divided by squared stature in meters), mother´s educational attainment (high-school or less (low), vocational training (medium) or any tertiary qualifications (high), history of cardiovascular disease, diabetes or hypertension in the nearest family (yes/no). Model 1: including age, sex, sexual maturity, intervention status (intervention/control) and a random intercept to account for school class membership. Model 2: as model 1, but including cardiorespiratory fitness. Model 3: as model 2, but waist-circumference is removed from composite risk score and included as a covariable. Our models took the form; $Y_2 = \beta cons + \beta X_2 + \beta X_1 + \beta Y_1 + \beta_i C_i$, where Y_2 and Y_1 represent the respective outcome at time 1 and 2, X_2 and X_1 are the muscle-fitness phenotype of interest at time 1 and 2, and C_i represents the set of relevant covariates. The term βX_2 from this model can be interpreted as the association between changes in the muscle-fitness phenotype and changes in the composite risk score, controlled for their baseline values. The models included time-variant covariates at the child level (sexual maturity, cardiorespiratory fitness, and waist-circumference) at baseline and follow-up. We report cross-sectional and prospective associations between baseline muscle-fitness phenotypes and two-year changes in cardiometabolic risk factors in Table S1 and S2 in the online only material. We added a sex-by-composite muscle-fitness interaction term to statistically evaluate evidence of sex-specific associations and re-analysed data stratified by sex if the p-value for the interaction term was ≤ 0.10.

As the optimal scaling of handgrip strength to achieve independence of body weight is unclear, we re-calculated handgrip strength in two ways; 1) divided by body weight$^{2/3}$ (theoretical scaling constant) [25], and 2) divided by body weight^{p}, where p represents a sample-, time- and sex-specific power to make handgrip strength independent of body weight (empirical scaling constants) [26]. Handgrip strength divided by body weight was used in the composite muscle-fitness score. We did not scale muscular power or agility to body weight as these tests control for weight by design [25]. The partial correlations at baseline (controlling for age, and sex) between body weight and strength, power and endurance were -0.33, 0.01, and 0.10, respectively. Statistical analysis was conducted in Stata version 14.0 (StataCorp, College Station, Texas, USA) with $\alpha = 0.05$ (two-sided).

RESULTS

Demographics, tracking and missing data

Descriptive characteristics at baseline for the analysed sample are shown stratified by sex in Table 1. The mean (SD) age at baseline was 8.4 (1.4) years and 50.6 % of participants were girls. Girls stature, body weight and waist-circumference increased more than boys (p-values <0.001). There were no statistically significant differences in development of biological risk factors, cardiorespiratory fitness or muscle-fitness indices between boys and girls (p-values ≥0.06). The analysed sample differed slightly from their consenting peers with key missing data (n=697). Refer to the online only material for comparison (Table S3), flowchart of included participants (Figure S1) and multiple imputation analysis including all 1209 consenting participants at baseline Table S4). Results from imputed data yielded similar conclusions as non-imputed data. The relative stability of single and composite muscle-fitness phenotypes during the follow-up period is

presented in Table 2. Tracking coefficients were highest for the composite phenotype (rho = 0.66) and lowest for muscular power (rho = 0.42).

[Table 1 inserts here]

[Table 2 inserts here]

Changes in muscle-fitness phenotypes and changes in cardiometabolic risk factors

Changes in the composite risk score were significantly (p-values ≤0.002) associated with changes in the composite muscle-fitness score -0.19 (-0.30 to -0.07), muscular strength -0.15 (-0.26 to -0.06), and agility 0.14 (0.04 to 0.23), but not muscular power (-0.06 (-0.14 to 0.03), p=0.17) as shown in Table 3. Following addition of changes in cardiorespiratory fitness to models, only muscular strength remained significantly associated with the composite risk score (-0.10 (-0.19 to -0.003), p=0.04), but this association was lost completely when removing waist-circumference from the composite risk score and including changes in waist-circumference to the model (0.01 (-0.10 to 0.11), p=0.93). Re-analyzing the associations between the composite risk score and composite muscle-fitness and muscular strength using the empirical scaling constants did not materially change interpretations or effect sizes for the empirical scaling constants, but muscular strength was no longer associated with the composite risk score following adjustment for cardiorespiratory fitness when using the theoretical scaling constant (-0.03 (-0.14 to 0.07, p=0.51). We report full details of associations using alternative scalings to body weight in Table S5 in the online only material.

[Table 3 inserts here]

Evidence to suggest a sex-by-composite muscle-fitness interaction was found for HOMA-IR and TC:HDL-c ratio (p-values ≤0.10). We therefore re-analysed the prospective associations and display the associations for composite muscle-fitness and the composite risk score and the cardiometabolic risk factors for boys and girls separately in Table 4. Associations for individual muscle-fitness phenotypes are shown in Table S6 and S7 in the online only material. In girls, the composite risk score, triglyceride, TC:HDL-c ratio and waist-circumference were significantly and inversely associated with composite muscle-fitness (p-values <0.02) with standardized betas ranging from -0.22 (-0.40 to -0.04) for triglyceride to -0.28 (-0.45 to -0.12) for the composite risk score. Following addition of cardiorespiratory fitness to these models, the association between composite muscle-fitness and triglyceride was no longer statistically significant (-0.18 (-0.38 to 0.02), p=0.08), and when further adding changes in waist-circumference, only TC:HDL-c ratio remained significantly associated with composite muscle-fitness (-0.21 (-0.33 to -0.09), p=0.001). In boys, prospective associations with composite muscle-fitness were significant for waist-circumference (-0.29 (-0.40 to -0.18), p<0.001) only.

[Table 4 inserts here]

DISCUSSION

This study suggests two-year changes in muscle-fitness phenotypes associates favorably with improvements in cardiometabolic risk factors in children, however only the association with muscular strength was independent of cardiorespiratory fitness. We observed sex-specific differences in the strength of associations but inclusion of waist-circumference to models greatly attenuated effect-sizes in both sexes. Increases in muscle-fitness were strongly associated with waist-circumference gains in both boys and girls.

Comparison with existing literature

Cross-sectional evidence coherently demonstrates an association between high muscle-fitness and cardiometabolic risk factor [19, 27, 28]. Conversely, the link between muscle-fitness and the risk factors presents itself less clearly in prospective intervention and observational studies. Prospective observational studies, such as the pan-European IDEFICS study, showed that favorable changes in cardiometabolic risk factors over two years were associated with changes in lower-body power but not muscular strength [12] in six-eleven year old children. In Australian adolescents, higher handgrip strength and back endurance were positively associated with systolic blood pressure, but negatively associated with insulin and triglyceride levels [13]. Finally, in ten-year-old children from the U.S. muscular strength changes over four years were not correlated with biological risk factors [11]. A recent systematic review of randomized controlled studies of resistance training in youth also showed mixed evidence for beneficial effects on biological risk factors with resistance training as only five of thirteen studies reported statistically significant improvements in a biological risk factor [10]. However, these observations are in contrast to the strong association between higher muscle-fitness in adolescence and lower risk of metabolic morbidity and mortality in young adulthood [29-32]. We extend existing literature on muscle-fitness by showing favorable associations with cardiometabolic risk factors in a sample of preadolescent children from a healthy population, which is particularly underrepresented in experimental evidence [10]. A distinction between muscle-fitness in adolescence and in pre-adolescent children, with lower circulating androgens, may be important as muscle-fitness adaptations in children are explained primarily by neural adaptations and less by increased muscle fiber volume [33]. Gains in lean body mass and the concomitant increase in resting metabolic rate and/or increased capacity for glucose and free-fatty acid clearance may represent mechanisms explaining beneficial metabolic adaptations with higher muscle-fitness [34]. Another potential mechanism, supported by the removal of most of the statistically significant associations

when controlling for waist-circumference, is that muscle-fitness represents a proxy for sustained physical activity engagement thereby favorably influencing energy balance. Our data suggested sexual dimorphic associations between muscle-fitness and cardiometabolic risk factors in children, which was also observed in a comparable study [13]. We are unable to explain the biological rationale behind this observation, however our sample size was relatively modest so results from especially sex-stratified models should be cautiously interpreted and we emphasize that confidence intervals were wide. Accordingly, this finding warrants further scrutiny.

The present study and existing literature is consistent in 1) the attenuation of muscle-fitness effect sizes following controlling for cardiorespiratory fitness and/or body composition indices [12, 13, 29, 30], and 2) changes in muscle-fitness are associated with changes in waist-circumference in both boys and girls [11, 12]. Body composition in youth has similar predictive capability of advanced atherosclerosis, metabolic syndrome and type 2 diabetes in adulthood than clustering of the biological risk factors [5] underpinning the importance of promoting behaviors, such as engagement in resistance-type exercise, which aids in preventing excess adipose tissue accumulation. The explanatory role of body weight in the muscle-fitness to risk factor association was reiterated in our analysis of other scaling approaches where, compared to scaling by dividing with body weight, some associations were lost. Five different approaches to model out the potential confounding effect of body weight were employed in as many prospective studies [12, 13, 29, 30, 35] limiting comparability between the studies. The potential for evidence synthesis is thus reduced and we suggest authors provide, minimally, a rationale for their modelling strategy, and preferably make available results of different approaches.

Associations with cardiometabolic risk factors were not universal across muscle-fitness phenotypes which is consistent with other studies [12, 30]. Selective effects could represent phenotype-specific adaptations but could also be partly explained by a non-identical degree of measurement error. Measurement error in exposure assessment will bias associations towards the null [36]. Handgrip strength is a valid and reliable marker of upper body and global muscular strength [37-39], while validity and reliability of the vertical jump and short-shuttle tests as markers of muscular power and agility are less described and usually with lower test-retest and criterion-measure correlations than is observed for handgrip strength [3, 37, 38]. In a subsample (n=94) of the CHAMPS-study DK, the standard error of the mean (as percentage of mean) for same-day repeated tests were 6.6, 7.6, and 2.5 % for handgrip strength, vertical jump and the short shuttle-run, respectively [18] but these estimates are suboptimal as they do not account for day-to-day variation. Measurement error may also partly explain the differences in tracking of muscle-fitness. Further, residual confounding from e.g. cardiorespiratory fitness may also differentially impact phenotype specific associations.

Potential public health relevance of population-wide muscle-fitness gains in young people

An analysis of individual-level changes in a population-based sample provides useful information about theoretical effects of a population-wide shift in the distribution of muscle-fitness. We estimated that a 1 SD change (corresponding to 0.088 kg/kg body weight) in muscular strength was associated with a 0.10 SD lower composite risk score at follow-up. With a mean of 0.54 kg/kg body weight at baseline, a 0.10 SD reduction in the population distribution of this composite risk score would theoretically be achieved by population-wide engagement in habitual physical activity of sufficient intensity and frequency to increase muscular strength by 16 %. Importantly, this effect on

population risk is after eliminating any additional effect from concurrent increases in cardiorespiratory fitness. Data to predict the life-time risk of chronic disease based on cardiometabolic risk factors in youth is limited [40]. However, a 1 SD higher composite risk score in adolescence has been associated with a 12 to 78 % higher risk of advanced atherosclerosis and type 2 diabetes diagnosis in young adulthood [41]. Promotion of resistance exercise in secondary schools have shown to be feasible and with some efficacy in improving upper body strength [42]. Further, body-mass index gains were lower in overweight/obese individuals participating in the resistance training group [42].

Limitations

We do not present a global assessment of muscle-fitness phenotypes as we, e.g. have no data on muscular endurance. We did not mutually adjust our models of individual muscle-fitness phenotypes, so we are unable to disentangle any "independent" role of these distinct traits. Cardiorespiratory fitness was indirectly assessed by a 15-second run/15 second break test. The intermittent nature of this test, although with reasonable criterion validity against maximal oxygen uptake (r-squared approximately 0.5) [20, 21] and test-retest reliability (r-squared approximately 0.7 – 0.8) [20, 21], may slightly favor individuals with high anaerobic capacity, thus potentially overcontrolling estimates in cardiorespiratory fitness adjusted models. Our analysis is based on the assumption that above average gains or losses in muscle-fitness is a result of engagement in a distinct type of physical activity behavior. However, it is possible that a genotype predisposing to fitness adaptations from exposure to physical activity [43] could also predispose to "metabolic-fitness" by e.g. enzymatic capacity, hence explaining any fitness-biological risk factor association. We also acknowledge the possibility of unmeasured or residual confounding from e.g. diet quality and

quantity or intra-uterine exposures restricting growth or muscle function. Strengths of our study include the prospective design in a population-based sample with the appropriate temporal relationship as muscle-fitness at follow-up was assessed prior to assessment of follow-up outcomes, reducing the possibility of reverse causation bias. Further, detailed measurements at two time-points allowed us to control for the confounding effect of cardiorespiratory fitness changes.

CONCLUSION

In conclusion, this study suggests increases in muscular-fitness results in favorable adaptations in cardiometabolic risk factors in children, and particularly in girls. In both sexes, gains in muscle-fitness were strongly associated with favorable changes in waist-circumference. The present study thus supports current public health messages that children should incorporate muscle-strengthening activities in their activity behavior.

Acknowledgments

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Data sharing

Data are available upon request from the CHAMPS Study Steering Committee due to legal and ethical restrictions. Interested parties may contact Dr Niels Wedderkopp (nwedderkopp@health.sdu.dk), and the following information will be required at the time of application: a description of how the data will be used, securely managed, and permanently deleted.

Table 1. Baseline characteristics of the sample

	Girls (n=259)	Boys (n=253)	Change score (Time 2 – Time 1)	P-value[a]
Age (years)	8.4 (1.4)	8.5 (1.4)	2.0 (0.03)	0.64
Stature (cm)	132.0 (9.4)	134.4 (10.4)	12.2 (2.3)	<0.001
Body weight (kg)	27.8 (24.4 - 33.6)[b]	28.6 (24.9 - 33.6)[b]	7.7 (3.1)	<0.001
Sexual maturity (% stage 1)	66	65	-	
Cardiorespiratory fitness (meters)	875 (90)	936 (102)	66 (81)	0.21
Mother´s educational attainment (low/medium/high - %)	20 / 36 / 43	26 / 30 / 43	-	-
Family history of CVD, hypertension or diabetes (% reporting a history)	37	37	-	-
Mother´s BMI (kg/m^2)	23.3 (21.7 - 26.4)	23.2 (21.5 - 25.6)	-	-
Composite muscle-fitness (z-scores)	-0.22 (0.92)	0.23 (1.03)	0 (1)	0.22
Muscular strength (kg/kg)	0.52 (0.09)	0.57 (0.10)	-0.01 (0.09)	0.59
Muscular power (cm)	27.2 (5.9)	28.2 (7.2)	3.0 (5.8)	0.49
Muscular agility (seconds)	25.8 (3.2)	24.8 (3.3)	-2.5 (2.5)	0.39
Composite risk score (z-scores)	0.00 (1.04)	0.00 (0.96)	-	-
Systolic blood pressure (mm Hg)	100.9 (6.9)	101.2 (8.3)	0.5 (7.7)	0.55
Waist-circumference (cm)	57.0 (53.5 - 61.5)[b]	57.0 (54.0 – 61.0)[b]	5.2 (4.1)	<0.001
HOMA-IR	0.69 (0.48 - 0.96)[b]	0.58 (0.43 - 0.83)[b]	0.26 (0.51)	0.13
Triglyceride (mg/dl)	57.0 (45.0 – 72.0)[b]	47.0 (40.0 – 58.0)[b]	-0.6 (28.6)	0.06
TC:HDLc-ratio	2.74 (2.37 - 3.22)[b]	2.58 (2.22 - 2.91)[b]	-0.01 (0.40)	0.20

Values are mean (SD) unless otherwise noted. BMI: body mass index, CVD; cardiovascular disease, HOMA-IR: homeostasis model assessment of insulin resistance, TC: total cholesterol, HDLc: High-density-lipoprotein-cholesterol. For conversion of triglyceride in mg/dl to mmol/l multiply with 0.0113. [a]P-value for difference in change score between boys and girls. [b]Median (25th-75th percentile). Mother´s education (high-school or less, vocational training or tertiary qualifications), mother´s BMI and family history of cardiovascular disease, hypertension or diabetes was obtained by a questionnaire distributed to parents by mail.

Table 2. Coefficients of tracking and longitudinal interdependence of single and composite muscle-fitness phenotypes

		2010			
		Composite muscle-fitness	Muscular strength	Muscular power	Muscular agility
2008	Composite muscle-fitness	0.66 (0.61 to 0.71)	0.54 (0.48 to 0.60)	0.41 (0.34 to 0.48)	-0.44 (-0.51 to -0.37)
	Muscular strength	0.48 (0.41 to 0.54)	0.57 (0.51 to 0.63)	0.23 (0.15 to 0.31)	-0.19 (-0.27 to -0.11)
	Muscular power	0.45 (0.38 to 0.52)	0.26 (0.18 to 0.34)	0.42 (0.35 to 0.49)	-0.27 (-0.35 to -0.19)
	Muscular agility	-0.43 (-0.50 to -0.36)	-0.26 (-0.34 to -0.18)	-0.20 (-0.28 to -0.12)	0.47 (0.40 to 0.53)

Partial correlations (Pearson´s product-moment correlation coefficient (rho)) adjusted for age and sex. 95% CIs are derived using Fishers transformation.

Table 3. Associations between two-year changes in single and composite muscle-fitness phenotypes and a composite risk score.

			Model 1				Model 2				Model 3	
		Std. beta	95% CI	p-value		Std. beta	95% CI	p-value		Std. beta	95% CI	p-value
Composite muscle-fitness		-0.19	-0.30 to -0.07	0.001		-0.10	-0.22 to 0.03	0.12		0.06	-0.07 to 0.19	0.39
Muscular strength		-0.15	-0.25 to -0.06	0.002		-0.10	-0.19 to -0.003	0.04		0.005	-0.10 to 0.11	0.93
Muscular power		-0.06	-0.14 to 0.03	0.16		-0.01	-0.09 to 0.08	0.84		0.06	-0.03 to 0.15	0.16
Muscular agility		0.14	0.04 to 0.23	0.004		0.06	-0.05 to 0.16	0.28		0.02	-0.09 to 0.13	0.72

n=512. HOMA-IR: homeostasis model of insulin resistance, TC: total cholesterol, HDLc: High-density-lipoprotein-cholesterol. Betas represent the expected change (in SD's) in the composite risk score for a one SD change in the exposure phenotype. The composite risk score is comprised of age- and sex standardized values of log HOMA-IR, log triglyceride, systolic blood-pressure (also standardized for stature), log TC:HDLc ratio and log waist-circumference (also standardized for stature). Results for muscular agility (shuttle-run) are multiplied by -1 in the composite muscle-fitness score.

Model 1: Adjusted for age, sex, school-type (intervention/control), family history of cardiovascular disease, diabetes or hypertension, sexual maturity, mother´s BMI, mother´s educational attainment, sexual maturity in 2008 and 2010, and including a random effect for school class.

Model 2: as model 1 including cardiorespiratory fitness in 2008 and 2010.

Model 3: as model 2, but waist-circumference is removed from composite risk score and included as a covariable (using 2008 and 2010).

Table 4. Associations between two-year changes in composite muscle-fitness score and biological risk factors by sex.

			Model 1				Model 2				Model 3	
		Std. beta	95% CI	p-value		Std. beta	95% CI	p-value		Std. beta	95% CI	p-value
Girls (n=259)												
Composite risk score		-0.28	-0.45 to -0.12	0.001		-0.20	-0.39 to -0.03	0.02		-0.07	-0.26 to 0.12	0.48
HOMA-IR		-0.09	-0.29 to 0.11	0.37		0.01	-0.21 to 0.23	0.92		0.07	-0.15 to 0.29	0.53
Systolic blood pressure		-0.05	-0.25 to 0.15	0.62		-0.04	-0.25 to 0.18	0.75		0.03	-0.19 to 0.25	0.79
Triglyceride		-0.22	-0.40 to -0.04	0.02		-0.18	-0.38 to 0.02	0.08		-0.13	-0.33 to 0.08	0.22
TC:HDL-cholesterol		-0.23	-0.34 to -0.12	<0.001		-0.23	-0.35 to -0.11	<0.001		-0.21	-0.33 to -0.09	0.001
Waist-circumference		-0.28	-0.43 to -0.13	<0.001		-0.21	-0.37 to -0.05	0.009				
Boys (n=253)												
Composite risk score		-0.13	-0.28 to 0.02	0.10		-0.03	-0.19 to 0.14	0.74		0.14	-0.04 to 0.32	0.13
HOMA-IR		-0.12	-0.30 to 0.05	0.17		-0.02	-0.20 to 0.17	0.86		0.02	-0.17 to 0.21	0.81
Systolic blood pressure		0.07	-0.09 to 0.24	0.40		0.06	-0.12 to 0.24	0.53		0.12	-0.07 to 0.30	0.20

Triglyceride		0.01	-0.17 to 0.19	0.94		0.11	-0.09 to 0.31	0.27		0.17	-0.03 to 0.37	0.09
TC:HDL-cholesterol		-0.08	-0.19 to 0.04	0.18		-0.02	-0.14 to 0.10	0.75		0.04	-0.09 to 0.16	0.54
Waist-circumference		-0.29	-0.40 to -0.18	<0.001		-0.23	-0.35 to -0.11	<0.001				

HOMA-IR: homeostasis model of insulin resistance, TC: total cholesterol, HDLc: High-density-lipoprotein-cholesterol. Betas represent the expected change (in SD's) in the respective outcome for a one SD change in the composite muscle-fitness score. Results for muscular agility (shuttle-run) are multiplied by -1 in the composite muscle-fitness score.

Model 1: Adjusted for age, sex, school-type (intervention/control), family history of cardiovascular disease, diabetes or hypertension, sexual maturity in 2008 and 2010, mother´s BMI, mother´s educational attainment, and including a random effect for school class.

Model 2: as model 1 including cardiorespiratory fitness in 2008 and 2010

Model 3: as model 2, but waist-circumference is removed from composite risk score and included as a covariable (using 2008 and 2010)

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Online only material

Muscle-fitness changes during childhood associates with improvements in cardiometabolic risk factors: A prospective study

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Cross-sectional associations between single and composite muscle-fitness phenotypes and biological risk factors

Supplementary Table S1. Cross-sectional associations between single and composite muscle-fitness phenotypes and biological risk factors.

		Model 1			Model 2			Model 3		
	Std. beta	95% CI	p-value	Std. beta	95% CI	p-value	Std. beta	95% CI	p-value
Composite risk score									
Composite muscle-fitness	**-0.37**	**-0.48 to -0.27**	**<0.001**	**-0.30**	**-0.41 to -0.18**	**<0.001**	**-0.14**	**-0.26 to -0.03**	**0.017**
Muscular strength	**-0.32**	**-0.40 to -0.23**	**<0.001**	**-0.67**	**-0.36 to -0.18**	**<0.001**	**-0.14**	**-0.23 to -0.05**	**0.003**
Muscular power	**-0.13**	**-0.24 to -0.02**	**0.018**	-0.07	-0.18 to 0.04	0.195	0.03	-0.07 to 0.14	0.542
Muscular agility	**0.23**	**0.12 to 0.34**	**<0.001**	**0.13**	**0.01 to 0.24**	**0.032**	**0.11**	**0.000 to 0.23**	**0.050**
HOMA-IR									
Composite muscle-fitness	**-0.17**	**-0.28 to -0.06**	**0.003**	-0.12	-0.23 to 0.003	0.06	-0.05	-0.17 to 0.07	0.43
Muscular strength	**-0.20**	**-0.29 to -0.11**	**<0.001**	**-0.17**	**-0.26 to -0.08**	**<0.001**	**-0.12**	**-0.21 to -0.02**	**0.01**
Muscular power	-0.03	-0.14 to 0.08	0.63	0.01	-0.10 to 0.12	0.87	0.05	-0.06 to 0.16	0.38
Muscular agility	0.05	-0.06 to0.16	0.36	-0.02	-0.14 to 0.09	0.72	-0.03	-0.15 to 0.08	0.58
Systolic blood pressure									
Composite muscle-fitness	-0.10	-0.20 to 0.009	0.07	**-0.14**	**-0.25 to -0.02**	**0.02**	-0.08	-0.20 to 0.03	0.17
Muscular strength	**-0.11**	**-0.20 to -0.02**	**0.01**	**-0.13**	**-0.22 to -0.04**	**0.005**	-0.09	-0.18 to 0.008	0.07
Muscular power	0.03	-0.07 to 0.14	0.53	0.03	-0.08 to 0.14	0.62	0.06	-0.05 to 0.17	0.28

Muscular agility		0.10	-0.005 to 0.21	0.06		**0.14**	**0.02 to 0.25**	**0.02**		**0.13**	**0.01 to 0.24**	**0.03**
Triglyceride												
Composite muscle-fitness		**-0.19**	**-0.30 to -0.08**	**0.001**		**-0.13**	**-0.25 to -0.01**	**0.03**		-0.10	-0.23 to 0.02	0.10
Muscular strength		**-0.11**	**-0.20 to -0.02**	**0.02**		-0.07	-0.17 to 0.02	0.13		-0.05	-0.15 to 0.05	0.33
Muscular power		-0.11	-0.22 to 0.004	0.06		-0.07	-0.18 to 0.04	0.23		-0.05	-0.16 to 0.06	0.37
Muscular agility		**0.14**	**0.03 to 0.25**	**0.01**		0.08	-0.04 to 0.19	0.22		0.07	-0.05 to 0.19	0.24
TC:HDLc ratio												
Composite muscle-fitness		-0.24	-0.35 to -0.13	**<0.001**		**-0.18**	**-0.30 to -0.06**	**0.002**		**-0.12**	**-0.24 to -0.01**	**0.04**
Muscular strength		-0.17	-0.26 to -0.08	**<0.001**		**-0.14**	**-0.23 to -0.05**	**0.003**		-0.09	-0.18 to 0.004	0.06
Muscular power		-0.07	-0.18 to 0.04	0.23		-0.02	-0.13 to 0.09	0.70		0.02	-0.09 to 0.13	0.74
Muscular agility		0.20	0.09 to 0.31	**<0.001**		**0.13**	**0.02 to 0.25**	**0.03**		**0.13**	**0.01 to 0.24**	**0.03**
Waist-circumference												
Composite muscle-fitness		**-0.42**	**-0.52 to -0.32**	**<0.001**		**-0.32**	**-0.42 to -0.21**	**<0.001**				
Muscular strength		**-0.33**	**-0.42 to -0.25**	**<0.001**		**-0.27**	**-0.35 to -0.19**	**<0.001**				
Muscular power		**-0.24**	**-0.34 to -0.14**	**<0.001**		**-0.17**	**-0.27 to -0.07**	**0.001**				
Muscular agility		**0.20**	**0.09 to 0.30**	**<0.001**		0.05	-0.06 to 0.16	0.36				

n=512. Outcome variables are standardized for age and sex (z-scores) with betas representing the expected SD change in the respective risk factor associated with a one SD change in the muscle-fitness phenotype in question. Muscular agility (shuttle-run) is inverted in the composite muscle-fitness score. HOMA-IR, triglyceride, TC:HDL-c ratio, and waist-circumference were transformed by the natural logarithm prior to standardization. Waist-circumference and systolic blood pressure are also standardized for stature.

Model 1: includes age, sex, school-type (intervention/control), family history of cardiovascular disease, diabetes or hypertension, sexual maturity, mother's BMI and mother's educational attainment.

Model 2: as model 1 including cardiorespiratory fitness.

Model 3: as model 2 including standardized waist-circumference (with waist-circumference removed from the composite risk score).

Associations between baseline muscle-fitness phenotypes and two-year changes in biological risk factors

Supplementary Table S2. Associations between baseline muscle-fitness phenotypes and two-year changes in biological risk factors.

			Model 1				Model 2				Model 3	
		Std. beta	95% CI	p-value		Std. beta	95% CI	p-value		Std. beta	95% CI	p-value
Composite risk score												
Composite muscle-fitness		**-0.12**	**-0.21 to -0.03**	**0.007**		-0.08	-0.18 to 0.02	0.11		-0.03	-0.13 to 0.08	0.60
Muscular strength		-0.05	-0.12 to 0.03	0.24		-0.02	-0.10 to 0.06	0.62		0.04	-0.05 to 0.12	0.38
Muscular power		-0.04	-0.13 to 0.05	0.37		-0.01	-0.10 to 0.07	0.79		-0.001	-0.09 to 0.09	0.99
Muscular agility		**0.16**	**0.07 to 0.24**	**<0.001**		**0.12**	**0.02 to 0.21**	**0.015**		**0.10**	**0.005 to 0.20**	**0.04**
HOMA-IR												
Composite muscle-fitness		**-0.16**	**-0.26 to -0.06**	**0.002**		-0.10	-0.21 to 0.01	0.07		-0.07	-0.18 to 0.04	0.22
Muscular strength		-0.06	-0.15 to 0.03	0.17		-0.02	-0.11 to 0.06	0.58		0.01	-0.08 to 0.10	0.90
Muscular power		-0.07	-0.18 to 0.03	0.15		-0.04	-0.14 to 0.06	0.45		-0.02	-0.12 to 0.08	0.73
Muscular agility		**0.19**	**0.09 to 0.29**	**<0.001**		**0.14**	**0.03 to 0.24**	**0.02**		**0.13**	**0.02 to 0.24**	**0.02**
Systolic blood pressure												
Composite muscle-fitness		0.00	-0.09 to 0.10	0.99		-0.01	-0.12 to 0.09	0.79		0.00	-0.10 to 0.11	0.93
Muscular strength		0.00	-0.08 to 0.08	0.99		-0.01	-0.09 to 0.08	0.87		0.01	-0.08 to 0.10	0.83
Muscular power		-0.02	-0.12 to 0.07	0.67		-0.03	-0.13 to 0.07	0.57		-0.02	-0.12 to 0.08	0.73
Muscular agility		-0.03	-0.13 to 0.06	0.53		-0.02	-0.13 to 0.08	0.69		-0.02	-0.13 to 0.08	0.65

Triglyceride												
Composite muscle-fitness		-0.08	-0.18 to 0.02	0.10		-0.03	-0.13 to 0.0805	0.63		0.00	-0.11 to 0.112	0.97
Muscular strength		0.02	-0.07 to 0.10	0.70		0.05	-0.03 to 0.135	0.25		0.08	-0.007 to 0.167	0.07
Muscular power		-0.05	-0.15 to 0.04	0.28		-0.03	-0.13 to 0.0721	0.60		-0.01	-0.11 to 0.0875	0.82
Muscular agility		**0.16**	**0.06 to 0.25**	**0.002**		**0.12**	**0.01 to 0.223**	**0.03**		**0.11**	**0.008 to 0.218**	**0.04**
TC:HDLc ratio												
Composite muscle-fitness		-0.02	-0.08 to 0.04	0.56		0.01	-0.06 to 0.0751	0.82		0.02	-0.05 to 0.09	0.58
Muscular strength		-0.01	-0.06 to 0.042	0.71		0.00	-0.05 to 0.0547	0.94		**0.01**	**-0.04 to 0.07**	**0.68**
Muscular power		0.03	-0.03 to 0.09	0.29		0.05	-0.01 to 0.111	0.13		0.06	-0.006 to 0.12	0.08
Muscular agility		0.05	-0.01 to 0.11	0.10		0.03	-0.03 to 0.100	0.33		0.03	-0.03 to 0.10	0.33
Waist-circumference												
Composite muscle-fitness		**-0.10**	**-0.17 to -0.02**	**0.009**		-0.07	-0.15 to 0.005	0.07				
Muscular strength		**-0.09**	**-0.15 to -0.03**	**0.006**		**-0.07**	**-0.14 to -0.01**	**0.02**				
Muscular power		0.02	-0.05 to 0.09	0.63		0.03	-0.04 to 0.10	0.38				
Muscular agility		**0.10**	**0.03 to 0.17**	**0.004**		**0.08**	**0.003 to 0.15**	**0.04**				

n=512. Models include only the baseline (2008) level of the respective muscle-fitness phenotype but both follow-up and baseline composite risk score. Outcome variables are standardized for age and sex (z-scores) with betas representing the expected SD change in the respective risk factor associated with a one SD change in the muscle-fitness phenotype in question. Muscular agility (shuttle-run) is inverted in the composite muscle-fitness score. HOMA-IR, triglyceride, TC:HDL-c ratio, and waist-circumference were transformed by the natural logarithm prior to standardization. Waist-circumference and systolic blood pressure are also standardized for stature.

Model 1: includes age, sex, school-type (intervention/control), family history of cardiovascular disease, diabetes or hypertension, sexual maturity (in 2008), mother's BMI and mother's educational attainment.

Model 2: as model 1 including cardiorespiratory fitness in 2008.

Model 3: as model 2, including standardized waist-circumference (using 2008, with waist-circumference removed from the composite risk score

LOSS TO FOLLOW-UP

Supplementary Table S3. Baseline characteristics of the analysed sample versus those lost to follow-up.

	N=	Analysed sample		N=	Non-analyzed sample		p-value[c]
Gender (% girls)	512	51		697	55		0.16
Age (years)	512	8.4 (1.4)		697	8.4 (1.4)		0.27
Stature (cm)	512	133.2 (9.4)		661	132.1 (9.9)		0.0498
Body weight (kg)[a]	512	28.2 (24.7 - 33.6)		660	28.4 (24.3 - 32.7)		0.65
Sexual maturity (% stage 1)	512	34		635	27		0.008
Cardiorespiratory fitness (meters)	512	906 (100)		609	879 (112)		<0.001
Mother's educational attainment (low/medium/high - %)	512	23 / 33 / 43		502	24 / 31 / 45		0.80
Family history of cardiovascular disease, hypertension or diabetes (% reporting a history)	512	37		524	40		0.39
Mother's BMI (kg/m^2)	512	23.3 (21.6 – 25.9)		480	23.6 (21.4 – 25.9)		0.59
Composite muscle-fitness (z-scores)[a]	512	0.08 (0.96)		633	-0.07 (1.03)		0.01
Muscular strength (kg/kg)	512	0.54 (0.09)		638	0.54 (0.10)		0.24
Muscular power (cm)	512	27.7 (6.6)		652	26.9 (6.6)		0.046
Muscular agility (seconds)	512	24.6 (23.1 – 27.1)		651	25.3 (23.3 – 27.8)		0.002
Composite risk score (z-scores)[a]	512	-0.05 (0.94)		447	0.06 (1.07)		0.08

Systolic blood pressure (mm Hg)	512	101.1 (7.6)		627	101.3 (7.9)		0.66
Waist-circumference (cm)[b]	512	57.0 (54.0 – 61.5)		659	57.5 (54.0 – 62.0)		0.50
HOMA-IR[b]	512	0.63 (0.45 – 0.90)		477	0.66 (0.45 – 0.97)		0.15
Triglyceride (mg/dl) [b]	512	51 (41.5 – 65.5)		477	51.0 (42.0 – 66.0)		0.70
TC:HDLc-ratio[b]	512	2.64 (2.29 – 3.06)		477	2.68 (2.28 – 3.12)		0.46

Values are mean (SD) unless otherwise noted. At baseline, 1209 participants provided written, informed consent from a parent or legal guardian.

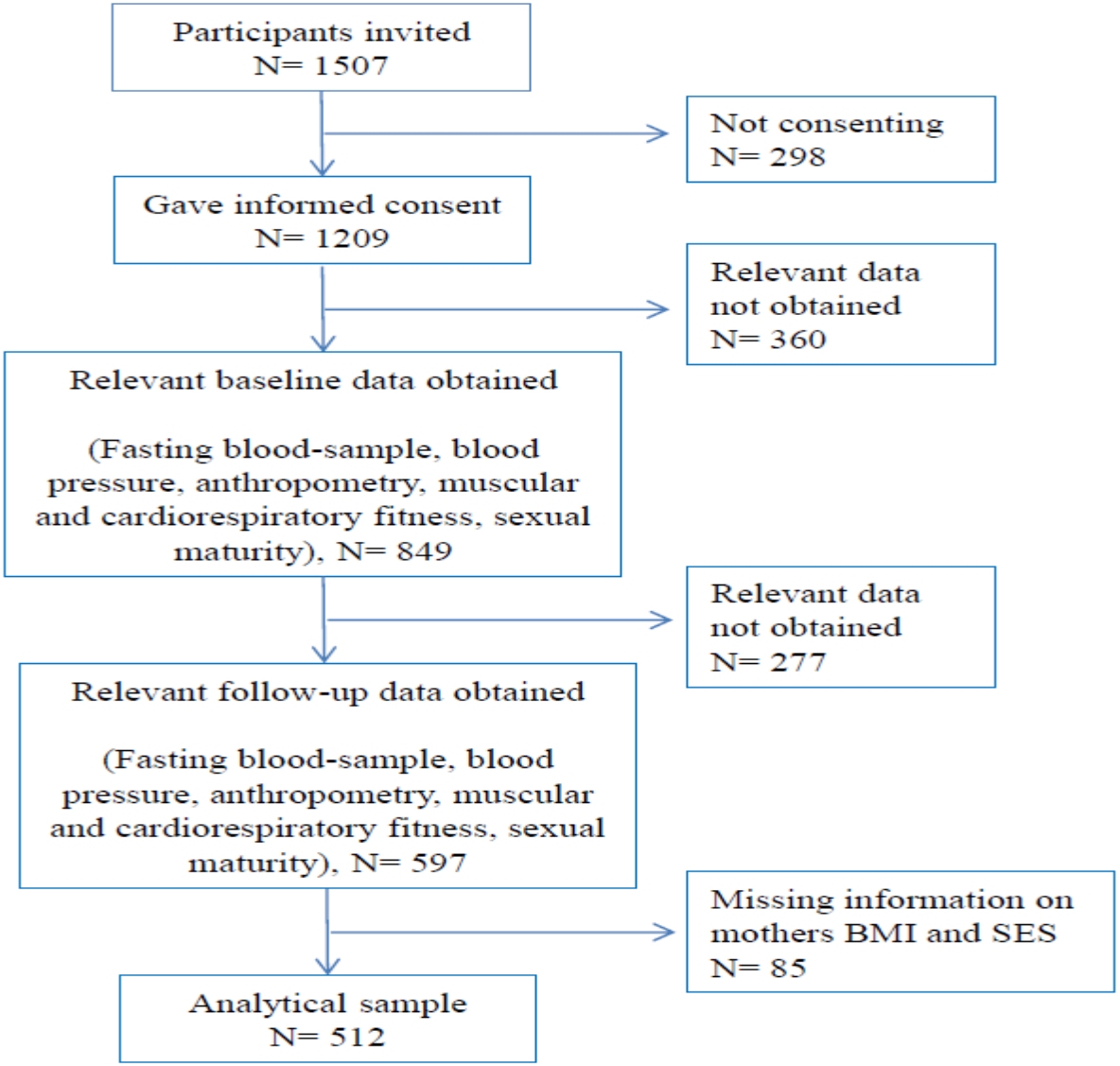
[a] Standardized to distribution in sample available at baseline

[b]Median (25th-75th percentile)

[c]Chi^2 for categorical variables, t-test or ranksum for continuous data as applicable.

Mother's education (high-school or less, vocational training or tertiary qualifications), mother's BMI and family history of cardiovascular disease, hypertension or diabetes was obtained by a questionnaire distributed to parents by mail.

Supplementary Figure S1. Flowchart of included participants



Multiple imputation analysis

Imputation of variables was performed by using chained equations ("mi impute chained") in Stata v14.0. Beta coefficients and standard errors were obtained based on 20 imputed datasets. All consenting students (n=1209) at baseline were included ("mi estimate"). The number of Imputed values ranged from n=296 (composite risk score at follow-up) to n=45 (muscular power at baseline), corresponding to 24 and 4 % of total observations, respectively. Imputation models were visually checked for convergence and the reproducibility of the estimates where inspected by Monte Carlo errors. The imputation analyses are based on the assumption of data being missing at random conditional on the observed covariates. Imputation was based on all variables included in models (including both baseline and 2010 data) except an indicator for school (ten different schools) was used instead of class, and we added an indicator for being in the analytical sample (n=512) or not (n=697) to the imputation models.

Results are presented in Supplementary Table S4.

Supplementary Table S4. Sensitivity analysis based on multiple imputation of missing observations. Cross-sectional and prospective (two-year change) associations between single and composite muscle-fitness phenotypes and a composite risk score.

		Model 1			Model 2				Model 3		
		Std. beta	95% CI	p-value	Std. beta	95% CI	p-value		Std. beta	95% CI	p-value
Cross-Sectional											
Composite muscle-fitness		**-0.34**	**-0.42 to -0.26**	**<0.001**	**-0.24**	**-0.33 to -0.16**	**<0.001**		-0.08	-0.17 to 0.01	0.10
Muscular strength		**-0.31**	**-0.38 to -0.24**	**<0.001**	**-0.25**	**-0.31 to -0.18**	**<0.001**		**-0.12**	**-0.19 to -0.05**	**0.001**
Muscular power		**-0.16**	**-0.24 to -0.08**	**<0.001**	**-0.08**	**-0.16 to -0.00**	**0.02**		0.05	-0.04 to 0.13	0.28
Muscular agility		**0.20**	**0.12 to 0.29**	**<0.001**	**0.07**	**-0.02 to 0.16**	**0.14**		0.06	-0.04 to 0.15	0.24
Prospective											
Composite muscle-fitness		**-0.21**	**-0.30 to -0.13**	**<0.001**	**-0.13**	**-0.23 to -0.03**	**0.01**		0.02	-0.09 to 0.13	0.74
Muscular strength		**-0.16**	**-0.23 to -0.09**	**<0.001**	**-0.11**	**-0.18 to -0.03**	**0.007**		0.01	-0.07 to 0.01	0.82
Muscular power		**-0.12**	**-0.19 to -0.05**	**0.001**	-0.07	-0.14 to 0.002	0.06		0.01	-0.07 to 0.08	0.89
Muscular agility		**0.10**	**0.01 to 0.19**	**0.03**	0.00	-0.10 to 0.10	0.98		-0.03	-0.13 to 0.08	0.61

n=1209. Betas represent the expected change (in SD's) in the composite risk score for a one SD change in the exposure phenotype. The composite risk score is comprised of age- and sex standardized values of log HOMA-IR, log triglyceride, systolic blood-pressure (also standardized for stature), log TC:HDLc ratio and log waist-circumference (also standardized for stature). Results for muscular agility (shuttle-run) are multiplied by -1 in the composite muscle-fitness score.

Model 1: Adjusted for age, sex, school-type (intervention/control), family history of cardiovascular disease, diabetes or hypertension, sexual maturity, mother´s BMI, mother´s educational attainment, and including a random effect for school class. Prospective models additionally adjusted for sexual maturity in 2008 and 2010.

Model 2: as model 1 including cardiorespiratory fitness. Prospective models additionally adjusted for sexual maturity and cardiorespiratory fitness in 2008 and 2010.

Model 3: as model 2, but waist-circumference is removed from composite risk score and included as a covariable. Prospective models additionally adjusted for sexual maturity, cardiorespiratory fitness, and waist-circumference in 2008 and 2010.

Cross-sectional and prospective (two-year change) associations using other scalings for body weight

Supplementary Table S5. Cross-sectional and prospective associations between muscle strength and composite muscle-fitness score with composite risk score using different scalings for body weight

		Model 1			Model 2			Model 3		
	Std. beta	95% CI	p-value	Std. beta	95% CI	p-value	Std. beta	95% CI	p-value
Cross-sectional										
Composite muscle-fitness	**-0.34**	**-0.45 to -0.23**	**<0.001**	**-0.26**	**-0.38 to -0.15**	**<0.001**	**-0.12**	**-0.24 to -0.004**	**0.04**
Muscular strength (empirical)	**-0.27**	**-0.36 to -0.18**	**<0.001**	**-0.22**	**-0.31 to -0.13**	**<0.001**	**-0.11**	**-0.21 to -0.02**	**0.02**
Muscular strength (theoretical)	**-0.22**	**-0.31 to -0.12**	**<0.001**	**-0.17**	**-0.26 to -0.07**	**0.001**	-0.10	-0.19 to 0.0003	0.051
Prospective										
Sexes combined										
Composite muscle-fitness	**-0.19**	**-0.31 to -0.08**	**0.001**	-0.10	-0.22 to 0.03	0.12	0.06	-0.08 to 0.19	0.40
Muscular strength (empirical)	**-0.15**	**-0.25 to -0.06**	**0.002**	**-0.10**	**-0.19 to -0.0001**	**0.050**	0.004	-0.10 to 0.11	0.93
Muscular strength (theoretical)	-0.09	-0.19 to 0.01	0.09	-0.03	-0.14 to 0.07	0.51	0.04	-0.06 to 0.15	0.42
Girls										
Composite muscle-fitness	**-0.29**	**-0.46 to -0.13**	**0.001**	**-0.22**	**-0.340 to -0.03**	**0.02**	-0.07	-0.27 to 0.12	0.46
Muscular strength (empirical)	**-0.22**	**-0.35 to -0.08**	**0.002**	**-0.18**	**-0.31 to -0.04**	**0.01**	-0.08	-0.23 to 0.07	0.29
Muscular strength (theoretical)	-0.14	-0.29 to 0.005	0.06	-0.11	-0.25 to 0.04	0.15	-0.04	-0.19 to 0.12	0.65
Boys										

Composite muscle-fitness		-0.13	-0.28 to 0.03	0.11		-0.02	-0.19 to 0.14	0.80		0.15	-0.03 to 0.33	0.11
Muscular strength (empirical)		-0.08	-0.20 to 0.05	0.22		-0.01	-0.13 to 0.12	0.93		0.10	-0.03 to 0.24	0.14
Muscular strength (theoretical)		-0.04	-0.17 to 0.09	0.57		0.03	-0.10 to 0.17	0.65		0.13	-0.02 to 0.27	0.08

n=512. Muscle strength (handgrip strength) was divided by body weigh scaled to 1) a theoretical scaling constant of 2/3 (theoretical), and 2) an empirically derived sex- and time (2008 and 2010) specific constant (theoretical). The empirical constants were 0.79 and 0.85 at baseline for girls and boys, respectively and 0.91 for both genders at follow-up. Muscle strength divided by body weight scaled to the empirical constants was included in the composite muscle-fitness score. Results for muscular agility (shuttle-run) are multiplied by -1 in the composite muscle-fitness score. Betas represent the expected change (in SD's) in the composite risk score for a one SD change in the exposure phenotype. The composite risk score is comprised of age- and sex standardized values of log HOMA-IR, log triglyceride, systolic blood-pressure (also standardized for stature), log TC:HDLc ratio and log waist-circumference (also standardized for stature).

Model 1: Adjusted for age, sex, school-type (intervention/control), family history of cardiovascular disease, diabetes or hypertension, sexual maturity, mother's BMI, mother's educational attainment, and including a random effect for school class. Prospective models additionally adjusted for sexual maturity in 2008 and 2010.

Model 2: as model 1 including cardiorespiratory fitness. Prospective models additionally adjusted for sexual maturity and cardiorespiratory fitness in 2008 and 2010.

Model 3: as model 2, but waist-circumference is removed from composite risk score and included as a covariable. Prospective models additionally adjusted for sexual maturity, cardiorespiratory fitness, and waist-circumference in 2008 and 2010.

Associations between two-year changes in single muscle-fitness phenotypes and changes in biological risk factors

Supplementary Table S6. Associations between two-year changes in single muscle-fitness phenotypes and changes in biological risk factors in girls.

		Model 1				Model 2				Model 3	
	Std. beta	95% CI	p-value		Std. beta	95% CI	p-value		Std. beta	95% CI	p-value
Composite risk score											
Muscular strength	**-0.22**	**-0.36 to -0.08**	**0.001**		**-0.18**	**-0.32 to -0.04**	**0.009**		-0.09	-0.24 to 0.06	0.26
Muscular power	-0.08	-0.21 to 0.04	0.17		-0.04	-0.16 to 0.09	0.55		0.03	-0.10 to 0.16	0.65
Muscular agility	**0.15**	**0.03 to 0.28**	**0.02**		0.09	-0.05 to 0.23	0.23		0.06	-0.09 to 0.21	0.43
HOMA-IR											
Muscular strength	-0.14	-0.30 to 0.03	0.10		-0.09	-0.26 to 0.08	0.29		-0.03	-0.20 to 0.14	0.76
Muscular power	-0.01	-0.15 to 0.14	0.92		0.04	-0.10 to 0.19	0.57		0.06	-0.09 to 0.20	0.45
Muscular agility	0.08	-0.07 to 0.23	0.30		0.00	-0.17 to 0.17	0.98		-0.02	-0.18 to 0.15	0.86
Systolic blood pressure											
Muscular strength	-0.15	-0.31 to 0.01	0.07		-0.15	-0.31 to 0.02	0.09		-0.10	-0.27 to 0.07	0.23
Muscular power	0.07	-0.07 to 0.21	0.33		0.08	-0.07 to 0.22	0.29		0.11	-0.04 to 0.25	0.14
Muscular agility	0.01	-0.14 to 0.16	0.89		0.00	-0.17 to 0.17	0.99		-0.01	-0.18 to 0.16	0.88
Triglyceride											
Muscular strength	-0.11	-0.26 to 0.04	0.14		-0.08	-0.23 to 0.07	0.30		-0.02	-0.17 to 0.14	0.83

Muscular power		-0.08	-0.22 to 0.05	0.21		-0.05	-0.19 to 0.08	0.43		-0.04	-0.18 to 0.09	0.55
Muscular agility		0.13	-0.003 to 0.27	0.06		0.11	-0.05 to 0.27	0.17		0.10	-0.0546 to 0.256	0.20
TC:HDLc ratio												
Muscular strength		**-0.12**	**-0.21 to -0.03**	**0.01**		**-0.11**	**-0.20 to -0.01**	**0.03**		**-0.10**	**-0.19 to -0.004**	**0.04**
Muscular power		**-0.10**	**-0.18 to -0.02**	**0.02**		**-0.08**	**-0.17 to -0.002**	**0.04**		-0.07	-0.15 to 0.01	0.09
Muscular agility		**0.12**	**0.03 to 0.20**	**0.006**		**0.10**	**0.005 to 0.20**	**0.04**		0.10	-0.0006 to 0.19	0.051
Waist-circumference												
Muscular strength		**-0.17**	**-0.29 to -0.04**	**0.009**		**-0.14**	**-0.26 to -0.01**	**0.03**				
Muscular power		**-0.13**	**-0.24 to-0.03**	**0.01**		-0.09	-0.20 to 0.02	0.10				
Muscular agility		**0.12**	**0.01 to 0.23**	**0.03**		0.04	-0.08 to 0.16	0.50				

n=259. Outcome variables are standardized for age and sex (z-scores) with betas representing the expected SD change in the respective outcome for a one SD change in the muscle-fitness phenotype in question.

Model 1: Adjusted for age, sex, school-type (intervention/control), family history of cardiovascular disease, diabetes or hypertension, sexual maturity in 2008 and 2010, mother's BMI, mother's educational attainment, and including a random effect for school class.

Model 2: as model 1 including cardiorespiratory fitness in 2008 and 2010

Model 3: as model 2, including standardized waist-circumference (using 2008 and 2010, with waist-circumference removed from the composite risk score).

Supplementary Table S7. Associations between two-year changes in single muscle-fitness phenotypes and changes in biological risk factors in boys.

		Model 1			Model 2			Model 3		
	Std. beta	95% CI	p-value	Std. beta	95% CI	p-value	Std. beta	95% CI	p-value
Composite risk score										
Muscular strength	-0.09	-0.21 to 0.04	0.17	-0.01	-0.14 to 0.11	0.83	0.10	-0.04 to 0.24	0.17
Muscular power	-0.04	-0.16 to 0.07	0.47	0.00	-0.11 to 0.12	0.97	0.10	-0.03 to 0.22	0.13
Muscular agility	**0.17**	**0.03 to 0.32**	**0.02**	0.09	-0.07 to 0.25	0.25	0.06	-0.10 to 0.23	0.46
HOMA-IR									
Muscular strength	-0.07	-0.22 to 0.07	0.31	0.00	-0.15 to 0.15	0.98	0.03	-0.12 to 0.18	0.71
Muscular power	-0.03	-0.16 to 0.11	0.70	0.04	-0.09 to 0.17	0.55	0.06	-0.07 to 0.20	0.37
Muscular agility	**0.20**	**0.03 to 0.36**	**0.02**	0.12	-0.06 to 0.30	0.20	0.11	-0.07 to 0.29	0.24
Systolic blood pressure									
Muscular strength	0.02	-0.11 to 0.15	0.77	0.01	-0.13 to 0.15	0.89	0.05	-0.09 to 0.19	0.50
Muscular power	0.09	-0.04 to 0.22	0.17	0.09	-0.04 to 0.22	0.19	0.12	-0.008 to 0.25	0.07
Muscular agility	0.05	-0.11 to 0.20	0.56	0.06	-0.11 to 0.24	0.49	0.05	-0.13 to 0.22	0.59
Triglyceride									
Muscular strength	-0.01	-0.16 to 0.13	0.86	0.06	-0.09 to 0.21	0.44	0.11	-0.05 to 0.26	0.18
Muscular power	-0.01	-0.15 to 0.13	0.91	0.03	-0.12 to 0.17	0.72	0.06	-0.09 to 0.20	0.45
Muscular agility	0.04	-0.13 to 0.21	0.64	-0.03	-0.23 to 0.16	0.73	-0.05	-0.24 to 0.14	0.61

TC:HDLc ratio												
Muscular strength		-0.06	-0.15 to 0.03	0.20		-0.02	-0.12 to 0.07	0.65		0.01	-0.08 to 0.11	0.79
Muscular power		-0.01	-0.10 to 0.08	0.79		0.01	-0.08 to 0.10	0.83		0.04	-0.05 to 0.13	0.35
Muscular agility		0.08	-0.02 to 0.19	0.13		0.04	-0.08 to 0.17	0.47		0.03	-0.09 to 0.14	0.68
Waist-circumference												
Muscular strength		**-0.19**	**-0.28 to -0.10**	**<0.001**		**-0.15**	**-0.24 -0.05**	**0.002**				
Muscular power		**-0.17**	**-0.26 to -0.08**	**<0.001**		**-0.13**	**-0.22 to -0.05**	**0.002**				
Muscular agility		**0.14**	**0.03 to 0.25**	**0.01**		0.05	-0.07 to 0.17	0.38				

n=253. Outcome variables are standardized for age and sex (z-scores) with betas representing the expected SD change in the respective outcome for a one SD change in the muscle-fitness phenotype in question.

Model 1: Adjusted for age, sex, school-type (intervention/control), family history of cardiovascular disease, diabetes or hypertension, sexual maturity in 2008 and 2010, mother's BMI, mother's educational attainment, and including a random effect for school class.

Model 2: as model 1 including cardiorespiratory fitness in 2008 and 2010

Model 3: as model 2, including standardized waist-circumference (using 2008 and 2010, with waist-circumference removed from the composite risk score).

Study 5

TITLE

Long-term follow-up on biological risk factors, adiposity, and cardiorespiratory fitness development in a physical education intervention: a natural experiment (CHAMPS-study DK)

AUTHORS

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LIST OF ABBREVIATIONS

NCD: non-communicable disease; CHAMPS-study DK: Childhood Health and Motor Performance School study Denmark; TC: total cholesterol; HDL-c: high-density-lipoprotein cholesterol; MVPA: moderate-to-vigorous physical activity; HOMA-IR homeostasis model assessment of insulin resistance; CI: confidence interval.

DECLARATIONS

Ethics approval and consent to participate

The CHAMPS-study DK was approved by the ethics committee of the region of southern Denmark (S-20080047 and S-20140105) and written informed consent was obtained from a parent or legal guardian at both time-points.

Consent for publication

Not applicable

Availability of data and materials

Data are available upon request from the CHAMPS Study Steering Committee due to legal and ethical restrictions. Interested parties may contact Dr Niels Wedderkopp (nwedderkopp@health.sdu.dk) or Dr Anna Bugge (anbugge@health.sdu.dk), and the following information will be required at the time of application: a description of how the data will be used, securely managed, and permanently deleted.

Competing interests:

The authors declare they have no competing interests.

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Authors' contributions

JT, NCM, NW and AB conceived the study. JT, EJ and HK collected the data. BW analysed the biological samples. All authors formulated the analytical approach. JT performed the analyses and wrote the first draft. All authors read, edited and approved the final manuscript.

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ABSTRACT

Background: Schools are a key setting for large-scale primordial non-communicable disease prevention in young people, but little data on sustainability of impacts on cardiometabolic risk markers is available.

Methods: Six and a half year follow-up of a natural experiment. In 2008, six public schools in the municipality of Svendborg (Denmark) augmented their curricular physical education (intervention) and four matched schools served as controls. At long term follow up in 2015 n=312 participants aged 5 – 11 years had complete data (33 % of children providing necessary baseline data). The intervention, that consisted of a trebling of weekly physical education lessons and courses provided to physical education teachers, was provided at intervention schools up until 6th grade. Participants attended 6th to 10th grade at follow-up. Differences in the homeostasis model assessment of insulin resistance, blood pressure, triglycerides, cholesterol ratios, cardiorespiratory fitness and waist-circumference, and a composite score of these, between participants attending intervention and

control schools were analysed by mixed linear regression models. Differences in physical activity at follow-up was analysed cross-sectionally (no baseline available) in n=495.

Results: Compared to controls, children at intervention schools had a non-significant -0.07 (-0.32 to 0.18) standard deviations lower composite risk score 6.5 years after project initiation. Likewise, no statistically significant differences between intervention and control schools were found for any of the other outcomes (p-values ≥ 0.41). However, six of seven outcomes were in a direction favouring intervention schools. No statistically significant differences between intervention and control schools were observed for physical activity outcomes (p-values ≥ 0.13).

Conclusions: An augmented physical activity program including 270 minutes of weekly physical education provided for three to seven years did not materialize in statistically significant differences in established risk markers in children from intervention compared to control schools. As the intervention was discontinued after 6th grade, the post-intervention effect of augmented physical education throughout adolescence is unknown. School-based physical activity programs may benefit from incorporating instruments for behaviour translation to leisure time in their intervention models to increase the probability of achieving public health relevance.

KEYWORDS

Physical activity, school, prevention, cardiometabolic, blood pressure, insulin resistance, lipids

INTRODUCTION

Excess adipose tissue accumulation and dysregulated metabolic homeostasis in young people are potentially key markers of increased risk of non-communicable diseases (NCDs) such as cardiovascular disease or type 2 diabetes in adulthood [1-3] and may even predict premature mortality [4]. Physical activity should be considered part of a preventive effort because of its role in energy balance and metabolic regulation [5, 6]. However, trends indicate that physical activity and cardiorespiratory fitness levels in young people may be declining [7-9] and possibly accelerated in low socioeconomic segments of society [9]. These trends are alarming as a lack of physical activity is a leading cause of premature mortality in the adult population [5] and physical activity levels track from childhood to adulthood [10].

Population-wide initiatives to increase physical activity levels and ameliorate obesity related disorders are warranted [11]. As 70 % of obese adults were not obese in their youth [12] preventive actions in high-risk individuals will not, even if effective for the individual, prevent the majority of disease burden in the population [13]. School-based approaches have the potential to reach near population-wide coverage as school attendance is mandatory, thus representing an ideal setting for large-scale primordial prevention based on a structural strategy. Structural strategies are appealing as socioeconomic gradients in health inequity may widen with individual-agency approaches [14, 15]. Physical education led by teachers trained in class-management and provision of quality physical education lessons is associated with relatively high physical activity levels during classes [16, 17], which may carry to higher physical activity patterns on physical education days [17]. Accordingly, provision of additional physical education led by professionals could be a viable option for increasing physical activity levels of school-aged children.

Population-based physical activity interventions initiated in schools (implemented as a physical activity only or part of a multicomponent approach) have shown positive benefits on biological risk factor in young people when evaluated immediately post-intervention [18, 19] However, there is a scarcity of data on long-term benefits on the risk factors [20]. Long-term evaluation is needed to create evidence informed practice. By implementing an augmented school-based physical education program in 2008 the Childhood Health and Motor Performance School study DK (CHAMPS study-DK) achieved reductions in clustering of biological risk factors [21] and lowered overweight/obesity prevalence at intervention schools as compared with control schools when evaluated after two years [22]. The purpose of this study is to investigate long-term (6.5 years) differences between children attending control and intervention schools in 1) risk factor clustering and 2) single risk-factors.

METHODS

Setting and study design

The CHAMPS-study DK is a natural experiment [23] implemented as a controlled intervention study including children from ten public schools (age range: 5 – 12 years old) in the municipality of Svendborg, Denmark at inception [16, 24]. The school-years covered are the first year of school (comparable to U.S. kindergarten) to the 4th grade. The CHAMPS-study DK was designed to evaluate a trebling of curricular physical education (270 minutes per week distributed across at least three school days), initiated by the municipality of Svendborg. Six of nineteen schools in the municipality were willing and able to fund the additional physical educations classes and became intervention schools. Four schools, matched on size, rural/urban and sociodemographic uptake area, agreed to serve as controls. Control schools maintained physical education at the national curricular

90 minutes per week. The additional physical education was implemented from the start of the school year in August 2008 and evaluated for effects on biological risk factors and adiposity two years later [21, 22]. After 2010, the six intervention schools have maintained their additional physical education from kindergarten to the 6th grade (children approximately 12 – 14 years old). Thus, from 7th to 9th grade (final mandatory school year in Denmark) the "standard" two physical education lessons per week were provided both at intervention and control schools. Hence, intervention-school participants could receive from three (4th grade in 2008) to seven (Kindergarten in 2008) years of additional physical education (detailed in table 1).

Intervention content

In addition to increased physical education, all physical education teachers (in Denmark physical education is mainly taught by physical education specialists) attended a 40-lessons skill developing course based on an Age-related Training Concept developed by the Danish organization for elite sports (Team Denmark) [25]. The purpose of this program is to augment development of body and motor skills in children and adolescents by considering their physical, physiological, mental and social development. Shortly put, the program is based on play, exercise and games with an increased focus on technical and coordinative skills in adolescence. Control schools maintained national guidelines.

Participants

In 2008, all children and parents from the ten schools (n=1507) were invited to participate in the study [21]. In 2015, 1278 adolescents attending 6th to 9th grade at the now nine schools (due to

merging of schools) were invited to participate in a follow-up study. Recruitment in 2015 was based on handouts at schools, postal mail of study material to parents, and telephone calls by study staff. Additionally, 10th grade students with prior participation in the study (n=179) were sought via postal mail and telephone calls. This study reports long-term effects of the CHAMPS study DK in 312 participants with 6.5 years of follow-up. The CHAMPS-study DK was approved by the ethics committee of the region of southern Denmark (S-20080047 and S-20140105) and written informed consent was obtained from a parent or legal guardian at both time-points.

Outcomes

Data collection for variables used in this study took place during August-October in 2008 (baseline) and February-May in 2015 (follow-up). At both time-points measurements were performed at schools by trained research staff following standardized procedures but not blinded to control/intervention status. Collection of data on 10th grade participants took place in weekends. Blood samples were obtained following an overnight fast between 08.00 and 10.00 am and analysed for total cholesterol (TC), triglyceride, HDL-cholesterol (HDL-c), glucose and insulin in a ISO 9001:2008 certified routine laboratory associated with the University of Vienna, Austria as detailed elsewhere [21]. Briefly, TC, triglycerides, HDL-c and glucose were assessed on a Cobas C System (Roche Diagnostics GmbH, Mannheim, Germany) while insulin was assessed on an Access Immunoassay System (Beckman Coulter, Inc. Brea, CA, USA) using the respective kits. Intermediate precision was determined using human samples and controls in an internal protocol according to the manufacturers. Coefficients of variation for the controls and samples ranged from 0.6 to 0.8 % for TC, from 0.6 to 0.9 % for triglycerides, from 0.5 to 0.8 % for HDL-c, from 0.5 to 0.8 % for glucose, 3.1 to 5.6 % for insulin. Resting blood pressure was measured using appropriate

sized cuffs by a Vital Signs Monitor 300 series with Flexiport™ Blood Pressure *(*Welch Allyn, New York, NY, USA) in 2008 and the Omron 705IT (Omron, Kyoto, Japan) in 2015. Participants sat resting in the sitting position for 5 min before monitoring. At least five subsequent values were recorded with 1-min intervals until the last three values had become stable. The mean of the last three recordings of systolic blood pressure was used in analysis. Waist-circumference was measured by a measurement band (Seca 201, Seca Corporation, Hamburg, Germany) to the nearest 0.5 centimetre across the umbilical cord following a gentle expiration. At least two measurements were performed with a third undertaken if the two differed by more than one centimetre. Cardiorespiratory fitness was assessed using a field-test (Andersen-test) lasting ten minutes with fifteen seconds of intermittent running and pausing. Total distance covered was used to represent cardiorespiratory fitness. Criterion validity (r-squared approximately 0.5 against directly measured maximal oxygen uptake) [26, 27] and test-retest reliability (r-squared approximately 0.7 – 0.8) of the Andersen-test are acceptable and have been validated in a subsample of the cohort [26].

Other variables

Body mass was measured to the nearest 0.1 kg on an electronic scale (Tanita BWB-800S, Tanita Corporation, Tokyo, Japan) with participants wearing light clothes. Stature was measured to the nearest 0.5 cm using a portable stadiometer (Seca 214, Seca Corporation, Hamburg, Germany or Harpenden stadiometer (West Sussex, UK). Both measures were conducted barefoot. Sexual maturity was self-reported by indicating resemblance on five drawings (progressive rating 1 – 5) of secondary sex characteristics as described by Tanner [28]. Pubic hair was used in boys and breast development in girls. Parents returned mailed questionnaires in 2008 and in 2015. These inquired on the educational attainment of the parents or legal guardians, birthweight of the child, and any

(biological) family history of NCDs. When available, data from 2015 was used but in case of non-response or missing answers to the 2015 questionnaire, the 2008 data was used. Physical activity levels were assessed by questionnaires and accelerometry in 2015 only and presented as participation in leisure-time structured physical activity (yes/no), % moderate-to-vigorous physical activity per day (%MVPA/day), and mean counts/minute (protocol and data-reduction details available in Additional file 1.

Data reduction

The primary outcome of this study is a standardized (mean 0 and standard deviation of 1) composite risk score [29] consisting of the homeostasis model assessment of insulin resistance (HOMA-IR) calculated as (insulin in IU/l x glucose in mmol/l)/22.5) [30], triglyceride, TC:HDLc-ratio, systolic blood pressure, waist-circumference, and cardiorespiratory fitness. A higher composite score represents an unfavourable risk profile. All variables were standardized by age and sex in separate linear regressions and the residuals averaged. Cardiorespiratory fitness was multiplied by -1 in the composite score. Systolic blood pressure and waist-circumference were additionally standardized for height. Blood chemistry variables in 2015 were additionally standardized for week-day of ascertainment [31] but this information was not available at baseline. Because only 3 % of the sample defined themselves in Tanner-category 3-5 at baseline, the sample was re-coded as being either pre-pubertal (stage 1) or pubertal (stages 2-5). At follow-up, 6 % defined themselves as Tanner-category 1-2 so this was recoded as stages 1-3, stage 4, or stage 5. Body mass index was calculated as body weight (kg)/stature (meters)^2 and used to define participants according to IOTF weight-status categories [32]. The mother or female guardian's highest completed education was used as a marker of socioeconomic position and recoded (from an abbreviated seven-level

instrument based on a Danish adaptation of the International Standard Classification of Education 2011) to; 0 (no tertiary qualifications) or 1 (any tertiary qualifications). Indication of family history of diabetes (any type), cardiovascular disease (any type) or hypertension in siblings, biological parents or grandparents, were combined into; 0 (no) or 1 (yes). Birthweight was used in continuous form.

STATISTICS

Participants included are those providing fasting values of insulin, glucose and lipids in 2008 and 2015 in addition to information on self-reported sexual maturity at both these time-points. Blood chemistry was required as these variables constituted the bulk of missing follow-up data. Sexual maturity was required because of its key role in insulin-resistance during adolescence [33, 34]. Missing values (n=3 to 33) of variables other than blood chemistry and sexual maturity were imputed by chained equations (MICE), which is detailed in Additional file 2.

Baseline characteristics between intervention and control schools were compared using an unpaired t-test for normal distributed continuous data or Wilcoxon rank-sum test for non-normal distributed data. A chi-squared test was used for categorical data. Details of comparisons between the analytical sample and those lost to follow-up using data obtained in 2008, 2010, 2012, 2013, and 2015 are available in Additional file 2. Differences between intervention and control schools are presented as composite risk score at follow-up, using control schools as reference, analysed in linear mixed models controlled for baseline values of the respective outcome. In secondary analyses, individual outcomes are analysed using the same approach. Results are presented with 95% confidence intervals (CI). Models additionally included the covariates age, sex, sexual maturity (in

2008 and 2015), educational attainment of the mother or female legal guardian, birthweight, and family history of NCDs. A random intercept for school-class at baseline was included. Random intercepts for school-class or school at follow-up were not included as little variance was explained by these terms. Physical activity was analysed using the same control variables but without baseline-adjustment as this data was not available. These analyses used a logistic regression model for structured leisure-time physical activity, and included indicators for the number of total days and weekdays included in models of % MVPA/day and mean counts/min. Participants were included in analysis of physical activity if they; had provided informed consent during the initial phase of the study (2008-2010), at the follow-up in 2015, provided either subjective or objective physical activity data, and had anthropometrical or physical performance data to inform imputation models (n=495, full details available in Additional file 1). Linear regression diagnostics were performed by visually inspecting normality and homoscedasticity of model residuals (assumptions met), checking influential observations by calculating the dfbeta (interpretation of results not affected), and visually verifying normal distribution of random intercepts (assumption met). HOMA-IR, triglyceride, TC:HDL-c ratio, waist-circumference and cardiorespiratory fitness were skewed and transformed by the natural logarithm prior to standardization. To explore if additional physical education had a distinct effect among those with the least favourable metabolic profiles, participants at control and intervention schools were stratified at the baseline median and analysed separately in secondary analyses. Stratification was performed for each outcome.

Analysis was performed based on participant's intervention status at baseline irrespective of their follow-up school membership. Six of ten schools covered kindergarten to 9th grade (no reallocation from primary to secondary school), while four schools (two control and two intervention) included kindergarten to 6th grade only. Of these four schools, students from intervention schools were allocated to other intervention schools, while students from one control school were allocated to an

intervention school while students from the other control school were allocated to a school outside of the project. As follow-up data is collected as an extension of the original study, no power calculations were performed prior to participant recruitment. TREND and TIDieR checklists are available as table S4 in Additional file 3 and table S5 in Additional file 4. Analysis was conducted in Stata v.15.0 (StataCorp, College Station, TX, USA). A two-sided alpha at the 0.05 level was used to indicate statistical significance.

RESULTS

Missing data

In 2008, 1209 participants provided informed consent of which a fasting blood sample was available in 959 participants (64 % of invited). In 2015, 745 participants consented to the study and 580 (40 % of invited) provided a fasting blood sample. A total of 312 participants, with a fasting blood sample and information on sexual maturity at both time-points, were available for long-term follow-up (analytical sample: 21 % of invited and 33 % of those who obtained fasting blood samples in 2008). Of invited in 2008, 18 and 31 % of participants were available for long-term follow-up at control and intervention schools, respectively. Please refer to figure 1 for flow-chart as well as table S1 in Additional file 2 for school-year specific loss to follow-up. The analytical sample differed from their peers unavailable for follow-up, in that the former was younger -0.75 (-0.57 to -0.93) years, and characterized by more favourable anthropometric and cardiorespiratory fitness characteristics (baseline body mass index -0.40 (-0.68 to -0.12) points, waist-circumference -1.30 (-2.11 – 0.49) centimetre, and cardiorespiratory fitness 19 (5 – 32) meters. Indications of non-identical missingness characteristics between intervention and control schools were observed for

family history of NCDs (p=0.07), educational attainment of the mother or female legal guardian (p=0.03), and pubertal development in 2012 (p=0.02). No evidence to suggest differential missingness characteristics were found for anthropometric variables, the composite risk score, or cardiorespiratory fitness at any time-point (p-values >0.34).

Sample characteristics

Baseline (2008) characteristics of the analytical sample are shown in table 2. At intervention schools 56 % of the 217 participants were girls while 44 % of the 95 participants were girls at control schools (p=0.06). At baseline, participants at intervention schools had a 1.79 (0.06 to 3.53) mmHg higher systolic blood pressure, but a 19 % lower HOMA-IR score. The prevalence of overweight or obesity was 8.4 % in 2008 and 6.8 % in 2015 with no difference between intervention and control schools (p-values ≥0.21).

Primary and secondary outcomes

Figure 2 presents differences in the composite score at follow-up between intervention and control schools. The difference did not reach statistical significance (standardized beta with 95 % CI) -0.07 (-0.32 to 0.18). Likewise, differences were non-significant for the individual risk factors (p-values ≥0.41) with standardized betas ranging from -0.10 (-0.39 to 0.20) for cardiorespiratory fitness to -0.03 (-0.25 to 0.19) for waist-circumference. All associations, although not significant, favoured intervention schools, except for cardiorespiratory fitness. In un-transformed scales, differences between intervention and control schools were -0.3 (-2.1 to 1.5) mmHg, -0.2 (-1.6 to 1.2) centimetre, and -9 (-39 to 20) meters for systolic blood pressure, waist-circumference and

cardiorespiratory fitness, respectively. For blood chemistry variables, differences expressed in untransformed scales were -0.03 (-0.12 to 0.06) mmol/l, -0.08 (-0.24 to 0.08), and -0.10 (-0.33 to 0.14) for triglycerides, TC:HDL-c ratio and HOMA-IR, respectively. Figure 3 depicts a visual illustration of the 2 and 6.5 year (analysed in this study) development of the biological risk factors in the CHAMPS-study DK.

In analyses with outcomes stratified at the median baseline-level (figure 4), the least favourable half at intervention schools presented, compared to the least favourable half at control schools, a -0.17 (-0.55 to 0.20) standard deviation lower composite score at follow-up albeit the difference did not reach statistical significance. No statistically significant differences were observed for the individual risk factors. Effect-sizes for (standardized) outcomes stratified at the median ranged from -0.32 (-0.67 to 0.02) for HOMA-IR and -0.26 (-0.62 to 0.10) for waist-circumference to 0.05 (-0.31 to 0.41) for systolic blood pressure. When analysing physical activity levels as assessed in 2015 from n=495, neither structured participation in leisure-time physical activity (odds ratio: 0.79 (0.46 to 1.36)), %MVPA/day (unstandardized beta: -0.17 (-0.67 to 0.33)), or mean counts/minute (unstandardized beta: -25 (-58 to 8)) differed statistically significant between intervention and control schools.

DISCUSSION

This study evaluated a natural experiment based on a trebling of curricular physical education from 90 to 270 weekly minutes at intervention schools compared to controls. In spite of a demonstrated 2-year efficacy of the intervention, the data did not support sustainability of favourable clustered or

single risk factor adaptations comparing intervention with control schools after 6.5 years. However, in general, the direction of associations favoured the intervention.

Existing literature

At the two-year follow-up of the CHAMPS study-DK, the intervention was associated with a favourable composite risk score and lower triglyceride and systolic blood pressure levels [21]. Short-term or immediate post-intervention positive benefits on biological risk factors may be achieved with school-based physical activity interventions [35-39], although the literature is not without its limitations [20] or exceptions [40, 41]. It is noticeable that in some studies a positive benefit appears without concomitant effects on adiposity indices [35, 37-39]. Other positive post-intervention benefits of school-based physical activity initiatives may include higher cardiorespiratory fitness [42] and lower obesity levels [43].

Post-interventional successes apart, our data did not support maintained intervention benefits over time. This is in contrast with two similar studies with data on long-term follow-up on biological risk factors, which do provide tentative support of positive sustainable benefits. These studies were based on provision of additional physical education and have evaluated both immediate post-intervention and long-term follow-up difference between intervention and control schools [36, 37, 44]. In a non-randomized controlled trial based on a doubling of weekly physical education from 90 to 180 minutes for three years and provision of outdoor play-ground equipment, Bugge et al., 2012 found higher insulin sensitivity in intervention school boys post-intervention and an approximately 3 mmHg lower systolic blood pressure change in boys when followed-up four years after the intervention had ceased [37]. No differences between intervention and control school girls were observed at any time point. At the four-year follow-up of the KISS randomized controlled trial

which included nine-months of augmented (lesson content) and expanded (from 135 to 225 minutes weekly) physical education curriculum at its core, participants at intervention schools had a substantially higher cardiorespiratory fitness (5 % difference) and greater participation in leisure-time sports compared to participants at control schools [44]. Only one risk factor being affected was in contrast to the post-intervention evaluation were several metabolic markers (composite score, HDL-cholesterol, triglyceride, glucose), cardiorespiratory fitness, and body composition were favourably modified by the intervention [36]. Long-term differences between intervention and control schools are particularly interesting in the light of the discontinued interventional support in both of these studies. However, even though these sustainable differences would have public health relevance if further maintained over time, statistical significance of results amounts to only one of eleven [37] and one of fourteen [44] investigated outcomes. Thus, in conjunction with high rates of attrition (439 of initially 694 participants [37] and 293 of initially 502 participants [36] available for long-term follow-up, (these losses apart from post-randomization but pre-baseline drop-out)), a cautious interpretation of cause and effect is warranted. The Cretan Health and Nutrition Education Programme provide further support that sustainable benefits of quality physical activity in school can be achieved. Following six years of a comprehensive school health programme (diet, physical activity, and risk behaviours), in which augmented physical education (two lessons per week) was one component, differences between intervention and control schools in blood cholesterol and blood pressure were maintained four years post-intervention [45, 46]. Selection bias (attrition and non-randomization) appears the major threat to the validity of this long-term follow-up. Noticeably, control schools in the Cretan study did not receive any structured physical education for the first half the intervention period. Conversely, an Australian twenty-week randomized controlled trial including daily twenty minutes fitness sessions did not results in immediate or six-months post-intervention effects on blood pressure or cholesterol levels in high- or low risk children [47].

Public health relevance

Effect sizes presented in this manuscript can be interpreted as standardized effect-sizes (Cohen's d) which for all outcomes, following Cohen's suggestions, are interpreted as small (and statistically insignificant). A key question is how large a difference in a population-level risk factor is needed to achieve a meaningful public health impact, and by extension, what is the necessary size of effects from school-based initiatives for this to be considered a viable adjunct to a multisectoral population-wide policy change? In a model-based study, even a one mmHg population-wide systolic blood pressure reduction in adults may have a profound effect on the total number of cardiovascular disease cases, surpassing that of reducing the prevalence of uncontrolled hypertension by 10% [48]. As hypertension control programmes operates at sizeable costs [49] and high blood pressure is a leading cause of disability-adjusted life-years [50], even fairly small reductions in blood pressure from school-based approaches are likely public health relevant. When considering the totality of evidence from school-based obesity prevention programs in the general population (including diet, physical activity and combined interventions), two recent meta-analyses reported comparable short term systolic blood pressure reductions of 1.64 (95% CI: 2.56 to 0.71) [19] and 1.95 (2.85 to 1.05)[51] mmHg. Our long-term follow-up did not support such effect-sizes as the systolic blood pressure at intervention schools was 0.22 mmHg lower (statistically insignificant) as compared to control schools.

Do physical activity interventions increase overall physical activity levels in young people?

Even though the CHAMPS study-DK was associated with favourable metabolic profiles and higher in-school physical activity level at intervention schools, no difference in total physical activity

between intervention and control schools was evident [16]. This was also observed in the cross-sectional analysis of physical activity levels in 2015 which unfortunately lacked baseline data to provide a longitudinal interpretation. As the additional physical education lessons were discontinued after the 6th grade it is possible that the lack of long-term differences between intervention and control schools may owe to the intervention not having resulted in the necessary motivation and/or skills to compensate for the removal of physical education lessons (e.g. by intervention schools students increasing out-of-school physical activity). The creation of lasting habits is likely necessary for school-based initiatives to result in meaningful health benefits. In this light, strictly school-based interventions build solely on increased provision of structured physical activity but without an instrument for translation of behaviour into discretionary time may, although efficacious in increasing physical activity when the intervention is delivered [16, 17, 52], not provide the necessary impetus to increase physical activity external to the intervention. Data to facilitate short-term intervention efficacy is increasing [53] but without evaluation of which intervention components associates with long-term behavior modification, information to guide public health policy will remain limited. In the short term, particularly promising effects of school-based interventions including a family component have previously been highlighted, which could e.g. relate to a supportive environment [42, 54, 55] for adapting leisure-time physical activities. With regards to physical activity levels evidence from twelve randomized controlled trials in youth above ten years of age in fact suggests objectively measured overall physical activity levels are not increased by current school-based interventional approaches, although some heterogeneity exists [56]. A review also including subjective physical activity assessments came to the reverse conclusion, but given the possibility of recall and social-desirability bias in self-reported data and the generally unconvincing quality of included studies, these results should be interpreted with caution [20]. In a comprehensive meta-analysis pooling thirty studies including an objective

physical activity assessment (accelerometry only) increases in physical activity from interventions were in the order of four minutes of MVPA/day [57]. The results of two recent meta-analyses of interventions including a "long-term" follow-up, defined as minimum six months and four weeks post-intervention, both conclude there is a lack of evidence to suggest sustainable effects on physical activity levels [58, 59]. The apparent lack of even short-term effects on physical activity is in contrast to interventional effects on the biological risk factors and may relate to measurement error and larger attrition rates for physical activity outcomes. Alternatively, changes in dietary behaviours as a consequence of the intervention (intended or not), may explain the discrepancy.

Limitations

The study should be interpreted in the light of several limitations. The non-randomized design precludes a causal interpretation, and although matching of schools was performed, the possibility of selection bias remains. Analyses were adjusted for potential demographic and biological confounders but information on e.g. nutritional behaviours was not available. Thereby potential confounding from dietary habits or other unmeasured and unbalanced variables cannot be eliminated. Selection bias may also be introduced as missing data was frequent, particularly for the oldest participants. Comprehensive analyses comparing included and non-included samples at baseline and over time revealed favourable anthropometric and cardiorespiratory fitness profiles in the included sample. This could have reduced the potential for beneficial intervention-related adaptations to occur as, in the 2-year evaluation of the study, particularly strong effects were observed in the half presenting the least favourable metabolic profile [21]. Importantly, no evidence of differential missingness characteristics in these variables across intervention and control participants was observed. Because the intervention was embedded in the school curriculum it was

not possible to collect true pre-intervention data. Instead this was obtained up to two months after intervention initiation. Accordingly, differences in baseline clinical characteristics, and particularly insulin sensitivity, between intervention and control participants could be the result of early adaptations to the increased physical education lessons. If this is the case, controlling for baseline values could be considered conservative [60]. When analyses of follow-up HOMA-IR and composite-score were repeated without including the baseline values, coefficients were roughly doubled (in favour of intervention), but remained statistically insignificant (data not shown). Although the available sample size was similar to previous studies with a long-term follow-up, it must be considered low with only 95 participants in the control group available. Hence, the possibility of a type II error cannot be ruled out. Sample size consideration was also the reason no subgroup analysis according to years of intervention exposure was pursued. Multiple imputation was used to retain sample-size, but the number of individuals with missing data on blood samples at follow-up was too large to meaningfully impute this information. From the school-year starting 2014 a nationwide school-reform mandating 60 minutes of physical activity on each school day was implemented. This could reduce heterogeneity in school children's exposure to physical activity and mask effects of an earlier intervention by affecting activity patterns at both control and intervention schools. However, when performing research within the educational system, and particularly when including a follow-up period spanning more than six years, changes to school-policies or the school environment (e.g. ban of purchasing sugar-sweetened beverages at schools, new safer roads for active commuting, new playground equipment) mandated at the school or national level is likely the norm. Finally, because the municipality of Svendborg considered the intervention to be highly successful, in 2012 the additional physical education was implemented at all schools in the municipality (including control schools). However, because the additional physical education was

provided to children starting in kindergarten from the school year 2012, children serving as controls in this study would not be directly affected.

CONCLUSIONS

Despite 2-year intervention efficacy, a trebling of curricular physical activity from kindergarten to 6th grade did not result in statistically significant reductions in clustered or single biological risk factors between intervention and control schools, when evaluated after 6.5 years of follow-up. Future research featuring school-based physical activity interventions should 1) pre-plan for long-term follow-up, 2) actively seek to minimize attrition over time, and 3) incorporate instruments for behaviour translation to leisure time into their physical activity intervention models to increase the probability of long-term effects on population health.

Additional files

Additional file 1 (.pdf). Description of methodology used for physical activity assessment, data-reduction, and imputation of missing variables in cross-sectional analysis of follow-up physical activity levels.

Additional file 2 (.pdf). Information on multiple imputation procedure, missing data by school-year (table S1), and comparison between participants available for long-term follow-up and those unavailable for follow-up (table S2 and S3).

Additional file 3 (.pdf). TREND checklist in table S4.

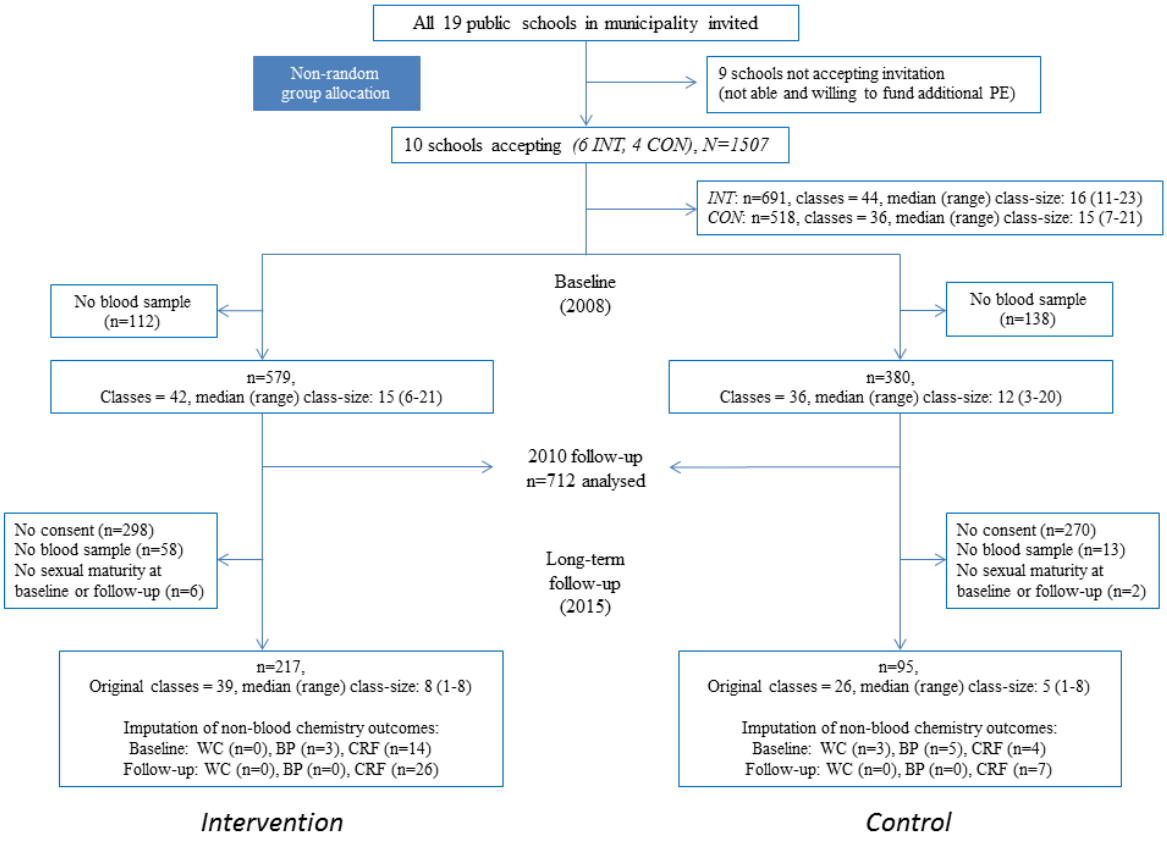
Additional file 4 (.pdf). TIDier checklist in table S5.

Table 1. Years of physical education exposure in the CHAMPS-study DK cohort by school-year at baseline and intervention status (2008 – 2015)

School-year at baseline	2008/09	2009/10	2010/11	2011/12	2012/13	2013/14	2014/15	School-year at follow-up
Intervention schools								
Kindergarten (5-6 years old)	Six weekly PE lessons							6th grade (12-13 years old)
1st grade	Six weekly PE lessons							7th grade
2nd grade	Six weekly PE lessons					Two weekly PE lessons		8th grade
3rd grade	Six weekly PE lessons				Two weekly PE lessons			9th grade
4th grade (10-11 years old)	Six weekly PE lessons			Two weekly PE lessons				10th grade (16 – 17 years old)
Control schools								
All	Two weekly PE lessons							6th to 10th grade

Grey boxes indicate six weekly lessons of physical education. Dark boxes indicate two weekly lessons. PE: Physical education.

Figure 1. Participant flow-chart



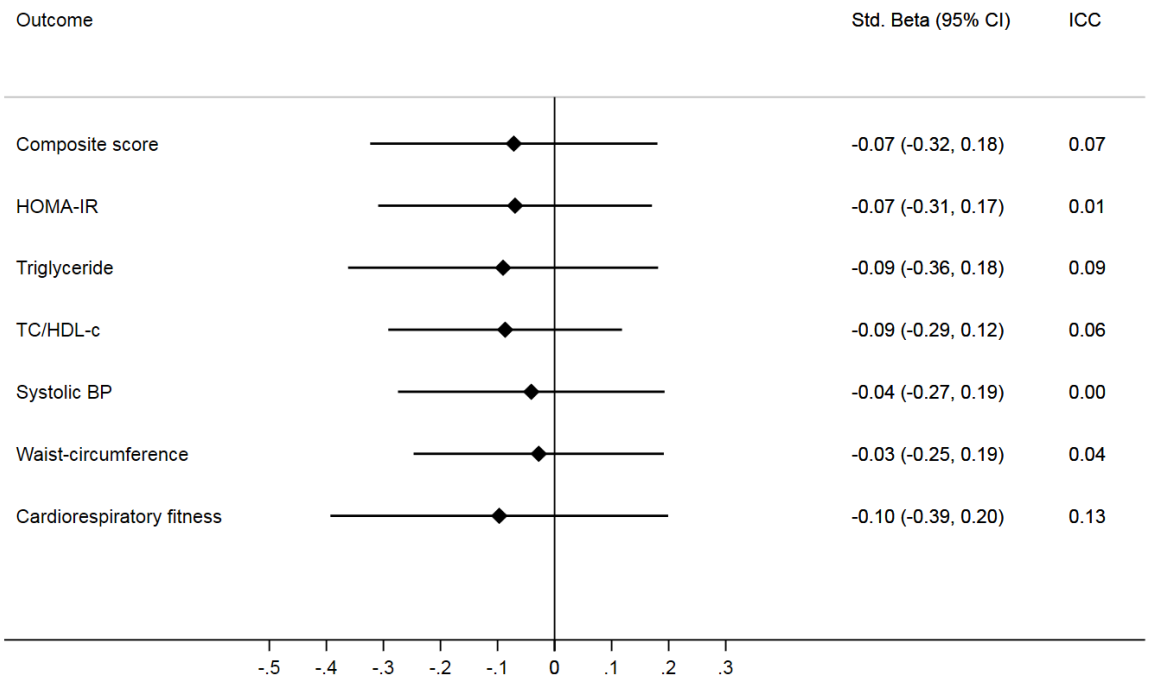
Consenting participants at baseline are lower (1209) than reported in an earlier publication (1218) [21]. INT; intervention, CON; control.

Table 2. Baseline characteristics of participants

	n	Intervention	n	Control		p-value for between school-type difference
Age (years)	217	7.8 (1.3)	95	7.8 (1.3)		0.98
Sex (% girls)	217	56	95	44		0.06
Stature (cm)	216	129.2 (8.8)	92	128.8 (9.7)		0.71
Body weight (kg)[a]	217	26.8 (5.4)	92	26.9 (5.9)		0.94
Sexual maturity (% tanner stage 1)	217	75	95	79		0.38
Mothers educational attainment (% any tertiary)	210	65	92	58		0.24
Family history of NCDs (% yes)	211	50	92	53		0.58
Birthweight (gram)	298	3495 (711)	91	3481 (550)		0.87
Composite score (z-sores)	200	-0.07 (0.97)	89	0.17 (1.05)		0.06
Cardiorespiratory fitness (meters)	203	887 (101)	91	885 (107)		0.84
Systolic blood pressure (mmHg)	214	100.1 (6.5)	90	98.3 (8.2)		0.04
Waist-circumference (cm)[a]	217	55.0 (52.5 - 58)	92	56.5 (53 - 61.3)		0.08
HOMA-IR[a]	217	0.54 (0.37 - 0.75)	95	0.65 (0.47 - 0.89)		0.004
Triglyceride (mmol/l)[a]	217	0.58 (0.46 – 0.71)	95	0.56 (0.47 – 0.71)		0.79
TC:HDL-c-ratio[a]	217	2.6 (2.3 - 3.0)	95	2.6 (2.4 - 3.0)		0.51

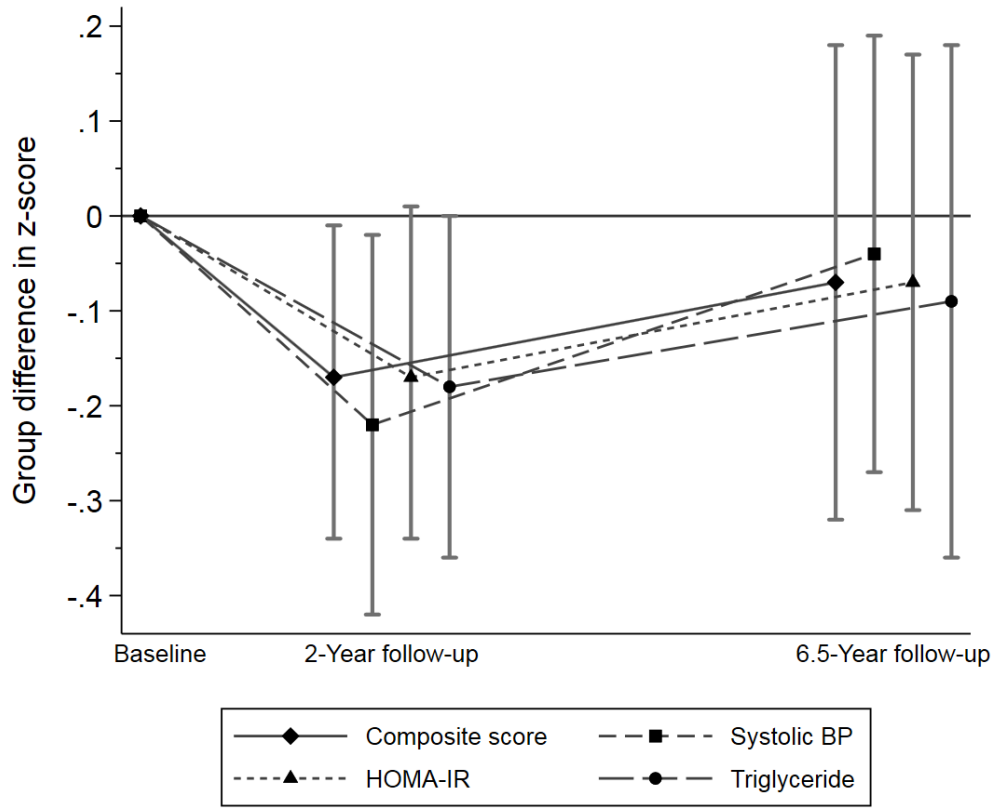
Mean (standard deviation) is given unless otherwise noted. [a]median (25th-75th centile). NCD= non-communicable diseases. HOMA-IR= homeostasis model assessment of insulin resistance. TC= total cholesterol. HDL-c= High-density lipoprotein cholesterol.

Figure 2. Long-term difference in risk factors between intervention/control schools



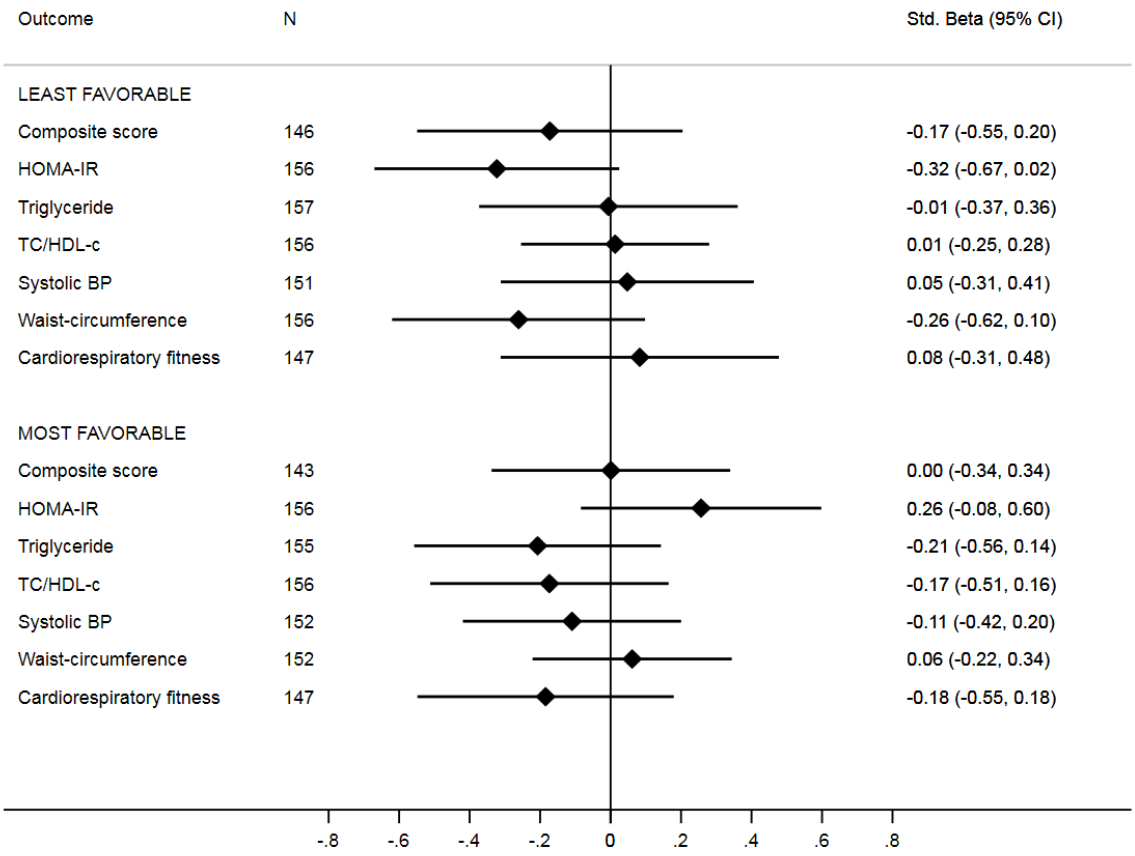
Estimates are standardized mean differences at follow-up (in z-scores with 95% CI), using control schools as the reference. Negative values are in favour of intervention schools except for cardiorespiratory fitness where a positive value favours intervention. ICC= intra-class correlation coefficient, BP = blood pressure; TC = total cholesterol, HDL-c = high-density-lipoprotein cholesterol

Figure 3. Illustration of difference in biological risk factor development between intervention/control schools in the CHAMPS study-DK (2008, 2010, and 2015).



Estimates are mean difference between intervention and control school with a 95 % CI. Negative values favour intervention group. Two-year follow-up estimates are from an earlier publication with 712 participants analysed [21]. 6.5 year follow-up are estimates from the 312 participants in this manuscript. Confidence intervals not overlapping zero are statistically significant. Baseline group-differences set to zero as results are presented as adjusted difference in change including baseline-value of outcome, which provides the interpretation that group-mean values are identical at baseline.

Figure 4. Long-term difference in cardiometabolic risk factors between intervention/control, stratified at the median of baseline values



Estimates are standardized mean differences (in z-scores), at follow-up, using control schools as the reference, with 95% CI. Differences are for groups stratified by the respective outcome at baseline (above or below the intervention/control-specific median value). Negative values are in favour of intervention except for cardiorespiratory fitness where a positive value favours intervention. The number of observations differs across outcomes due to missing data at baseline. ICC= intra-class correlation coefficient, BP = blood pressure; TC = total cholesterol, HDL-c = high-density-lipoprotein cholesterol

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Additional File 1. Physical activity assessment

Physical activity was assessed by a questionnaire and accelerometry in 2015. Participants were included in analysis of physical activity if they; had provided informed consent during the initial phase of the study (2008-2010), at the follow-up in 2015, provided either subjective or objective physical activity data, and had anthropometrical or physical performance data to inform imputation models. This left 495 participants for analysis. Physical activity data was only analysed cross-sectionally as no appropriate baseline data was available (see [1] for details of physical activity assessment in the CHAMPS-study DK). Questionnaires were completed by participants at schools under the supervision of research staff. Students were asked to indicate their participation in structured leisure-time physical activity. Answers were dichotomized as yes/no. Hip-mounted accelerometers (Actigraph GT3X and GT3X+, Pensacola, FL, USA) were distributed allowing for a minimum of seven consecutive days of measurement. Accelerations were recorded in 30 Hz (GT3X+) or 2 seconds (GT3X – due to memory limitations) epoch, but data was downloaded using a 10-second epoch. Accelerometers were set to start recording at 06:00 on the day after participants received the device, with participants instructed to remove the device only when performing aquatic activities, showering and at night. Because missing data was expected, we issued a screening protocol to maximize data availability. Participants not providing at least four days with ten hours of wear-time including at least one weekend day were asked to re-wear the monitor (no differences in wear-time, moderate physical activity or vigorous physical activity (p-values >0.10) were observed between participants providing sufficient data at the first distribution of accelerometers and those providing sufficient data at the re-wear period). The measurement period lasted from February to May 2015. The percentage of participants meeting the screening criterion increased from 57 to 75 % of eligible participants after including re-wear. Accelerometer data was analyzed using open-source software (Propero, University of Southern Denmark, Odense, Denmark).

Consecutive strings of zero counts of ≥60 min were considered "monitor not worn" and discarded from summation of physical activity and wear-time. Accept criteria for analysis were set at three days (not requiring a weekend-day) of at least 8 h of worn time collected from 06:00 to 24:00. Data from the first distribution period and the potential re-wear period was included if available. Days with vigorous physical activity > 3 standard deviations of the mean were discarded to remove influence from potential accelerometry malfunctions (e.g. vigorous physical activity for >15 % of the day). Physical activity was expressed as mean counts/min and %MVPA/day, with MVPA defined as counts/min ≥2296 counts/min [2] but rescaled to match the 10 seconds epoch. Physical activity levels in 2015, comparing intervention with control schools, were analysed using linear and logistic mixed regression models for %MVPA/day and sport-participation, respectively. Models were controlled for age, sex, sexual maturity (Tanner stages), educational attainment of the mother or female guardian, family history of NCD's, and including a random intercept for school-class membership in 2015. Accelerometry data was further controlled for number of included days, and number of included weekend-days.

Multiple imputation by chained equations (MICE) was used to impute missing data (n=29 for %MVPA/day and n=5 for leisure time sports or physical activity) using 2015 information on stature, body weight, waist-circumference, cardiorespiratory fitness, systolic blood pressure, sexual maturity, blood-sample available (yes/no), number of valid total and weekend accelerometry days, school membership and accelerometry/questionnaire data. Birthweight, educational attainment of the mother or female guardian, and family history of NCD's from the 2015 parental questionnaire was used and updated with the 2008 questionnaire if missing. Coefficients end their standard errors were based on twenty imputed datasets. Imputation models were visually checked for convergence and the reproducibility of the estimates where inspected by Monte Carlo errors. The imputations are

based on the assumption of data being missing at random conditional on the observed variables (MAR).

In non-imputed data (n=466), the median (25-75th percentile) included days and weekend-days were 7 (6 – 8) and 2 (1 – 2) at intervention and 7 (6 – 7) and 2 (1 – 2) at control schools, respectively with no statistically significant difference between schools types (p-values ≥0.36). Median (25-75th percentile) wear-time was 13.7 (13.0 – 14.3) and 13.7 (13.0 – 14.4) hours at intervention and control schools, respectively with no statistically significant difference between school types (p = 0.97).

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Additional file 2. Missing data

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Multiple imputation using chained equations

Missing values of variables other than blood chemistry and sexual maturity were imputed by chained equations (MICE). Beta coefficients and standard errors were obtained based on twenty imputed datasets. Imputation models were visually checked for convergence and the reproducibility of the estimates where inspected by Monte Carlo errors. The imputations are based on the assumption of data being missing at random conditional on the observed variables (MAR). Imputations were based on all variables included in analytical models except an indicator for school was used instead of class and the addition of stature, body weight and waist-circumference in 2010 as data was near complete for these variables. The number of imputed observations ranged from three (waist-circumference in 2008) to 33 (cardiorespiratory fitness in 2015). Data on sexual maturity could not be imputed due to non-convergence of models, hence participants not having this information were considered lost to follow-up (n=8).

Table S1. School-year specific and total loss-to-follow-up stratified by intervention and control schools

	School-year at follow-up					
	Year 6	Year 7	Year 8	Year 9	Year 10	Total
Intervention schools (n)	103	124	122	120	110	579
Analysed (% of n)	54	47	45	32	9	37
Non-analysed (% of n)	46	53	55	68	91	63
Control schools (n)	47	76	79	87	91	380
Analysed (% of n)	60	28	32	17	7	25
Non-analysed (% of n)	40	72	68	83	93	75

Numbers (n) refers to the 959 participants with a fasting blood sample at baseline.
Percentages are calculated within intervention/control columns.

Comparison of participants available for long-term follow-up with participants unavailable for analysis in the CHAMPS-Study DK

Table S2 and S3 presents comparisons of the sample included in the manuscript (analysed sample) with participants from the CHAMPS-study DK not available for long-term evaluation of biological risk factors. The non-analysed group includes participants with (n= 647) and without (n= 250) data on fasting blood samples at baseline. Comparisons are made using data from 2008, 2010, 2012, 2013, and 2015 CHAMPS- Study DK data collections [1, 2]. Comparison of characteristics between the analysed sample and those with missing data was conducted by mixed effects linear or logistic regression, as applicable, including participant age and a random intercept for school-class. In secondary analyses, evidence of non-identical characteristics between non-analysed participants at intervention and control schools is investigated by adding an evaluating an intervention arm (coded as 0 or 1)-by-availability of follow-up data (coded as 0 or 1) interaction term. Estimates are presented as

unstandardized betas (1=analysed sample) for continuous variables or prevalence for categorical variables unless indicated otherwise. Models are adjusted for age and a random intercept for school-class. Family history of cardiovascular disease, hypertension, or diabetes, birthweight, mother or female guardian's educational attainment, and body mass index of the mother are included from the baseline questionnaire only.

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Table S2. Adjusted differences between analysed and non-analysed participants in the CHAMPS-Study DK, by year of data availability.

	N (non-analysed)	N (analysed)	Mean difference (95% CI) OR crude prevalence (1=analysed OR first number= not in sample)	p-value[a]
Gender (% girls)	897	312	53 vs 52	0.99
Age (years)	897	312	-0.75 (-0.57 to -0.93)	<0.001
From 2008 questionnaire				
Mothers BMI (kg/m^2)	712	279	-0.25 (-0.82 to 0.33)	0.40
Family history of CVD, diabetes, hypertension (yes - %)	747	289	38 vs 38	0.99
Birthweight (grams)	605	239	-81 (-183 to 20)	0.12
Educational attainment of mother (% any tertiary)	729	285	43 vs. 48	0.15
2008				
Stature (cm)	865	308	-0.54 (-1.30 to 0.20)	0.16
Body weight (kg)	863	309	-0.99 (-1.69 to -0.29)	0.005
BMI (kg/m^2)	863	308	-0.40 (-0.68 to -0.12)	0.004
Sexual maturity[b]	835	312	0.94 (0.62 to 1.41)	0.76
Waist-circumference (cm)	862	309	-1.30 (-2.11 to -0.49)	0.002
Cardiorespiratory fitness (meters)	827	294	18 (5 to 32)	0.007
Systolic blood pressure (mmHg)	835	304	-0.79 (-1.74 to 0.16)	0.10
Composite score[c]	647	312	-0.05 (-0.19 to 0.09)	0.47
2010				
Stature (cm)	800	309	-0.52 (-1.38 to 0.34)	0.24
Body weight (kg)	799	309	-1.10 (-2.03 to -0.17)	0.02
BMI (kg/m^2)	799	309	-0.38 (-0.69 to 0.06)	0.02
Sexual maturity[b]	781	305	0.91 (0.66 to 1.27)	0.59

Waist-circumference (cm)	800	309	-0.96 (-1.94 to 0.01)	0.053
Cardiorespiratory fitness (meters)[d]	698	271	27 (13 to 41)	<0.001
Systolic blood pressure (mmHg)	800	309	-0.14 (-1.16 to 0.88)	0.79
Composite score[c]	607	269	-0.07 (-0.22 to 0.08)	0.36
2012				
Stature (cm)	579	287	-0.45 (-1.42 to 0.53)	0.37
Body weight (kg)	575	286	-1.65 (-2.77 to -0.53)	0.004
BMI (kg/m^2)	575	286	-0.55 (-0.90 to 0.20)	0.002
Sexual maturity[e]	574	284	0.76 (0.57 to 1.02)	0.07
Waist-circumference (cm)	576	286	1.59 (-2.68 to -0.50)	0.004
Cardiorespiratory fitness (meters)	484	246	22 (6 to 38)	0.008
2013				
Stature (cm)	479	272	0.06 (-1.05 to 1.17)	0.92
Body weight (kg)	475	272	-1.16 (-2.44 to 0.12)	0.08
BMI (kg/m^2)	475	272	-0.43 (-0.80 to -0.06)	0.02
Sexual maturity[e]	477	272	0.95 (0.68 to 1.33)	0.76
Waist-circumference (cm)	472	267	-1.17 (-2.27 to -0.07)	0.04
Cardiorespiratory fitness (meters)	396	236	18 (1 to 36)	0.04
2015				
Stature (cm)	312	150	0.42 (-1.14 to 1.99)	0.60
Body weight (kg)	311	150	-1.12 (-3.04 to 0.78)	0.25
BMI (kg/m^2)	311	150	-0.44 (-0.96 to 0.08)	0.10
Sexual maturity[e]	312	149	1.02 (0.64 to 1.61)	0.94
Waist-circumference (cm)	312	150	-1.18 (-2.71 to 0.34)	0.13
Cardiorespiratory fitness (meters)	279	119	20 (-4 to 44)	0.10
Systolic blood pressure (mmHg)	308	148	-0.22 (-1.85 to 1.42)	0.79

Composite score[c]	312	97	-0.29 (-0.52 to -0.06)	0.01

OR: odds ratio, BMI: body mass index; CVD; cardiovascular disease,

Estimates are unstandardized (unless noted otherwise) betas (0=not in sample) adjusted for age and a random intercept for school-class OR absolute prevalence (first number= not in sample)

[a]P is for between-sample difference

[b]Mixed effects logistic regression – odds ratio of being >stage 1

[c]Composite scores is standardized and includes standardized values of lnHOMA-IR, lnTriglyceride and lnTC:HDLc ratio

[d]using data from spring 2011 (6 months later) as cardiorespiratory fitness was not assessed fall 2010

[e]Mixed effects ordinal logistic regression – odds ratio of being in a higher tanner stage

Table S3. Investigation of non-identical missingness characteristics at intervention and control schools by year of data availability.

	Control		Intervention		p-value[a]
	non-analysed	analysed	non-analysed	analysed	
Sex (% girls)[b]	48	56	46	44	0.18
Age in 2008 (years)	8.6 (1.5)	7.8 (1.3)	8.6 (1.4)	7.8 (1.3)	0.91
From 2008 questionnaire					
Mothers BMI (kg/m^2)	24.0 (3.9)	24.0 (3.7)	24.4 (4.1)	24.0 (4.0)	0.66
Family history of CVD, Diabetes, hypertension (% yes)[b]	38	47	39	35	0.07
Birthweight (grams)	3582 (623)	3481 (557)	3506 (675)	3468 (688)	0.55
Educational attainment of mother (% any tertiary)[b]	48	43	38	50	0.03
2008					
Stature (cm)	133.6 (10.1)	128.8 (9.7)	134.0 (9.7)	129.2 (8.8)	0.95
Body weight (kg)	30.1 (7.3)	26.9 (5.9)	30.2 (7.0)	26.8 (5.4)	0.92
BMI (kg/m^2)	16.7 (2.2)	16.0 (1.7)	16.6 (2.3)	15.9 (1.7)	0.71
Sexual maturity (% > tanner stage 1)[c]	31	25	36	21	0.09
Waist-circumference (cm)	59.4 (7.0)	57.2 (5.5)	59.1 (7.0)	56.1 (5.6)	0.78
Cardiorespiratory fitness (meters)	890 (112)	885 (107)	895 (107)	887 (101)	0.79
Systolic blood pressure (mmHg)	101.5 (8.1)	98.3 (8.2)	102.0 (7.9)	100.1 (6.5)	0.79
Composite score[d]	0.03 (0.99)	0.14 (0.94)	-0.01 (1.02)	-0.12 (0.97)	0.37
2010					
Stature (cm)	145.4 (10.5)	140.6 (10.2)	146.4 (10.5)	141.2 (9.1)	0.65
Body weight (kg)	37.6 (9.0)	33.9 (8.0)	38.1 (9.2)	33.8 (6.9)	0.57
BMI (kg/m^2)	17.6 (2.4)	16.9 (2.2)	17.5 (2.6)	16.8 (2.0)	0.89
Sexual maturity (% > tanner stage 1)[c]	74	67	76	62	0.25
Waist-circumference (cm)	64.1 (8.4)	61.5 (7.4)	64.4 (8.2)	61.3 (6.9)	0.62

Cardiorespiratory fitness (meters)[e]	978 (103)	976 (84)	980 (113)	977 (99)	0.79
Systolic blood pressure (mmHg)	102.8 (7.8)	102.3 (7.6)	101.6 (8.2)	100.3 (7.4)	0.34
Composite score[d]	0.07 (1.03)	0.13 (1.13)	-0.03 (0.96)	-0.10 (0.95)	0.52
2012					
Stature (cm)	152.3 (10.6)	149.4 (11.3)	154.6 (10.8)	149.8 (9.7)	0.72
Body weight (kg)	43.1 (10.5)	39.4 (8.9)	44.4 (10.5)	39.7 (8.4)	0.94
BMI (kg/m^2)	18.4 (2.7)	17.5 (2.3)	18.3 (2.7)	17.5 (2.1)	0.62
Sexual maturity (% stage 1, 2, 3, 4+5)[f]	19 / 36 / 31 / 14	21 / 41 / 29 / 9	16 / 29 / 33 / 22	28 / 40 / 25 / 7	0.01
Waist-circumference (cm)	68.5 (8.6)	65.7 (6.8)	67.2 (8.1)	64.2 (7.4)	0.68
Cardiorespiratory fitness (meters)	1010 (118)	1022 (127)	1022 (106)	1021 (97)	0.97
2013					
Stature (cm)	157.2 (11.0)	155.3 (12.1)	160.8 (10.7)	156.3 (10.1)	0.48
Body weight (kg)	46.3 (10.7)	43.9 (10.9)	50.0 (11.5)	44.7 (9.1)	0.92
BMI (kg/m^2)	18.5 (2.5)	18.0 (2.5)	19.1 (2.9)	18.1 (2.1)	0.81
Sexual maturity (% stage 1+2, 3, 4+5)[f]	40 / 33 / 36	45 / 28 / 27	29 / 28 / 43	46 / 35 / 19	0.22
Waist-circumference (cm)	68.6 (7.8)	66.8 (7.3)	70.5 (8.4)	67.4 (6.7)	0.94
Cardiorespiratory fitness (meters)	1010 (109)	1023 (110)	1034 (123)	1026 (114)	0.98
2015					
Stature (cm)	163.9 (10.1)	166.4 (10.8)	166.7 (9.3)	167.4 (9.3)	0.74
Body weight (kg)	53.0 (12.0)	54.6 (13.2)	56.2 (11.6)	54.9 (9.8)	0.86
BMI (kg/m^2)	19.5 (2.8)	19.5 (3.0)	20.1 (3.2)	19.5 (2.4)	0.68
Sexual maturity (% stage 1-3, 4, 5)[f]	52 / 44 / 4	41 / 43 / 16	40 / 44 /15	39 / 48 / 13	0.81
Waist-circumference (cm)	72.3 (9.0)	72.2 (8.3)	73.0 (9.3)	71.3 (7.1)	0.70
Cardiorespiratory fitness (meters)	1078 (110)	1119 (112)	1075 (113)	1096 (112)	0.75
Systolic blood pressure (mmHg)	106.9 (8.4)	107.4 (8.8)	107.5 (9.7)	107.6 (8.4)	0.63
Composite score[d]	0.25 (0.87)	0.08 (1.17)	0.21 (1.21)	-0.14 (0.86)	0.63

OR: odds ratio, BMI: body mass index; CVD; cardiovascular disease,

Estimates are unstandardized betas adjusted for age and a random intercept for school-class unless indicated otherwise.

[a]p-value is from intervention arm-by-availability of follow-up data interaction term

[b]Logistic regression model

[c]Mixed effects logistic regression model

[d]Composite scores is standardized and includes standardized values of lnHOMA-IR, lnTriglyceride and lnTC:HDLc ratio

[e]using data from spring 2011 (6 months later) as cardiorespiratory fitness was not assessed fall 2010

[f]Mixed effects ordinal logistic regression model

Table S4. TREND Statement Checklist

Paper Section/Topic	Item No.	Descriptor	Reported? ✓	Reported? Pg #
TITLE and ABSTRACT				
Title and Abstract	1	• Information on how units were allocated to interventions		Abstract
		• Structured abstract recommended		Abstract
		• Information on target population or study sample		Abstract
INTRODUCTION				
Background	2	• Scientific background and explanation of rationale		Introduction
		• Theories used in designing behavioral interventions		None applied
METHODS				
Participants	3	• Eligibility criteria for participants, including criteria at different levels in recruitment/sampling plan (e.g., cities, clinics, subjects)		Setting and study design + Statistics
		• Method of recruitment (e.g., referral, self-selection), including the sampling method if a systematic sampling plan was implemented		Setting and study design
		• Recruitment setting		Setting and study design
		• Settings and locations where the data were collected		Outcomes
Interventions	4	• Details of the interventions intended for each study condition and how and when they were actually administered, specifically including:		
		○ Content: what was given?		Setting and study design + Table 1
		○ Delivery method: how was the content given?		Setting and study design + Intervention content
		○ Unit of delivery: how were subjects grouped during delivery?		Setting and study design
		○ Deliverer: who delivered the intervention?		Setting and study design + Intervention content
		○ Setting: where was the intervention delivered?		Setting and study design + Intervention content
		○ Exposure quantity and duration: how many sessions or episodes or events were intended to be delivered? How long were they intended to last?		Setting and study design + Table 1
		○ Time span: how long was it intended to take to deliver the intervention to each unit?		Setting and study design + Table 1
		○ Activities to increase compliance or adherence (e.g., incentives)		
Objectives	5	• Specific objectives and hypotheses		Introduction
Outcomes	6	• Clearly defined primary and secondary outcome measures		Data reduction
		• Methods used to collect data and any methods used to enhance the quality of measurements		Methods
		• Information on validated instruments such as psychometric and biometric properties		Methods

Table S4. TREND Statement Checklist

Sample size	7	• How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules		Setting and study design + Statistics
Assignment method	8	• Unit of assignment (the unit being assigned to study condition, e.g., individual, group, community)		Setting and study design
		• Method used to assign units to study conditions, including details of any restriction (e.g., blocking, stratification, minimization)		Setting and study design
		• Inclusion of aspects employed to help minimize potential bias induced due to non-randomization (e.g., matching)		Setting and study design
Blinding (masking)	9	• Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to study condition assignment; if so, statement regarding how the blinding was accomplished and how it was assessed		Setting and study design + Outcomes
Unit of Analysis	10	• Description of the smallest unit that is being analyzed to assess intervention effects (e.g., individual, group, or community)		Statistics
		• If the unit of analysis differs from the unit of assignment, the analytical method used to account for this (e.g., adjusting the standard error estimates by the design effect or using multilevel analysis)		Statistics
Statistical methods	11	• Statistical methods used to compare study groups for primary methods outcome(s), including complex methods for correlated data		Statistics
		• Statistical methods used for additional analyses, such as subgroup analyses and adjusted analysis		Statistics
		• Methods for imputing missing data, if used		Statistics
		• Statistical software or programs used		Statistics
RESULTS				
Participant flow	12	• Flow of participants through each stage of the study: enrollment, assignment, allocation and intervention exposure, follow-up, analysis (a diagram is strongly recommended)		Figure 1
		○ Enrollment: the numbers of participants screened for eligibility, found to be eligible or not eligible, declined to be enrolled, and enrolled in the study		Figure 1 + Setting and study design + Participants
		○ Assignment: the numbers of participants assigned to a study condition		Figure 1
		○ Allocation and intervention exposure: the number of participants assigned to each study condition and the number of participants who received each intervention		Figure 1
		○ Follow-up: the number of participants who completed the follow-up or did not complete the follow-up (i.e., lost to follow-up), by study condition		Figure 1 + Participants + Statistics
		○ Analysis: the number of participants included in or excluded from the main analysis, by study condition		Statistics + Figure 1
		• Description of protocol deviations from study as planned, along with reasons		
Recruitment	13	• Dates defining the periods of recruitment and follow-up		Outcomes
Baseline data	14	• Baseline demographic and clinical characteristics of participants in each study condition		Table 2
		• Baseline characteristics for each study condition relevant to specific disease prevention research		Table 2
		• Baseline comparisons of those lost to follow-up and those retained, overall and by study condition		Additional file 2
		• Comparison between study population at baseline and target population of interest		Not applied
Baseline equivalence	15	• Data on study group equivalence at baseline and statistical methods used to control for baseline differences		Sample characteristics + Statistics
Numbers analyzed	16	• Number of participants (denominator) included in each analysis for each study condition, particularly when the denominators change for different outcomes; statement of the results in absolute numbers when feasible		Sample characteristics + Figure 4
		• Indication of whether the analysis strategy was “intention to treat” or, if not, description of how non-compliers were treated in the analyses		Statistics
Outcomes and estimation	17	• For each primary and secondary outcome, a summary of results for each estimation study condition, and the estimated effect size and a confidence interval to indicate the precision		Primary and secondary

Table S4. TREND Statement Checklist

				outcomes
		• Inclusion of null and negative findings		Primary and secondary outcomes
		• Inclusion of results from testing pre-specified causal pathways through which the intervention was intended to operate, if any		Primary and secondary outcomes
Ancillary analyses	18	• Summary of other analyses performed, including subgroup or restricted analyses, indicating which are pre-specified or exploratory		Primary and secondary outcomes
Adverse events	19	• Summary of all important adverse events or unintended effects in each study condition (including summary measures, effect size estimates, and confidence intervals)		not reported
DISCUSSION				
Interpretation	20	• Interpretation of the results, taking into account study hypotheses, sources of potential bias, imprecision of measures, multiplicative analyses, and other limitations or weaknesses of the study		Discussion
		• Discussion of results taking into account the mechanism by which the intervention was intended to work (causal pathways) or alternative mechanisms or explanations		Discussion
		• Discussion of the success of and barriers to implementing the intervention, fidelity of implementation		Discussion
		• Discussion of research, programmatic, or policy implications		Discussion
Generalizability	21	• Generalizability (external validity) of the trial findings, taking into account the study population, the characteristics of the intervention, length of follow-up, incentives, compliance rates, specific sites/settings involved in the study, and other contextual issues		Discussion
Overall evidence	22	• General interpretation of the results in the context of current evidence and current theory		Discussion

From: Des Jarlais, D. C., Lyles, C., Crepaz, N., & the Trend Group (2004). Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: The TREND statement. *American Journal of Public Health*, 94, 361-366. For more information, visit: http://www.cdc.gov/trendstatement/

The TIDieR (Template for Intervention Description and Replication) Checklist*:

Information to include when describing an intervention and the location of the information

Table S5. TIDieR Checklist

Item number	Item	Where located **	
		Primary paper (page or appendix number)	Other † (details)
	BRIEF NAME		
1.	Provide the name or a phrase that describes the intervention.	Setting and study design + Intervention content	
	WHY		
2.	Describe any rationale, theory, or goal of the elements essential to the intervention.	Introduction	
	WHAT		
3.	Materials: Describe any physical or informational materials used in the intervention, including those provided to participants or used in intervention delivery or in training of intervention providers. Provide information on where the materials can be accessed (e.g. online appendix, URL).	Intervention content (with URL)	
4.	Procedures: Describe each of the procedures, activities, and/or processes used in the intervention, including any enabling or support activities.	Setting and study design + Intervention content	Study protocol: Wedderkopp et al., 2012 – *BMC Pediatrics* (doi: 10.1186/1471-2431-12-128)

	WHO PROVIDED		
5.	For each category of intervention provider (e.g. psychologist, nursing assistant), describe their expertise, background and any specific training given.	Intervention content	
	HOW		
6.	Describe the modes of delivery (e.g. face-to-face or by some other mechanism, such as internet or telephone) of the intervention and whether it was provided individually or in a group.	Setting and study design + Intervention content	
	WHERE		
7.	Describe the type(s) of location(s) where the intervention occurred, including any necessary infrastructure or relevant features.	Setting and study design	
	WHEN and HOW MUCH		
8.	Describe the number of times the intervention was delivered and over what period of time including the number of sessions, their schedule, and their duration, intensity or dose.	Setting and study design + Intervention content (with URL)	
	TAILORING		
9.	If the intervention was planned to be personalised, titrated or adapted, then describe what, why, when, and how.		Qualitative analysis: Nielsen et al., *in preparation*: "Factors influencing the implementation of a natural
	MODIFICATIONS		
10.‡	If the intervention was modified during the course of the study, describe the changes (what, why, when, and how).		
	HOW WELL		
11.	Planned: If intervention adherence or fidelity was assessed, describe how and by whom, and if any strategies were used to maintain or improve fidelity, describe them.		

12.‡	Actual: If intervention adherence or fidelity was assessed, describe the extent to which the intervention was delivered as planned.	experiment to triple the time spent on physical education at primary school: a qualitative study"

** **Authors** - use N/A if an item is not applicable for the intervention being described. **Reviewers** – use '?' if information about the element is not reported/not sufficiently reported.

† If the information is not provided in the primary paper, give details of where this information is available. This may include locations such as a published protocol or other published papers (provide citation details) or a website (provide the URL).

‡ If completing the TIDieR checklist for a protocol, these items are not relevant to the protocol and cannot be described until the study is complete.

* We strongly recommend using this checklist in conjunction with the TIDieR guide (see *BMJ* 2014;348:g1687) which contains an explanation and elaboration for each item.

* The focus of TIDieR is on reporting details of the intervention elements (and where relevant, comparison elements) of a study. Other elements and methodological features of studies are covered by other reporting statements and checklists and have not been duplicated as part of the TIDieR checklist. When a **randomised trial** is being reported, the TIDieR checklist should be used in conjunction with the CONSORT statement (see www.consort-statement.org) as an extension of **Item 5 of the CONSORT 2010 Statement.** When a **clinical trial protocol** is being reported, the TIDieR checklist should be used in conjunction with the SPIRIT statement as an extension of **Item 11 of the SPIRIT 2013 Statement** (see www.spirit-statement.org). For alternate study designs, TIDieR can be used in conjunction with the appropriate checklist for that study design (see www.equator-network.org).