Body Composition and Cardiovascular Health in School-aged Children.

The Childhood Health, Activity and Motor Performance School Study Denmark

An evaluation on the health effect of sport schools in the Svendborg Project

Heidi Klakk

Exercise Epidemiology

Institute of Sport Science and Clinical Biomechanics

Centre of Research in Childhood Health

and

University College Lillebælt

SYDDANSK UNIVERSITET
UNIVERSITY OF SOUTHERN DENMARK

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Content

Preface

This thesis was performed at the Institute of Sport Science and Clinical Biomechanics, Faculty of Health Sciences, University of Southern Denmark, Odense. Clinical Professor, MD, Niels Wedderkopp and Professor Dr. Med. Lars Bo Andersen provided supervision.

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Evaluation Committee

Clinical Associate Professor Henrik Steen Hansen, Cardiology, Department of Clinical Research, University of Southern Denmark (chairman)

Professor Sigmund Alfred Anderssen, Seksjon for idrettsmedisinske fag, The Norwegian School of Sport Sciences, Oslo

Professor Bente Klarlund, Department of Clinical Medicine, Section of Orthopaedics and Internal Medicine, University of Copenhagen

List of Papers

I. Wedderkopp N, Jespersen E, Franz C, Klakk H, Heidimann M, Christiansen C, Moller NC, Leboeuf-Yde C: **Study protocol. The Childhood Health, Activity, and Motor Performance School Study Denmark (The CHAMPS-study DK)**. *BMC Pediatr* 2012, **12**(1):128.

II. Klakk H, Chinapaw M, Heidemann MS, Andersen LB, Wedderkopp N: **Effect of Extra Physical Education Lessons on Body Composition in School Children aged 8-13 years.** *BMC Pediatr* 2013, **13**:170.

III. H Klakk, LB Andersen, MS Heidemann, NC Moller, N Wedderkopp: **Six Physical Education Lessons a Week – the effect on cardiovascular risk factors in School Children aged 6-13 years: The CHAMPS study-DK.** *Scand J of Public Health ,* 2013;0:1-9.

IV. H Klakk, A Grøntved, LB Andersen, MS Heidemann, NC Moller, N Wedderkopp: **Prospective association of adiposity and cardiorespiratory fitness with cardiovascular risk factors in healthy children.** Accepted for publication in *"Scandinavian Journal of Medicine & Science in Sports" - epub ahead of print* November 2013.

Thesis at a glance

	Study design	Sample and exposure	Methods	Aim	Conclusion
Paper I	Study protocol, Controlled prospective cohort study The CHAMPS-study.dk	Children attending 2nd to 4th grade in 10 public schools, 6 sports schools (intervention) and 4 normal (control) schools, Four extra physical education (PE) lessons/week at sports schools.	Questionnaire Anthropometrics, blood pressure, blood samples, CRF, motor performance and Accelerometers	To describe The CHAMPS study-DK that aims to evaluate the effect of implementing 4 extra Physical Education (PE) achieved through a number of studies.	The main areas of interest were 1. life-style diseases 2. Bone health 3. Musculoskeletal problems, and 4. Motor performance.
Paper II	Experimental prospective cohort study	739 children attending 2nd to 4th 2008, follow up 2010 Four extra PE lessons at sports schools.	Primary outcome: Body Mass Index and Total Body Fat (TBF) percentage derived from DXA.	To evaluate the effect of four extra PE lessons/week in primary schools on body composition and weight status	Four extra PE lessons at school significantly improved prevalence of OW/OB. A larger effect of intervention was observed in children who were OW/OB or adipose at baseline.
Paper III	Experimental prospective cohort study	1218 children attending pre-school to 4^{th} 2008, follow-up 2010 Four extra PE lessons at sports schools.	Blood pressure blood samples Anthropometry, Cardiorespiratory fitness (CRF)	To evaluate the effect of six PE lessons on children's CVD risk defined by a composite risk score	Four extra PE lessons at school can reduce children's cardio vascular risk measured as a composite risk score.
Paper IV	Observational prospective cohort study	739 children attending 2nd to 4th grade schools 2008 – 2010. Exposure was adiposity and CRF	Blood pressure blood samples Anthropometry, TBF% by DXA, CRF Questionnaire: Birth weight and parental education level	To examine the prospective association of three different measures of adiposity and CRF with CVD risk factors.	CRF and adiposity was linearly associated with CVD risk factor levels over the full spectrum. The association of CRF attenuated when adiposity was adjusted for.

Dansk Resume

Baggrund

I 2011 vurderede Verdens sundheds organisationen, WHO, at mere end 40 millioner børn under 5 år og 10% af verdens skolebørn var overvægtige. Udover at påvirke børnenes øjeblikkelige sundhed har overvægt i barndommen vist sig også at have konsekvenser for sundhed og sygdom i voksenlivet. Forskere og politikere bør derfor have særlig fokus på området, da det må anses som et stigende problem for folkesundheden. Fysisk aktivitet er vigtigt for børns velbefindende og naturlige vækst og anses for at spille en vigtig rolle i forebyggelsen af overvægt og fedme og relaterede sygdomme.

Danske skoler er potentielt effektive arenaer for sundhedsfremme og forebyggelse, da man her, uden at stigmatisere høj-risiko børnene, har adgang til størstedelen af børn i Danmark, uanset etnisk og socioøkonomisk baggrund. WHO har da også udpeget skoler som et særligt egnet område til fremme af øget fysisk aktivitet for børn og unge. Der er således gennem de sidste årtier foretaget en del skolebaserede studier for at fremme fysisk aktivitet og forebygge overvægt. Resultaterne af indsatserne har ikke ført til entydig konklusion. Særligt er der behov for nye fremadrettede og længerevarende studier, hvor både form, indhold og varighed af interventionen vurderes.

Denne afhandling er baseret på 4 videnskabelige artikler, som beskriver og evaluerer en sådan længerevarende indsats med mere idræt i skolen; Svendborg Projektet. Svendborg Projektet er et kommunalt tiltag i 10 folkeskoler i Svendborg Kommune. Tiltaget betyder at seks udvalgte skoler er blevet sports skoler og har øget deres obligatoriske antal idrætstimer fra 2 til 6 ugentlig lektioner. The Childhood Health, Activity and Motor Performance School study (CHAMPS study-DK) er navnet på det forskningsprojekt, der efterfølgende er blevet tilknyttet for at evaluere effekten af det kommunale tiltag.

Formål og problemstillinger i afhandlingen

Det overordnede formål med afhandlingen er at evaluere effekten af de fire ekstra idrætstimer på børns nuværende og fremtidige sundhed.

Problemstillingerne er:

1. At beskrive ideen og designet for Svendborg Projektet og CHAMPS study-DK (paper I).

1. At evaluere effekten af fire ekstra idrætstimer om ugen i folkeskolen på børnenes udvikling af BMI, fedt procent og overvægts prævalens (paper II).
2. At evaluere effekten af fire ekstra idrætstimer om ugen i folkeskolen på børnenes udvikling af hjerte-kar-sygdoms risikofaktorer(paper III).
3. At undersøge betydningen af kondition og kropssammensætning (direkte og indirekte målt) for hjerte-kar-sygdoms risikofaktorer hos raske danske børn i alderen 7 til 11 år over en to års periode. Samt at undersøge betydningen af ændringer i kondition og kropssammensætning i samme periode(paper IV).
4. At komme med anbefalinger for fremtidig forskning og folkesundhedstiltag på baggrund af de observerede resultater.

Metode

Dette studie er baseret på data fra ovennævnte longitudinelle studie i 10 folkeskoler, seks intervention og fire kontrol skoler. Interventions skolerne indførte fire ekstra idrætstimer om ugen, mens kontrol skolerne fortsatte med de sædvanlige to idrætstimer om ugen. I 2008 blev i alt 1507 børn (intervention n=773, kontrol n=734) fra børnehaveklasse til fjerde klasse inviteret til at deltage i forskningsdelen CHAMPS study-DK. 81% af børnene og deres forældre valgte at deltage. Højde, vægt, talje omkreds, kondition, blodtryk, pubertets status og fedtprocent blev målt og blodprøver gennemført i 2008 og 2010. Information om forældrenes uddannelse, indkomst og børnenes fødselsvægt blev indsamlet med spørgeskemaer i løbet af det første skoleår.

Resultater

Interventionen havde en gavnlig, omend ikke statistisk signifikant, effekt på gennemsnittet af BMI og fedt procent. Denne effekt var endnu større hos de børn, der var overvægtige ved start. Andelen af overvægtige og fede børn var signifikant lavere på interventions skolerne efter 2 år med mere idræt i forhold til kontrol skolerne. Idrætstimerne havde også en positiv effekt på en samlet hjerte-kar-sygdomsrisikofaktor score og de fleste enkelt risikofaktorer. Størrelsen af børnenes BMI, talje omkreds og fedtprocent ved start havde en lineær sammenhæng med niveauet af hjerte-kar-sygdomsrisikofaktorer efter to år. Denne sammenhæng blev svækket, men forsvandt ikke, når vi justerede for børnenes kondition, som også havde en lineær sammenhæng med risikofaktorerne.

Konklusion

Evalueringen af Svendborg Projektet viser at indførelsen af fire ekstra idrætstimer om ugen i folkeskolen kan forbedre børnenes sundhedstilstand i form a mindre grad af overvægt og fedme, samt ved bremse udviklingen af hjerte-karsygdomsrisikofaktorer. Særlig stor effekt sås hos de børn, som havde mest behov: dem med den højeste BMI, fedprocent eller risiko profil ved start. Den lineære sammenhængen mellem hjerte-kar-sygdoms risiko faktorer og børnenes kropsmål og/eller kondition peger på at en hvilken som helst ændring til det bedre i BMI, talje omkreds, fedt procent eller kondition hos børn vil være gavnlig for folkesundheden. Således viser evalueringen af Svendborg projektet at indførelsen af seks ugentlig idrætstimer er en effektiv strategi til forebyggelse af senere livsstilssygdomme hos børn.

English summary

Background

In 2011 the World Health Organization (WHO) estimated that more than 40 million children under the age of five were overweight and ten per cent of the world's school aged children are estimated to carry excess body fat. Childhood obesity is associated with a number of immediate cardiovascular health consequences and linked to subsequent morbidity and mortality in adolescence and adulthood. The issue is of growing concern for public health and therefore an important area for health researchers to address. Physical activity is essential for the wellbeing and normal growth of children and youth and plays an important role in the prevention of overweight and obesity and related morbidities.

Schools are recognized as potentially effective settings for public health initiatives, as they access a large population of children and youth across a variety of ethnic and socioeconomic groups without stigmatizing specific subgroups of high-risk children. The WHO specifically identified schools as a target setting for the promotion of physical activity among children and youth. During the last decades a considerable number of school-based, physical activity promotion and overweight prevention studies have been conducted, and their effectiveness on health outcomes evaluated. However, design and methods of these school-based studies differ, and results are not univocal, and more research is required on duration and volume of interventions in large-scale cohorts with long term follow up.

This thesis consists of 4 articles describing and evaluating a natural experiment, The Svendborg Project, in 10 public schools in the Municipality of Svendborg, Denmark. The experiment focused on increasing the amount of mandatory physical education (PE) lessons from two to six lessons per week. The Childhood Health, Activity and Motor Performance School study (CHAMPS study-DK) is the scientific research part of the Svendborg Project evaluating the initiative.

Purpose and objectives of the thesis

The overall aim of this thesis is to evaluate the effect of four extra PE lessons in primary school (pre-school to 6th grade) on health related outcomes in children.

The objectives are:

1. To describe the Svendborg Project and the CHAMPS study-DK (paper I).

2. To evaluate the effect of four extra PE lessons per week in primary schools on body composition and weight status in children aged 8 to 13 (paper II).
3. To evaluate the effect of four extra school-based PE lessons per week on future cardiovascular disease (CVD) risk factors in children aged 6 to 13(paper III).
4. To examine the prospective associations of cardio respiratory fitness (CRF) and direct and indirect indicators of adiposity with CVD risk factors in apparently healthy Danish children 7-11-years followed over 2 years. Furthermore, to examine the association of change in adiposity and CRF with change in CVD risk factor levels during follow-up (paper IV).
5. To recommend directions for future research and public health initiatives based on the results.

Methods

This study is based on prospective data from 10 public schools, six intervention and four control schools matched according to the uptake area of the schools and socio-economic position of the parents. Intervention schools provided four additional PE lessons per week, where as control schools continued as usual (two PE lessons per week). A total of 1507 children (intervention n=773, control n=734) attending pre-school to the 4th grade in 2008 were invited to participate in the CHAMPS study-DK and 1218 (81%) children and their parents accepted. Height, weight, waist circumference, DXA scans, Cardio respiratory fitness (CRF), blood pressure, pubertal stage and fasting blood samples were obtained at baseline (2008) and follow-up (2010). Information on parental education level, household income and birth weight were collected from questionnaires during the first school year.

Results

Intervention had beneficial, but non significant effect on mean BMI or mean Total Body Fat percentage (TBF%), but a significant beneficial effect on overweight and obesity prevalence, as children at intervention schools had a significant reduced risk of becoming overweight or obese after 2 school years compared to children at control schools. Also composite risk score and most single risk factors for CVD changed significantly more in favour of children attending intervention schools compared to children attending control schools. Baseline adiposity was independently and positively associated to increased composite CVD risk score after 2 years. Adjusted for CRF this association attenuated, but stayed significant and independent. The associations were linear across the entire distribution of adiposity and CRF.

Conclusion

Evaluation of this natural experiment showed that six PE lessons per week significantly changed prevalence of overweight and obesity after two years, also mean BMI and TBF% improved in intervention schools, though not significantly different from control schools. CVD risk factors significantly decreased in intervention schools compared to control schools. The intervention had a larger effect in children who were overweight and obese, or had the highest (over median) CVD risk scores at baseline. The shape and magnitude of associations of adiposity and CRF with CVD risk factors, suggest that any effort to shift the population distribution of adiposity downwards would be valuable for early CVD prevention in primary school children.

Overall, results support that a simple and relatively easily adaptable intervention, like the Svendborg Project, has the potential to positively affect future public health.

Abbreviations

BMI= Body mass index

CHAMPS study-DK= the Childhood Health, Activity and Motor Performance School Study in Denmark

CRF =cardiorespiratory fitness

CVD=cardiovascular disease

DXA= Dual Energy X ray Absorptiometry

HOMA-IR= Homeostasis Assessment Model for assessing insulin resistance

IASO= International Association for the Study of Obesity

IOTF = International Obesity Task Force

NW=normal weight

OB=obesity

OW=overweight

TBF%= Total Body Fat Percentage

TC= total cholesterol

TC:HDL = Total Cholesterol High Density Lipoproteins ratio

TG= triglycerides

WC= waist circumference

WHO= World Health Organisation

Introduction

This introduction will give a short description of key terms and existing knowledge in school based interventions and children's health in relation to adiposity, cardiorespiratory fitness and CVD risk factors.

Children and adiposity

Almost two decades ago (1997) The World Health Organisation (WHO) declared obesity (OB) to be a global epidemic. In 2011 WHO estimated that more than 40 million children under the age of five were overweight (OW) (1) and ten percentage of the world's school aged children were estimated to carry excess body fat (2). Childhood OW and OB not only has immediate social, psychological and physiological health consequences for the individual(3, 4), it also comes at increasing cost to the public and calls for prevention (2, 4). The condition is sustained over time and is a strong predictor of adult OW and OB (5) and linked to subsequent morbidity and mortality in adolescence and adulthood (6, 7). Thus childhood adiposity is a multicomponent condition with complex aetiology and consequences(8).

Measurement of adiposity in children occurs in a range of settings, using a range of methods and definitions, making comparisons of the magnitude of the problem between national and international groups challenging. Nevertheless, international comparison is possible and meaningful, as large cohort studies and national surveys almost by rule use the terminology overweight and obese (often taken together) measured by body mass index (BMI) and defined categorical by nationally or internationally established reference values and cut points. The issue of different measurements and definitions of adiposity, OW and OB will be addressed in the next section, after a brief overview of the magnitude and consequence of the obesity epidemic.

OW and OB prevalence between European and North American countries show large international variations. Rates of OW and OB range from 10-20% in Northern Europe and higher still in southern Europe - from 20% to as high as 36% in parts of Southern Italy, where the scale of the problem has been compared to that of the USA (9) and prevalence increasing by 0.5 to 1% every year (2)

In Denmark prevalence of OW and OB in children varies from 12% to 25% depending on age, area and choice of measurement (10-12) and whether both OW and OB are included (13). The prevalence of overweight and obesity in schoolchildren in Denmark might seem low and for the age of 10-16 years the prevalence is less than half the prevalence of the USA (13-16), but levels have increased since the midst of last century and are higher than ever(17).

The economic burden of overweight and obesity in Denmark is not fully elucidated through research, but economic analysis of this burden in other European countries is estimated as high as 0.61 % of gross domestic product (GDP) (18). These estimates depend on which method is used for economic assessment, and whether both direct and indirect costs are included(19).

One Danish economic analysis from 2007 (20) estimated that 2.8% (1.8-3.6%) of the running costs at Danish hospitals was spent on obesity related care. The numbers were calculated on the basis of registrations in 2003, where 177,703 hospital contacts in Denmark were related to obesity (in children and adults). These numbers are equivalent to the results from similar analyses from other European countries with comparable health care systems. The health-economic costs related to obesity in Denmark depend on the prevalence of obesity therefore any attempt to prevent the prevalence from increasing in the future population is worthwhile(20). It is suggested that even small reductions in weight gain or changing obese children to overweight will be cost effective (21).

In sum, the consequences of OW and OB in childhood are a major concern both for the individual and for society and therefore it is important to focus prevention and health promotion (6).

Measurements of overweight, obesity and adiposity in children

Ideally a measure of adiposity should be “accurate in its estimate of body fat; precise, with small measurement error; accessible, in terms of simplicity, cost and ease of use; acceptable to the subject; and well documented, with published reference values”(22). No existing measure satisfies all these criteria.

WHO defines obesity as a disorder of excess body fatness that is associated with an increased risk of disease(23), but the development of one simple index for the measurement of health related OW/OB or adiposity is challenged especially in children and adolescents, as their bodies undergo a number of physiological changes, as they grow. Health related cut points are difficult to establish, as associations with increased risk hazard might not be present at the time of onset of adiposity.

It might seem obvious that OW and OB should be defined by body fatness, as it is this, not weight, which is associated with the comorbid conditions(24), but often more indirect measures as body mass index (BMI; weight in kg/height in meters squared) and/or waist circumference (WC) is more feasible, and therefore commonly used in clinical and research settings instead of more direct measurements as dual energy x-ray absorptiometry (DXA), Computerized tomography (CT), Magnetic resonance imaging (MRI) or underwater weighing (hydro-densitometry). The direct measures are predominantly used for research and tertiary care settings, but may be used as a "gold standard" to validate anthropometric measures of body fatness(2, 25).

Body mass index

In the following section validity and feasibility of three commonly used adiposity measures will be described and compared. In the method section it will be made clear which specific methods and procedures were used for measuring and defining OW/OB and adiposity in the studies for this thesis.

BMI is the most commonly used indirect measure in studies on OW/OB. It is a weight for height measure and does not take the proportion or distribution of fat mass into account. Though BMI has been shown to strongly correlate with Total Body Fat (TBF) in children (26, 27), misclassification of OW and OB is evident when BMI is compared to more accurate measures of adiposity such as body fat percentage measured by DXA: high specificity but low sensitivity for BMI is found (28, 29).

International age and sex specific cut points for defining OW/OB in children, corresponding to a BMI ≥ 25 and ≥ 30 respectively for adults, have been established by Cole and colleagues(30), and are recommended by the International Obesity Task Force (IOTF).

The percentage of body fatness corresponding to BMI classifications for OW and OB in children varies considerably with age in growing children for both sexes(31, 32) and the accuracy varies with the degree of body fatness(33). Thus BMI as a measure of obesity is more accurate with increased degrees of body fatness(2).

In summary, BMI and the BMI reference cut points might not be accurate in estimating the individual's body fatness, but are suitable and recommended for research use and for monitoring and evaluating changes in populations(2).

Waist circumference

WC is another indirect and frequently used assessment of body composition. In adults it has been shown to capture metabolic disturbances better than BMI. Whether this is the case for children and adolescents, is still under discussion(22, 33, 34). WC and trunk fat, estimated from DXA in children aged 3-19 years, correlates fairly well (r=0.83 for girls and 0,84 for boys) and in addition WC correlates well with CT scans as measure of subcutaneous abdominal adipose tissue (r=0.93) and fairly well with intra-abdominal adipose tissue (r=0.84)(2). Two studies have suggested age and sex specific cut offs for WC in children(35, 36). However, the International Association for the Study of Obesity (IASO) still does not consider any cut-off values for WC for the classification of OW and OB in children as recommendable(2). IASO and IOTF recommend and encourage researchers to collect data on WC as well as BMI(2). In monitoring the obesity epidemic, use of WC is suggested to provide additional information to that of BMI, as WC appears to be more suitable for detecting small changes in the paediatric population over time (37).

Both indirect measures, BMI and WC, are easy to measure with simple, low cost equipment, have low observer error, and offer good reliability, validity and low measurement error. Both measures are more accurate when measured by a trained person, than self-reported.

Total body fat percentage measured by DXA

DXA scans are based on the principle that transmitted X-rays at two energy levels are differentially attenuated by bone mineral tissue and soft tissue. Using experimentally derived calibration equations, the soft tissue components can be divided into fat and lean tissue. Fat mass can be estimated by region, but DXA cannot distinguish between intra-abdominal and subcutaneous fat in the abdominal region as for example by MRI or CT scans. It correlates highly with CT scans estimating total body fat(38), but delivers lower radiation exposure and is thus more suitable for use in children and adolescents(39). The procedure must take place in a medical facility; the equipment is expensive and must be operated by a skilled technician. The procedure also requires some cooperation by the subject under investigation, making it mainly recommendable for subjects above the age of 6(2). DXA provides estimates for: absolute value (fat mass in grams or kilograms), and relative value of body fatness (total body fat percentage, TBF%).

Though some age and sex dependent inaccuracy and misclassification can occur in DXA assessment of body composition in children (31, 32, 40), it is considered an accurate and reliable measurement close to a "golden standard" for definition of adiposity in children (26, 31, 32, 39). Classification of excess body fatness has been attempted in several studies, either defining level of body fatness corresponding to the established cut points for OW and OB by BMI (31, 41), or from cross sectional associations with metabolic risk factors(42-44), as longitudinal studies on the relation of body fatness in childhood to adult disease risk is lacking(33). The most widely used of these classifications are the ones proposed by Williams *et al*(42), based on the association of skinfold-estimated body fat percentage, with adverse levels of lipids and blood pressure. Their proposed cut points for excess body fatness was 25% for boys and 30% for girls aged 5-18. Though widely used and recognized as a measure of unhealthy adiposity, a critique is that age modification was not taken into account; it is considered likely that excess body fatness should be defined relative to the child's sex and age peers in much the same way that BMI cut points for children have been constructed(33).

Summary on measurements for overweight, obesity and adiposity

In summary BMI is a screening tool, not a diagnostic tool. The cut points defined for OW and OB are statistically based, and are not based on health risk. In addition, because the relationship between BMI and adiposity varies by sex, age, and race/ ethnicity, risk based cut-points may also vary(45). Hence, it is not merely a question of how to define OW and OB in children, but how to establish useful cut-points that best predict future health risks and how to efficiently screen for such risks. These answers may be different for different populations. Further examination of the relation between various health measures and different adiposity measures, measured on a continuous basis, may provide additional valuable information(46, 47).

Health consequences of childhood adiposity

Though it has been claimed that childhood obesity has been looked upon as a merely cosmetic problem (6), research from the last decades have given solid evidence that childhood obesity is a complex and multi-factorial condition, with short-term and long-term health consequences of serious dimensions for the individual and for society(2, 6, 8, 9, 48, 49).

It is considered well established that high BMI levels have consistent associations with cardiovascular risk factors in both adults and children (33). As in adults, OW/OB in childhood does cause hypertension, dyslipidaemia, chronic low grade inflammation, increased blood clotting tendency, endothelial dysfunction and hyperinsulinaemia and/or insulin resistance(6, 8). It is less clear how early childhood adiposity affects the vascular system. Even though clinical manifestations of CVD do not usually appear before middle age, studies have shown that increased body weight is related to thicker and stiffer arteries as early in life as five years old (50).

A longitudinal study on a large Danish cohort (n>270.000, followed for 5.063.622 person-years) has shown that higher BMI levels in childhood (from the age of 7 or 13) were associated with greater risk of cardiovascular disease events in adulthood (earliest entry to analyse was 25 years of age). However, whether this association reflected association with adult adiposity could not be determined as data for this was not available(7). They found that for each 1-unit increase in BMI z-score, at every age from 7 to 13 years in boys and 10 to 13 years in girls, risk of an event significantly increased(7). Risks seemed to increase across the spectrum of BMI values and no cut points at which risk dramatically increased were identified. Whether this linear association is already present in childhood is less clear, but recently investigated by Lawlor et al in a prospective cohort of children aged 9-12 at baseline and follow up at age 15-16 years. They found that a linear association to CVD risk across the whole distribution of adiposity (regardless of direct or indirect measures) was present, and no threshold effect could be identified. Further more they found that children who "normalised" their weight category between baseline and follow up improved their cardiovascular risk factor profile (51). In a recent systematic review by Friedemann et al (52) the association and its magnitude between BMI category and CVD risk parameters in school aged children was described. They conclude that having a BMI outside the normal range significantly worsens immediate risk parameters. In another recent study, in a large prospective cohort of American children and adolescents (5 to < 20 years of age), obesity and the associated cardiovascular risk factors in childhood, were shown to be significantly associated to premature death in adulthood (mean follow up period 23.9 years)(53). Finally, some studies indicate psychosocial health consequences of childhood OW/OB like low self-esteem and behavioural problems (2, 6). Also fatty liver, early menarche, asthma and sleep disorders are observed more often in severely obese than normal weight children(2). These health consequences are well recognized by the writer, but are not with in the scope of this thesis and therefore not further addressed.

In summary, adiposity in childhood seems to have short- and long-term health consequences similar to those found in adults. It is still unclear whether these long-term consequences are due to the fact that both conditions, unhealthy bodyweight and increased CVD risk factors, seem to track into adulthood, causing morbidity and premature mortality (5, 7, 54). Studies to confirm the linearity and immediate associations in childhood are still warranted.

Children's cardiorespiratory fitness and health

Whether cardiorespiratory fitness (CRF) should be considered one of the traditional risk factors for metabolic unbalance is still under debate, but increasingly recognized, as there is growing evidence from several studies in adults (55), and an emerging number of studies in children and adolescents, that low CRF is associated with immediate and future increased CVD risk (55-60).

CRF is the overall capacity of the cardiovascular and respiratory systems and the ability to carry out prolonged strenuous exercise(61). Data from cross sectional studies suggest that boy's CRF increase almost linearly in relation to chronological age. Girl's data demonstrates a similar, but less consistent trend as some studies suggest a tendency to plateau at about 14 years of age(62). Estimations from regression equations on these data suggest that boys and girls from 8 to 16 years increase their CRF by 150% and 80% respectively(62). It is debated whether endurance exercise training is likely to have any considerable impact on CRF in healthy pre-pubertal children as shown improvements are often small (≈ 5%) (63). On the other hand, it has been shown that highly-trained child endurance athletes can demonstrate an approximately 30% higher CRF than their untrained counterparts(63).

CRF can be estimated using maximal or submaximal tests, by direct or indirect methods. In epidemiological studies involving young people, the most common test for assessing cardiorespiratory fitness has been the 20-m shuttle run test, or adaptations/modifications of this test. The CRF test used for this thesis is the Andersen test (64), which is an indirect measure of CRF, developed to provide teachers and health care professionals with an important tool to estimate CRF in children and adolescents in a fast, non-expensive and reliable way(64, 65).

CRF is in part genetically determined, but it can also be greatly influenced by environmental factors, making it one of the modifiable risk factors for future and present health(61). Longitudinal data have shown a significant relationship between adolescent CRF and later body fatness; cross sectional studies indicate that those individuals having a high CRF level also have significantly lower total adiposity(61). Even in overweight or obese children, those having higher CRF levels show lower overall adiposity(61, 66). Physical exercise of is considered one of the main determinants of CRF and concomitant or subsequent body composition (62, 63). In both youth and adolescence levels of physical activity have been decreasing in economically developed countries the last decades(67). Therefore it is considered important to implement strategies aiming at increasing children's physical activity levels, though there is an informed debate going on as to whether this perceived decline in physical activity levels could be expected to affect the temporal trends in children's CRF or if the increase in adiposity is more likely to be the predominant factor of such a decline (62, 63). Because CRF level to a large extent is explained by long-term engagement in physical activity, it is likely that adiposity is on the causal pathway between CRF and CVD risk. Currently there is an on going debate on the competing influences of CRF and adiposity on CVD risk. Research results are not univocal as to whether CRF and adiposity have an additive or combined effect on CVD risk factors, and which contribute the most as predictors of health in childhood, adolescence and adulthood (68-80).

Longitudinal studies are warranted to estimate the independent association of CRF and adiposity and to explore if alternative mechanisms can explain the relationships, as both exposures are putative intermediate variables in the causal pathway (i.e. adiposity is an intermediate variable between CRF and CVD risk) (68, 73, 81). In addition more research focusing on the mechanisms driving exercise-induced changes in aspects of aerobic fitness during growth and maturation is called for(62).

Clustering of risk factors

All CVD risk factors, including CRF and adiposity, interact and affect each other. Further more it has been shown that in apparently healthy children, level of single risk factors show large differences, and as they tend to fluctuate on a day-to-day basis a high level does not necessarily indicate an increased risk of future disease (82-84). However, when several risk factors are high in an individual, it might indicate that some underlying mechanism for metabolic imbalance has been turned on. This clustering of risk factors is more often found in children with high levels of BMI and/or low levels of CRF (33, 82, 84-86). As no hard endpoint such as manifest disease or death have yet occurred in children, being at high risk for CVD is difficult to define. Therefore a measure of the clustering of single risk factors for CVD has been proposed as a good measure of metabolic unbalance in apparently healthy children (82, 84). Furthermore, as opposed to the dichotomization of the risk factors in any definition of the metabolic syndrome (MetS), a composite risk score does not reduce the available data, but uses the full spectrum of knowledge available. This approach, constructing a composite risk score, sums up standardized values (z-scores) of selected single risk factors (i.e. blood pressure, insulin, glucose, cholesterol, triglycerides and a measure of adiposity and subtracting the value of CRF). No consensus exists in regard to which risk factors to include in the composite risk score, and a variety is seen in the literature. This makes comparison between studies difficult, but the outcome measure (composite risk score) less vulnerable to day to day fluctuation compared to single risk factors (82, 84).

School-based Interventions and Public Health

Given the apparent persistent nature of OW/OB, intervention strategies on treatment can seem infeasible and prevention in childhood appears to be a more realistic and cost-effective way to combat the international obesity epidemic(2). Based on results from several reviews and on behalf of the IASO International Obesity Taskforce, Lobstein and colleagues claim, that prevention is the only realistic and cost-effective strategy for dealing with the health consequences emerging from the rising trend in prevalence of obesity in the world(2). Preventing childhood obesity is by all means the most logical and probably most cost-effective public health strategy(2), though not the only answer to combat adult adiposity as many obese adults were not obese children (22). An evaluation is still required of whether the optimal intervention strategy for preventing increasing adiposity in the paediatric population is to target the children with more extreme values of adiposity, or to reduce childhood adiposity, using a broader public health strategy, or both (87).

Schools are recognized as potentially effective settings for public health initiatives, as they access a large population of children and youth across a variety of ethnic and socioeconomic groups without stigmatizing specific subgroups of high-risk children. The WHO specifically identified schools as a target setting for the promotion of physical activity among children and youth (88). Developing and testing new methods for prevention is stated as being more important than high risk behaviour surveys(89).

Over recent decades a considerable number of school-based physical activity promotion and overweight prevention studies have been conducted and their effectiveness on health outcomes evaluated and reviewed (88, 90-92). The conclusions of these reviews are divergent depending on setting, target group, intervention programs and choice of health outcomes, but there is mounting evidence that PE-based strategies within school are effective in increasing physical activity (93, 94) and hence may contribute to the prevention of OW and OB and low CRF. Furthermore, school-based PE interventions are theoretically appealing because adherence with the intervention is potentially high as PE lessons are mandatory in Danish schools. In order to make changes in the school physical activity curriculum adaptable and sustainable, it is recommended to involve stakeholders (politicians, parents, teachers and children) in the design and provide flexible and easily adaptable solutions. Such policies and solutions could potentially be incorporated and sustained on a population level if shown to be effective (94).

The CHAMPS study-DK, upon which this thesis is based, is a quasi experimental study evaluating such a natural experiment, the Svendborg Project, where a local municipality decided to increase the amount of PE lessons in public schools and to evaluate the effects on various health outcomes. The design and methods of the intervention is published in paper I, the Study Protocol. One important issue for the CHAMPS study-DK was to increase the amount of PE lessons to more than double (i.e. more than four lessons per week), as that volume had been proven not enough to affect important health outcomes in another recent Danish School study(95). Another important issue in the set up phase was that the intervention should be approved by stakeholders (children, parents, teachers and politicians) and easy to implement and adhere to. The specific methods and perspectives from the CHAMPS study-DK used for this thesis will be further elucidated in the following sections.

Summary and what this thesis aims to add to existing knowledge

In summary it is known that adiposity and low CRF in childhood are associated with each other and later morbidity and premature death. This it not only due to known tracking and stability of adiposity and other risk factors into adulthood, but also due to the fact that risk factors, already present in childhood, affect the progression of the atherosclerosis process (89). Reducing adiposity and increasing physical activity/ CRF in the general paediatric population might be one of the more efficient ways to prevent and reduce incidence of future CVD risk in the adult population. (Paper II).

It is also known that school-based interventions can affect children's current health, but thoughtful improvements to earlier tested strategies, considering settings, volume, implementation and feasibility, can contribute to the accumulation of evidence needed to design and implement the next generation of optimal interventions (Paper I, II, III, IV).

Finally, the concomitant effect of CRF and adiposity on current and future CVD risk in young children is still to be investigated in longitudinal studies (Paper IV).

Purpose and objectives of the thesis

The overall purpose of this thesis is to evaluate the effect of six PE lessons per week, compared to two PE lessons per week in primary school (pre-school to 6^{th} grade) on health related outcomes in children. Secondarily the established cohort gives the opportunity to investigate prospective associations of modifiable exposures and health outcomes in a general paediatric population.

The objectives are:

1. To describe the Svendborg Project and the CHAMPS study-DK (paper I).
2. To evaluate the effect of four extra PE lessons per week at primary schools on body composition and weight status in children aged 8 to 13 years (paper II).
3. To evaluate the effects of four extra School-based PE lessons per week on future CVD risk factors in children aged 6-13 years (paper III).
4. To examine the prospective association of three different measures of adiposity and CRF with 2-year change in CVD risk factors in children. Furthermore, to examine the association of change in adiposity and CRF with change in CVD risk factor levels during follow-up (paper IV).

5. To recommend directions for future research and public health initiatives based on the results.

Methods

The studies in this thesis are based on participants and measurements from the CHAMPS study-DK, the research part of the Svendborg Study, which is described and published in the study protocol (paper I) (96). The planning and design of both will briefly be described in the start of this method section followed by a detailed description of the dependent and independent variables used in the three other papers (paper II-IV).

The Svendborg Project

The original concept for the Svendborg Project aimed at establishing sport schools and was brought forth by the organization "Sport Study Svendborg", a secretariat in the Municipality of Svendborg, and they were responsible for setting up the Svendborg project. Initially all 19 public schools were invited to participate in the project. Six of these schools agreed to be a sport school.

The school- based PE program

The school leaders and PE teachers of the sport schools were invited to design the set-up for an optimal PE intervention. The number of children per PE teacher was on average 20, and girls and boys had PE together. The six intervention schools chose to implement four additional PE lessons per week to their usual PE program (resulting in a minimum of 4.5 hours PE per week divided over at least 3 sessions of at least 60 minutes), for all children attending Kindergarten to 4th grade starting out in 2008. Furthermore PE-teachers at sport schools were educated in specific age-related training principles. According to this concept it is important to train children in a biologically relevant manner depending on their physical and physiological maturity. The final concept was accepted by the city council that also provided funding for four extra PE lessons per week. The four control schools continued their regular PE curriculum (i.e. 2 PE lessons/week resulting in 1.5 hours/week) (96). Parents and children were unaware of the initiation of this project until two months before the following school year avoiding parents making an influenced school choice(96). The Svendborg Project can be described as a natural experiment in which the intervention is not undertaken for the purpose of research but where, nevertheless, the variations in exposure (i.e. the ' sports' schools versus the ' normal' schools) and outcomes can be analysed with the intent of making causal inferences(97).

The Childhood Health, Activity, and Motor Performance School Study Denmark (CHAMPS-study DK)

The CHAMPS study-DK can be described as a quasi-experimental study evaluating the natural experiment "The Svendborg Project". The decision of additional research was made after the 6 sports schools were selected. The role of the researcher was to measure the effect of the change introduced into the community but not to set up the experiment. It is community-based, meaning that its heterogeneity reflects that of the general population and not some possibly highly selected study sample. Funding for the research activities was obtained from external bodies.

The municipality was asked to provide six matched control schools but only four schools agreed to become a control school. The six intervention schools and the four control schools were matched based on school size and uptake area, urban-suburban/rural area and socio-economic position of the parents thus providing 10 schools for the research project. Though the town of Svendborg is the capital of the municipality, it is a small town with surrounding rural districts. The 10 participating schools represent half of the public schools in the municipality. Four schools were urban/suburban (two intervention/two control) and six were rural (four intervention/two control). Of the non-participating nine schools, six were urban/suburban and three were rural schools. Schools included in the project ranges in size from 120 -571 children.

The project with sport schools started in the school year 2008-2009 and is still running. Baseline testing for the research part was carried out in the beginning of the school year 2008 and follow up for this thesis at the same time points in the beginning of the school year 2010. Thus data in this thesis comprises two school years.

Quality control

The testing of more than a thousand children allocated on 10 schools and 76 classes within a relatively short time span on the day of testing (two school lessons) and within the same months in the curriculum did require a lot of testers for each test round. To reduce the risk of measurements errors, quality control was performed at different levels.

A detailed manual was written for all measurements obtained and the researchers trained all the test-personal every semester before testing the children in the project. The training was performed using children at the same age as the test subjects, but at a school outside the Municipality of Svendborg. Central variables, not regarded tested sufficiently for validity and reproducibility for this age group by others, were tested for before baseline (CRF, WC). Data was entered manually after each round of testing and double-checked by re-entering a subsample of data revealing less than 1% error. Each subject was given a unique ID-number and treated anonymous. Outliers were checked by manually checking nonsense values or those differing more than one standard deviation (SD). To stabilize variance and make data more normal distribution-like data were box cox transformed before 1 SD was set.

Measurements and participants

All measures for this thesis was obtained at baseline (autumn 2008) and follow up to years later (autumn 2010).

Participants

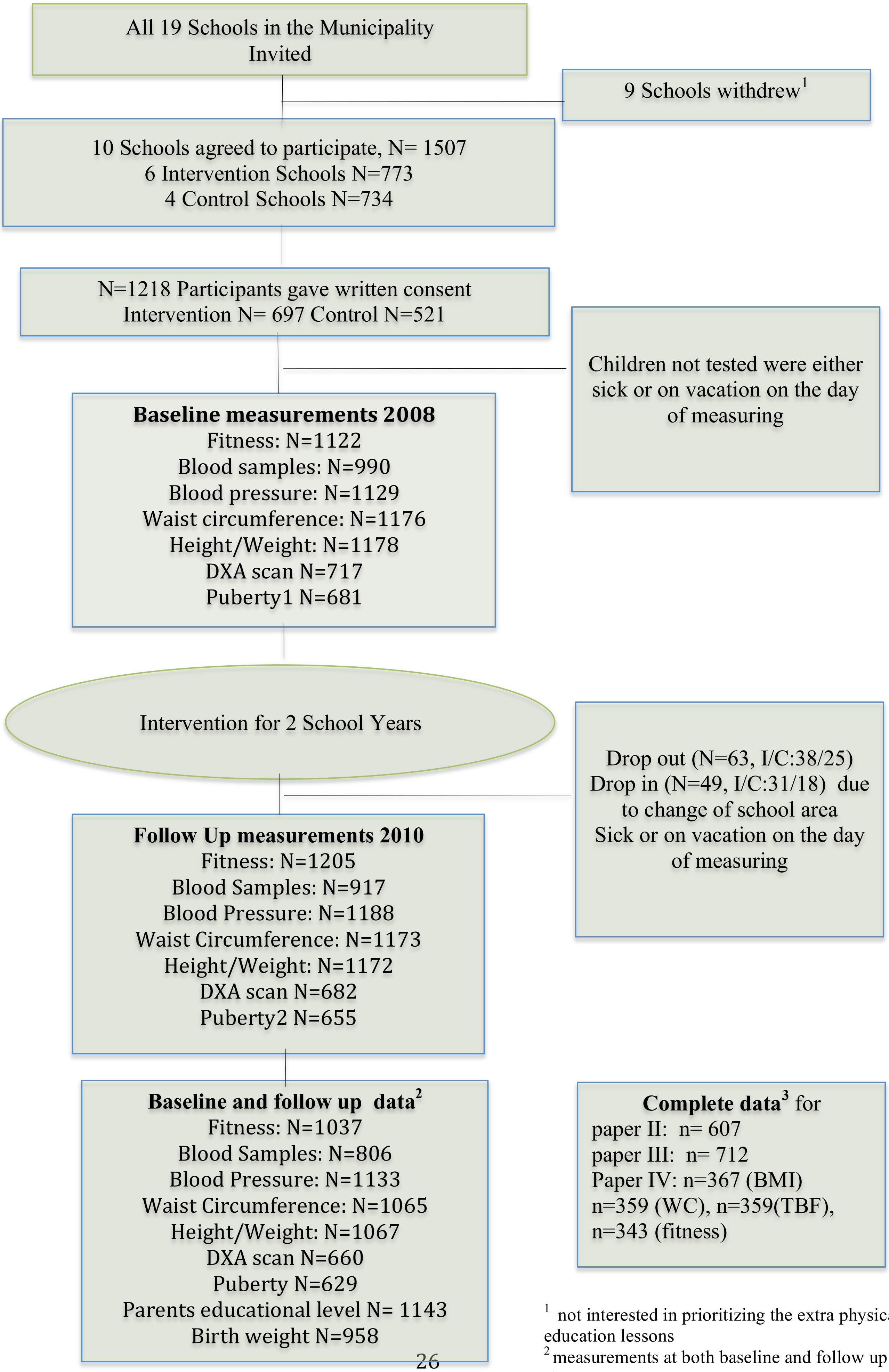
All children and parents from pre-school to 4th grade were asked to participate in the research program. In the sport schools n=697 (90%) and in the control schools n=521(71%) agreed to participate at baseline. The study was kept open, so that new children could enter and leave the study at any time. The number of children in the cohort and the specific analyses will therefore differ according to time and the research question asked. During the first three school years n=1328 unique ID numbers were given to children, who had one or more test.

The numbers of children entering and leaving the study the first three years are illustrated in the flowchart in Figure 1. During the first three years n=121 (intervention: n=86/ control: n=35) entered the project and n=113 (intervention: n=70/control: n=43) left the project. Most children who left the study did so because they moved from the municipality. Very few (n=9) changed school within the project. N=4 from one interventions school to another intervention school, n=2 from control to intervention and n=3 from intervention to control school (one of those left the research part). A priori it was decided that all analyses would be conducted on an intention-to-treat basis, thus minimizing the risk of selection bias.

Number of participants at baseline and follow up in the CHAMPS study-DK in relation to the papers of this thesis is illustrated in figure 1. Baseline characteristics of all eligible children from pre-school to 4th grade in 2008 are shown in Table 1. At baseline, boys were significantly higher, had better cardiorespiratory fitness, had lower score in composite risk and lower TBF %, were more likely to have stated their puberty stage above 1, less likely to be defined as overweight by WC and BMI, but not by TBF% than girls. There was no significantly difference in any of these values between school types except from WC; children at intervention schools on average having 0.9 centimetres less WC.

As the study is a natural experiment, conducted solely by the municipality and the participating schools, we did not as researchers have full information on the other schools. Based on data form the Danish Database of National Statistics (source: www.statistikbanken.dk) we compared income of parents between the participating schools and the remaining municipality. There were no significant differences in household income between control schools and intervention schools, while in non-participating schools parental income was 15% lower (p=0.04). Parental education level was not significantly different between participating and non-participating schools (p=0.70) or between intervention and control schools (p=0.80).

Figure 1: Flowchart of participants in the CHAMPS-study



[1] not interested in prioritizing the extra physical education lessons
[2] measurements at both baseline and follow up on the shown parameters
[3] numbers in the fully adjusted model

Table 1: Baseline characteristics of all eligible children from pre-school to 4th grade.

Variable	N all	Gender	Intervention Mean (SD)	N int	Control Mean (SD)	N con	P*
Age (years)	622	Boy	8.4(1.4)	349	8.5(1.5)	273	0.3
	706	Girl	8.4(1.4)	428	8.4(1.5)	278	*0.3*
Height (cm)	596	Boy	133.6(9.8)	349	133.6(10.3)	247	0.001
	627	Girl	131.6(9.5)	372	131.9(10.0)	255	*0.7*
Weight (kg)	552	Boy	29.4(6.6)	305	29.7(6.8)	247	0.3
	626	Girl	28.8(6.6)	372	29.4(7.6)	254	*0.2*
Body mass index (BMI)	551	Boy	16.2(2.1)	304	16.5(2.4)	247	0.08
	626	Girl	16.5(2.2)	372	16.5(1.8)	254	*0.11*
OW/OB defined by BMI[1]	551	Boy	21(7)/5(2)	304	24(10)/2(1)	247	0.01
	626	Girl	48(13)/7(2)	372	30 (12)/9(4)	254	*0.8*
Total Body Fat (TBF) (%)	343	Boy	17.4(8.0)	180	17.9(7.5)	163	<0.001
	374	Girl	22.7(7.3)	222	24.0(7.8)	152	*0.43*
OW/OB defined by TBF%[2]	343	Boy	29 (16.1)	180	31 (19.0)	163	0.4
	374	Girl	38 (17.1)	222	37 (24.3)	152	*0.09*
Waist Circumference (cm)	551	Boy	58.3(6.7)	304	59.1(6.0)	247	0.53
	625	Girl	58.0(6.8)	371	59.0(7.5)	254	*0.03*
OW/OB defined by WC[3]	551	Boy	29 (9.5)	304	29 (11.7)	247	0.04
	625	Girl	47(12.7)	371	44 (17.3)	254	*0.09*
Puberty[4]	556	Boy	217(71)/89(29)	306	177(71)/73(29)	250	<0.001
Pre-pubertal/ pubertal, N(%)	619	Girl	314(84)/58(16)	372	218(88)/29(12)	247	*0.6*
Fitness (m)	533	Boy	923.5(110.5)	287	926.4(108.4)	246	<0.001
	589	Girl	871.1(87.1)	345	853.5(99.6)	244	*0.45*
Composite Risk	428	Boy	-0.25(1.0)	242	-0.20(0.9)	186	<0.001
z-score	479	Girl	0.11(1.0)	305	0.37(1.1)	174	*0.06*

[1] according to IOTFs international cut points (30)

[2] according to cut points defined by Williams (42)

[3] according to Fredriks(35)

[4] puberty self-assessed by Tanner Stages. Due to very few >Tanner Stage 2 (less than 2%), puberty is dichotomized into pre-pubertal= Tanner Stage 1, pubertal=>Tanner Stage 1

* p values in normal font are for the difference between gender, p values in *italics* are for differences between school type (intervention/control)

Adiposity

Total Body Fat percentage (TBF%)

Total body fat mass was measured by Dual Energy X ray Absorptiometry (DXA), (GE Lunar Prodigy, GE Medical Systems, Madison, WI), ENCORE software (version 12.3, Prodigy; Lunar Corp, Madison, WI). The procedure took place at The Hans Christian Andersen Children Hospital, University Hospital Odense. The child was instructed to lie still in a supine position wearing underwear, a thin T-shirt, stockings and a blanket for the duration of the x-ray (typically 5 minutes). The instrument automatically altered scan depth depending on the size of the subject, as estimated from age, height, and weight (for more technical details see appendix to study protocol). All scans were performed by two different operators and analysed by one of them. The DXA machine was reset every day, following standardized procedures. TBF% was calculated for each participant from the equation: [(TBF (g) x 100)/ weight (g)].

TBF% was used as a continuous and a categorical variable in analyses. When used as a categorical variable OW/OB (adiposity) was defined according to cut offs suggested by Williams et al(42). These cut offs were defined as excessive body fat (measured by skinfolds) associated with elevated risk of CVD. The cut offs were not age related but related to sex. For boys and girls aged 5-18yrs the cut offs were ≥25 % and ≥30% total body fat respectively (42)

Body Mass Index (BMI)

BMI was calculated as [weight (kg)/height^2 (m)]. Weight was measured to the nearest 0.1 kg on an electronic scale, (Tanita BWB-800S, Tanita Corporation, Tokyo, Japan) with the child wearing light clothes. Height was measured to the nearest 0.5 cm using a portable stadiometer<, (SECA 214, Seca Corporation, Hanover, MD). Both anthropometrics were conducted barefoot.

BMI was used as a continuous and a categorical variable (OW/OB or not) in analyses. These categories were defined according to international cut points for OW and OB defined by BMI recommended by The International Obesity Task Force (IOTF)(30). This approach is age and sex related and conceptually equivalent to the adult definition of BMI ≥25

<30 defining overweight and BMI ≥30 defining obesity. Due to very few obese children in the cohort (< 2%) OW/OB was treated as one category.

Waist circumference (WC)

WC was measured using a tape measure with a standardized tension of the tape measure. WC was measured to the nearest 0.5 centimetre (cm) at umbilicus level after a gentle expiration. Two measurements were undertaken and a consecutive third measure performed if the difference between the first two measurements exceeded one cm. An average of the two nearest measurements was calculated and used in the analysis. WC is used as a continuous variable in analysis.

Cardiorespiratory Fitness (CRF)

CRF was assessed by the Andersen test, a 10 minutes intermittent running test validated against directly measured maximal oxygen uptake (64). Validity (r^2=0.85 compared to VO_{2max} treadmill test) and reliability (r^2=0.86 test/retest) of this field test was tested and described thoroughly for the age group of our cohort before testing (65).

The test was carried out indoors on one-half of a handball court (wood flooring), on 20-m running lanes marked by cones. Subjects ran from one line to the other, where they had to touch the floor behind the line with one hand, turn around and run back. After 15 seconds, the test leader blew a whistle and the subjects stopped as quickly as possible (within two steps) and rested for the next 15 seconds. Other staff counted the laps for each child, 6-10 children ran at the same time. This procedure was repeated during 10 minutes. Subjects ran as fast as they could in order to cover the longest possible distance during the test period. The test leader announced the end of the each resting period by counting backwards from 3 to 0. In addition all children wore heart rate monitors, making it possible to test the “degree of exhaustion” during the test. Staff counting the laps were instructed to write comments if the child for some reason were not able to “run as fast as possible”. During data generation outliers were checked, and children, subjectively assed not to have performed maximal for one reason or another, were excluded (n=6) at baseline. The total distance measured in meters was the test result.

Cardiovascular disease risk (CVD)

Blood samples

Fasting blood samples were obtained between 8.00 and 10.30 in the morning of testing. Samples were kept on ice and handed in to a laboratory within 4 hours, pipetted and centrifuged, and kept at -80 degrees Celsius until analysed. TC, TG, HDL-C and glucose were analysed by quantitative determination using enzymatic, colorimatic method on Roche/Hitachi cobas c systems. Insulin was analysed using solid phase enzyme-labelled chemiluminescent immunometric assay. The analyses were carried out at a certified laboratory in Austria at the Department of Sports and Exercise Physiology, Institute of Sports Sciences at the University of Vienna by experienced staff.

Total Cholesterol:HDL-C ratio (TC:HDL), was calculated. Homeostasis Assessment Model (HOMA-IR) assessed insulin resistance. The HOMA-IR score was calculated from insulin (μU/ml) x glucose (mmol/l)/22.5 as described by Matthews et al (98). The reliability of HOMA-IR, as a measure of insulin resistance, in a large scale and general population, has been shown by Bonora et al (99, 100).

Blood Pressure

Blood pressure was recorded with a suitable cuff size on the left arm using an automated blood pressure monitor (Welch Allyn®, Vital Signs Monitor, 300 Series with FlexiPort™ Blood Pressure). The child was resting in the sitting position for five minutes before monitoring. Five subsequent values were recorded with one-minute intervals or until the last three values had become stable. Mean of the three last recordings of systolic blood pressure (SBP) was used in the analysis.

Composite risk score

The composite risk score was constructed by summing standardized scores of logHOMA-IR, SBP, logTC:HD, logTG and logWC (if included) and subtracting CRF (if included)(see method section in the specific paper III and IV). CRF and WC are included as a risk factor in the composite CVD risk score for paper III. In paper IV those are exposure variables, and therefore not in the composite risk score. A low value of composite-risk score is considered healthier than high values of composite-risk score.

Other Covariates (independent variables)

Pubertal stage

The Tanner pubertal stages self-assessment questionnaire (SAQ) was used to determine pubertal status (101). Boys were presented with five pictures and text of Tanner staging for pubic hair development, whereas girls were presented with five pictures and text representing breast development and pubic hair(102). Explanatory text in Danish supported the self-assessment. The children were asked to indicate which stage best referred to their own pubertal stage. The procedure took place in a private space with sufficient time to self-assess the pubertal stage.

A validation study of the SAQ used in this study was performed in which n=63/120 invited children participated (n=31 girls and n=32 boys, aged 9.5 to 13.2). Agreement between self- assessment of pubertal maturation and the objective examination performed by an experienced paediatric endocrinologist was analysed using quadratic weighted kappa statistics. The conclusion was an almost perfect agreement for girls (weighted kappa =0.92; 95% CI: 0.8 to1.0) and a substantial agreement for boys (weighted kappa 0.74, 95% CI: 0.6 to 1.0) (unpublished data from this cohort).

For analysis pubertal stage was dichotomized (Tanner Stage 1 = pre-pubertal, Tanner Stage ≥2 = pubertal) as very few children were classified as Tanner Stage 3-5.

Parental level of education and children's birth weight

Parental educational level and birth weight were collected from questionnaires during the first school year.

Parental level of education was obtained by asking the parents what their highest degree of completed education was. These answers were categorized based on levels from the Danish Education Nomenclature (DUN) which is broadly comparable to international standard classification of education (ISCED (UNESCO 1997)) but for simplicity collapsed into 5 levels: 1) primary and lower secondary education, 2) general upper secondary education, 3) vocational education and training, 4) bachelor degree and 5) masters or PhD degree.

The child's birth weight was obtained by a simple question for the parents asking them to provide the weight of birth in grams.

Age, gender and school type

Age was calculated by subtracting day of birth from test date and expressed in years. Gender was reported by the child and verified by Central Personal Registration (CPR) number.

School type is referring to sports school (intervention) or normal school (control). Paper II and III is a study on effect of school type (exposure). Paper IV is a study on longitudinal associations of adiposity and fitness to CVD risk on pooled data from these schools and therefore school type is adjusted for.

Statistics

In this section general principles and considerations for the statistical approaches performed to comply with the cluster structure of a school-based design will be described. For detailed statistical explanations see method section in the papers included.

Sample size, power calculation and significance level

Sample size calculations should be and were performed before the start of the study. The power calculations were performed for the variable of interest with the highest variation (accelerometer data) to calculate the number needed for this, taking into account the clustering on schools and classes. A sample size of 1224 was calculated to make it possible to detect changes of less than 2% in blood pressure, fitness and body composition. Further, a change of less than 10% of the number of overweight children would be detectable, with this number of participants(96). None of the effect studies in this thesis comprises a sample size as big as n=1224, therefore analyses performed on subgroups, with a smaller number of subjects than the entire cohort, might suffer from lack of power. Therefore, results should also be interpreted by the observed absolute value of difference or change between groups, and not only by statistical significance level.

All analyses were carried out in STATA (version12.1) with $\alpha=0.05$ (two-sided).

Considerations in relation to clusters within the study sample

The study is school-based, and it is therefore possible, that there are more differences in students between schools and classes than within individual schools and classes (cluster effect). This means that special statistical methods are needed to take this into account. For the various measurements this requires the number of children in each group to be multiplied by a cluster effect of 3. This decision was based on findings in a previous study on the European Youth Heart Study and might be conservative(96, 103).

Multilevel multivariate mixed effect models were used for analyses on associations and inference between schools. These models were chosen to be able to take the hierarchical structure of data into account. Individual, class and school were considered random effects, other covariates as fixed effects. Intra class correlation (ICC) was calculated to compare the variation between school and school classes as a fraction of the total variance.

All analyses in the effect studies were based on the intention to treat principle (paper II and III).

Distribution, interaction and missing data

WC, HOMA-IR, TC:HDL ratio and TG, but not fitness and SBP, were slightly skewed, and therefore log transformed before used as single risk outcome variable and before z scores were made for the composite risk score.

Effect modification of relevant covariates was explored by adding an interaction term between the moderator and the exposure variable of interest (paper II and III: school type, paper IV: adiposity or fitness indicator). If the p-value of interaction term was <0.05, subgroup analyses were performed.

Sensitivity analysis were performed imputing missing values on the outcomes and covariates (see paper II, III and IV) using chained equations ("mi impute chained" in STATA) including all other covariates (respective outcome at baseline, age, gender, pubertal status, school type, and for paper IV also parental educational status and birth weight) and random effects (indicators for school class and school). Beta coefficients and standard errors (SE) were obtained based on 20 imputed datasets.

Ethical considerations

All children and parents from the participating schools received information about the study through school meetings and written information. Parents signed informed consent forms for joining the project. Participation was at all times voluntary. Permission to conduct The

CHAMPS study–DK was granted by the Regional Scientific Ethical Committee of Southern Denmark (ID S-20080047).

The children that were DXA scanned were from 2nd to 4th grade at the first scan. The reason for not scanning the younger children was the ethical issue of transporting 5-7 year old children 50 km to a different city and introducing them to new unknown environments, without their parents being present. Furthermore, as described in the introduction, the procedure requires some cooperation by the subject under investigation, making it mainly recommendable for subjects above the age of 6(2). The ethical committee's approval for DXA scanning only included the children from 2nd to 4th grade.

Results

Paper I is the study protocol and is included to give an overview of the CHAMPS study-DK in which the other three studies are nested. The main results from paper II, III and IV will be presented in the following section. For a more detailed description of results from each individual study, please see appendices.

Effect of extra PE lessons on Body Composition (paper II)

In the overall study, 1507 children from the preschool year to 4th grade (age range 5.4 – 11.5 years) were invited to participate in The CHAMPS study-DK from baseline in September 2008, of which 1218 (80%) accepted. All 800 children attending 2nd to 4th grade (7.7-11.5 years) at baseline were invited for a DXA scan providing a direct measure of adiposity; total body fat percentage. In total 742 children (93%) accepted the invitation, of these 739 (99.6%) children had a DXA scan at one time point, 717 children (97%) had a DXA scan at baseline and 682 (92%) at follow-up, 660 children (89%) had measurements at both time points, but when adjusting regressions for the chosen covariates this number was reduced to n=632 (86%)(please see figure 1 in paper II).

The aim of this study was to evaluate the effect of six PE lessons on children's body composition defined by BMI and adiposity level measured by DXA scan and expressed as total body fat percentage (TBF%). We found no significant differences between intervention or control schools regarding age, gender, anthropometry, and prevalence of OW/OB, adiposity and pubertal stages at baseline. Boys and girls at all ages were equally represented in the sample. Individuals with missing data or lost to follow up (n=between 85 and 107) had higher mean values of height, weight, BMI, TBF% and higher prevalence of OW/OB by BMI and TBF. School type was equally represented in children with missing data/lost to follow up (63% intervention, 47% control schools).

Multilevel linear regression analysis showed no significant intervention effect on the primary outcomes mean BMI or mean TBF%, (BMI: β -0.14, 95% CI: -0.33; 0.04; TBF%: β -0.08, 95% CI:-0. 65;0.49). Sensitivity analysis comparing the intervention based on the non-imputed sample (n=632) with the sample with imputed data (n=739) did not change effect estimates significantly (BMI: β -0.14, 95% CI: -0.45; 0.07; TBF%: β -0.12, 95% CI:-0.73;0.49).

The intervention had a significant beneficial effect on the prevalence of OW/OB. Children at intervention schools had a significant reduced risk of becoming OW/OB (OR 0.29, 95% CI: 0.11;0.72, p=0.01) after 2 school years compared to children at control schools. The intervention effect on prevalence of adiposity (defined by TBF%>25 for boys and TBF%>30 for girls) was smaller and borderline significant (OR 0.64, 95% CI:0.39; 1.05, p=0.08)(please see table 2 in paper II).

We observed no significant effect modification by age or gender (significance level for interaction set at p≤0.10). There was however, a significant moderating effect of being OW/OB (β -.48, p=0.07) on BMI. Additionally a significant moderating effect of being categorized as having excess adiposity defined by TBF% was found (β -.14, p=0.05). Therefore subgroup analyses were performed in OW/OB (n=67) versus NW children (n=565) and adipose (n=111) versus normal fat children (n=521). The intervention effect on BMI in OW/OB children was larger but not significant (β -0.5, 95% CI: -1.6; 0.6) compared to NW children (β -0.09, 95% CI: -0.24; 0.06). The intervention effect on TBF% was even larger although not significant in adipose children (β -1.18. 95% CI: -2.6; 0.2) compared to normal fat children (β 0.16, 95 % CI: -0.4; 0.74).

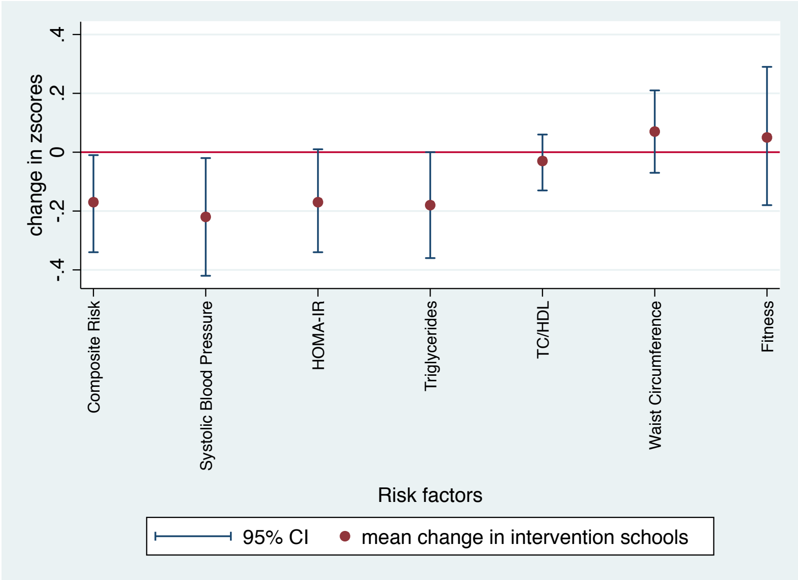
In summary: We observed a positive but non-significant effect on BMI and TBF% for children at sports schools compared to children at control schools. The preventive effect of intervention on prevalence of OW/OB defined by BMI was positive and significant. When OW/OB was defined by TBF% the effect was still in favour of the children at sports schools, but borderline significant. Estimates of effect size were, though not reaching significance, five to eight times larger for the children being OW/OB at baseline.

Effect of extra PE lessons on CVD risk factors (paper III)

The aim of this study was to evaluate the effect of six PE lessons on children's CVD risk. Out of 1507 invited children from the 10 public schools 1218 (81%), 697/773 (90%) from intervention schools and 521/734 (71%) from control schools gave written consent. At baseline 907 (75 %) of all participants had measurements of all single CVD risk factors and 878 (73 %) at follow up. In total, 712 (59 %) had complete data on the main outcome at both time points (see figure 1 in paper III). At baseline, the group of children included in the analyses, mean age 8.5 (range 5.7 to 11.4), were taller, had lower BMI and a higher proportion were pubertal compared to children not having complete data for composite risk score at both time points (see Table2). Children with non-complete data had significantly worse baseline values of TG, TC/HDL, WC and CRF, but not composite risk score, SBP, HOMA- IR, compared to children with complete data (in sample). There were no differences in any baseline values between children at sports schools and control schools in the sample (see Table 1, paper III). Sensitivity analysis imputing missing values in a multiple imputation did not change estimates of association for any of the CVD risk scores (estimates available in supplementary Table 1, appendix paper III).

Composite risk score changed significantly more in favour of children attending intervention schools compared to children attending control schools (β-0.17 SD, CI 95%:-0. 34 to - 0.01, ICC 0.15). SBP and TG changed significantly more favourable in children attending intervention schools compared to control schools (for SBP β-0.22 SD, CI 95%; -0.42 to -0.02) (for TG β -0.18 SD, CI 95%; -0.36 to 0.00). Also changes in HOMA-IR was in favour of the children attending intervention schools, but border line significant (β -0.17 SD, CI 95%;-0.34 to 0.01; ICC for class clusters 0.09). School clusters, as random effects, did not explain a significant fraction of the variation. Difference in changes between intervention and control for TC: HDL, WC and fitness were small and insignificant. An overview of the adjusted effect estimates on z scores of single and composite risk scores is displayed in Figure 2.

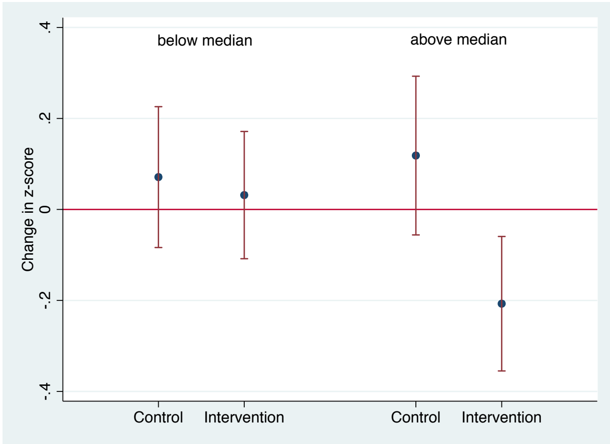
Figure 2: Difference in change in composite and single risk scores between intervention and control schools during two school years (expressed in z scores)



Estimates adjusted for age, puberty, gender and baseline values in a linear multilevel mixed effect regression model

No effect modification by age, gender or puberty was found. Interaction for those above versus below the median of composite risk score was significant (β -0.29 SD, CI 95%: -0.5 to -0.07, p=0.008). Analysis showed that intervention was significantly more beneficial for children at "higher risk" (composite risk score above the median), than to children not at risk (below the median) at intervention schools, and also compared to the children with the least favourable risk score at control schools (β -0.32 SD, CI 95%; -0.19 to -0.01).
See Figure 3 for illustration of differences in changes in z scores of composite risk score.

Figure 3: Difference in changes in risk scores between children with a composite risk score above or below the median at intervention and control schools



Circles representing mean. Vertical bars represents 95% CI. Estimates adjusted for age, puberty, gender and baseline values in a logistic multilevel mixed effect regression model

In summary: Four extra PE lessons a week significantly decreased CVD risk factors in children at intervention schools. The effect was significant for the composite risk score, but also for single risk factors as SBP, TG and border line significant for insulin sensitivity (HOMA-IR), but not for TC: HDL, WC and fitness. The children with the least favourable composite risk score (those above the median) had the greatest effect from intervention – both compared to the children with the least favourable composite risk score at control schools and with their counterparts with a better risk score (below median) at intervention schools.

Prospective association of adiposity and CRF with CVD risk (Paper IV)

In this study we investigated the prospective association of direct and indirect adiposity measures with CVD risk. Participants were recruited amongst the children from 2nd to 4th grade at baseline (see flowchart figure 1). When adjusting for possible confounders as covariates, the number of subjects in analyses was reduced to almost half. Therefore comparison between participants and non-participants were made and sensitivity analysis carried out on the main results.

In general, participants were normal weight, relatively fit and girls and boys equally represented at all ages. Characteristics of the study sample comparing the children with complete data (n=365) to those with non-complete data are shown in Table 2. Children with complete data for this paper were slightly higher, had better CRF, lower WC, TBF% and BMI and more children had entered puberty than those with non-complete data. For all other covariates and outcomes there were no significant differences between groups.

Pairwise correlation between the three adiposity measures was strong (TBF% and BMI r= 0.81, TBF% and WC r=0.74, BMI and WC r=0.84) and they were strongly correlated over time (age and sex adjusted tracking coefficients; TBF%: 0.91, BMI 0.93, WC 0.84, all p values <0.001).

Baseline adiposity by all three measures was independently and positively associated to composite CVD risk score after 2 years. TBF% was the strongest predictor of increased single and composite CVD risk factors. Estimates for the association of adiposity with CVD risk changed slightly downwards when adjusted for CRF at baseline (see Table 2 of paper IV). We did not see statistical evidence that any of these associations were modified by sex (p>0.05 for interaction). When we included a quadratic term of adiposity or fitness, we did not see evidence of a curve-linear relationship between adiposity and CVD risk factors (BMI^2 p=0.56, $TBF\%^2$ p=0.53, WC^2 p=0.66). Graphically evaluating the shape of associations of the three adiposity measures and CRF to CVD risk also gave no reason to reject assumption of linearity.

Baseline CRF was significantly and inversely associated with composite CVD risk factor score (β -0.12 SD, 95% CI: -0.21 to -0.02). When the model was adjusted for baseline adiposity, the association attenuated and became non-significant (see Table 2 in paper IV). Stability of CRF over time was lower than for adiposity (age and sex adjusted tracking coefficient: 0.67).

Table 2: Baseline Characteristics of Participants followed for 2 years and those lost to follow up/missing data on CVD risk factors

Variable Mean (SD)	n	Individuals with complete data n=365	n	Individuals with missing data or that were lost to follow up (n=374-185)	P*
Age (years)	365	9.4(0.8)	374	9.3(0.9)	0.60
Range (years)		7.7 to 11.4		7.4 to 11.6	
Gender	365		374		
Girls/boys n(%)		187(51)/178(49)		197(53)/177(47)	0.70
Height (cm)	365	138.8 (7.4)	345	137.0 (7.6)	0.01
Weight (kg)	365	32.3(6.2)	345	32.0(6.4)	0.90
BMI (point)	365	16.7(2.0)	345	16.9(2.3)	0.05
TBF(%)	365	19.9 (7.5)	352	21.2(8.7)	0.02
WC (cm)	365	59.8(6.4)	344	61(7.3)	0.01
CRF (m)	351	938 (97.8)	323	916.6(105.8)	0.02
HOMA-IR	365	0.85 (0.5)	256	0.89 (0.7)	0.60
TG (mmol/L)	365	0.66 (0.3)	256	0.64(0.3)	0.90
TC:HDL	365	2.7 (0.6)	256	2.8 (0.8)	0.05
SBP (mmHg)	365	103.3(7.0)	310	102.1 (8.5)	0.50
Composite Risk score (zscore)	365	0.19 (0.9)	220	0.10 (1.1)	0.80
Pre-pubertal/pubertal	365		316		
N (%)		215(59)/150(41)		217(68)/99(32)	0.01
School type	365		374		
Sports/normal – N/%		210(57)/155(43)		208(56)/166(44)	0.70
Overweight†	365		345		
N(%)		37 (10)		45 (13)	0.25
Obese†					
N(%)		3 (1)		8 (2)	0.13
Parental Educational level ‡	365		286		
N		175/190		146/140	0.44
%		48/52		51/49	
Birth Weight (kg)	365	3.5(0.7)	185	3.6(0.7)	0.40

* P is for difference between individuals with complete data and individuals with missing data or that were lost to follow-up.
†, Defined according to International Obesity Task Force (IOTF) criteria (Cole et al 2000)
‡Below /above bachelor degree
CRF = cardiorespiratory fitness, TBF=Total Body Fat, BMI= Body Mass Index, WC=Waist Circumference, HOMA-IR= Homeostasis Assessment Model for insulin resistance, SBP=systolic blood pressure, TC:HDL= Total Cholesterol/High Density Lipoprotein, TG= triglycerides.

TBF% was the strongest predictor for all single risk factors, BMI was fairly similar whereas WC only significantly predicted increase in HOMA-IR and TG, but not SBP and TC:HDL. Cardiorespiratory fitness was inversely associated with HOMA-IR, but not with the other three single risk factors, see Table 3.

Imputing missing values in a multiple imputation analysis did not change estimates of association for either cardiorespiratory fitness or adiposity indicators (see supplementary Table 1 for paper IV).

We also examined the association of change in adiposity and CRF with change in CVD risk factor levels. Changes in adiposity (Δ TBF%, ΔBMI or ΔWC) were all similarly associated with change in CVD risk factor levels (Table 3 paper IV). We observed that the associations of changes in WC and changes in CRF with the composite CVD risk score were stronger in boys compared with girls (WC: β 0.21, p=0.01 for interaction, CRF: β -0.19, p=0.02 for interaction). Associations were in the same direction but increasing WC for boys had stronger association to increasing CVD risk than for girls (β 0.36 SD, 95% CI: 0.22 to 0.50 compared to β 0.18 SD, 95% CI: 0.08 to 0.29). For CRF the sex difference was a more protective effect of increasing CRF level for boys than girls (β -0.27 SD, 95% CI: -0.39 to -0.14 compared to β -0.14 SD, 95% CI: -0.27 to -0.01). The association of change in CRF was independent of change in adiposity for boys; however, for girls the association was non-significant after this additional adjustment.

We found no indication of interaction between change in adiposity and change in CRF on the change in CVD risk factor levels (p values between 0.10 and 0.97). Also no interaction was found between these indicators as continuous variables (p values between 0.31 and 0.72) indicating an additive and not multiplicative effect of status or change in CRF to adiposity status or change.

In summary: We observed a significant association of adiposity and adverse CRF with increasing CVD risk factors. Associations appeared linear across the entire adiposity and fitness range. The combined association of CRF and adiposity was additive and not multiply. Results did not suggest any threshold or cut point where risk increased dramatically. We observed sex differences in association for changes in WC and CRF suggesting that boys benefitted most from positive changes in CRF and had stronger unfavourable associations with CVD risk with increased change in WC.

Table 3: Association of adiposity and cardiorespiratory fitness to CVD composite score and single risk factors

Outcome (1 SD)	Model 1*				Model 2†			
	N	β ‡	(95% CI)	P	N	β ‡	95% CI	P
Total Body Fat% (SD: 7.5 %)								
Composite risk	365	0.30	(0.21 to 0.39)	<0.001	349	0.28	(0.17 to 0.38)	<0.001
HOMA-IR (0.85)	386	0.36	(0.25 to 0.46)	<0.001	368	0.32	(0.20 to 0.44)	<0.001
SBP(7.0 mmHg)	443	0.18	(0.09 to 0.46)	<0.001	442	0.20	(0.11 to 0.30)	<0.001
TC:HDL (0.6)	386	0.10	(0.04 to 0.17)	0.002	368	0.09	(0.01 to 0.16)	0.02
TG (0.3mmol/L)	386	0.19	(0.09 to 0.28)	<0.001	368	0.17	(0.06 to 0.27)	0.003
BMI (SD:2.0 points)								
Composite risk	367	0.24	(0.15 to 0.33)	<0.001	351	0.21	(0.11 to 0.30)	<0.001
HOMA-IR (0.85)	387	0.27	(0.18 to 0.37)	<0.001	370	0.23	(0.12 to 0.34)	<0.001
SBP (7.0 mmHg)	445	0.15	(0.07 to 0.23)	<0.001	424	0.16	(0.07 to 0.25)	<0.001
TC:HDL(0.6)	387	0.09	(0.03 to 0.15)	0.003	370	0.07	(0.01 to 0.14)	0.04
TG (0.3 mmol/L)	387	0.15	(0.06 to 0.24)	0.001	370	0.12	(0.03 to 0.22)	0.01
Waist Circumference (SD:6.4 cm)								
Composite risk	367	0.20	(0.10 to 0.31)	<0.001	351	0.16	(0.05 to 0.28)	0.01
HOMA-IR (0.85)	386	0.27	(0.16 to 0.39)	<0.001	370	0.22	(0.09 to 0.35)	0.001
SBP (7.0 mmHg)	445	0.08	(-0.01 to 0.17)	0.09	424	0.09	(-0.01 to 0.20)	0.08
TC:HDL (0.6)	386	0.07	(-0.008 to 0.14)	0.08	370	0.04	(-0.05 to 0.12)	0.38
TG (0.3mmol/L)	386	0.16	(0.06 to 0.27)	0.003	370	0.15	(0.03 to 0.26)	0.02
Cardiorespiratory fitness (SD:97.8 m)								
Composite risk	351	-0.12	(-0.21 to -0.02)	0.02	349	0.009	(-0.11 to 0.09)	0.87
HOMA-IR (0.85)	370	-0.16	(-0.27 to -0.05)	0.05	368	-0.03	(-0.14 to 0.09)	0.66
SBP (7.0 mmHg)	424	-0.05	(-0.14 to 0.04)	0.30	422	0.05	(-0.05 to 0.15)	0.30
TC:HDL (0.6)	370	-0.06	(-0.12 to 0.01)	0.06	368	-0.02	(-0.10 to 0.06)	0.60
TG (0.3mmol/L)	370	-0.09	(-0.19 to 0.01)	0.09	368	-0.02	(-0.13 to 0.10)	0.77

* Model 1:analyses were adjusted for baseline values of risk, age, gender, school type, pubertal status, birth weight, and parental educational level (associations of WC also adjusted for height and height^2)

† Model 2: additionally adjusted for fitness (or TBF% when fitness was the exposure of interest)

‡ β coefficient represents change in risk factor (expressed in SD) per 1 SD in exposure(adiposity or fitness). The value of 1 SD is displayed in parenthesis after each outcome/exposure.

BMI= Body Mass Index, HOMA-IR= Homeostasis Assessment Model for insulin resistance, SBP=systolic blood pressure, TC:HDL= Total Cholesterol/High Density Lipoprotein, TG= triglycerides.

Numbers in parenthesis after risk factor and exposure is the values of 1 SD of the variable.

Discussion

This thesis is based on the results of two papers evaluating the effect of a school based intervention (The Svendborg Project) on body composition (paper II) and CVD risk factors (paper III) and one paper investigating the longitudinal associations of CRF and three different indicators of adiposity with CVD risk factors in school aged Danish children (paper IV).

Evaluation of the Svendborg Project showed that four extra mandatory PE lessons at school could positively affect children's body composition and CVD risk factor levels. The magnitude of effect of extra PE lessons was greater in children with the higher values of adiposity and CVD risk at baseline.

Investigating the magnitude and shape of association of adiposity and CRF with CVD risk showed strong association of CRF and adiposity, regardless of indicator of adiposity, and we found no reason to reject linearity across the full range of all exposure indicators (TBF%, BMI, WC and CRF). The combined effect of CRF and adiposity on CVD risk appeared additive as no interaction between CRF and adiposity was observed. The influence of change in CRF and adiposity on CVD risk factors levels appeared stronger in boys compared to girls.

Methodological considerations in relation to design of the CHAMPS study DK

Discussing the results of the Svendborg Project (paper II and III) is not only challenged by the enormous variety of aims, interventions, target groups and durability of school based studies to compare with, but also the big differences in ways of running primary and public schools internationally make generalizability and comparisons difficult. Furthermore the evaluation of implementation and effectiveness of public health interventions conducted as natural experiments is challenged in several ways (104). These challenges will be displayed and discussed in the next paragraph, in the perspective of general strengths and limitations for the study, before discussing the main findings of the studies and comparing results with other studies.

Danish schools and natural experimental design

More than 80% of all children in Denmark attend public schools (105). Funding from food or beverage companies is not allowed, and having a health policy is mandatory (e.g. for the school canteen, social behaviour and physical activity). Danish schools do normally not provide meals (except from in few deprived areas) so the custom is that parents or the child prepare a lunch box to bring, and the school canteens mostly provide healthy snacks and milk, juice or water for drinking. The Svendborg project did not specifically aim at changing nutritional behaviour, but the image of being a sports school (intervention) could have put emphasis on other parts of health behaviour among parents, children and teachers that we are not aware of and have not collected data on. This will lead to residual- and unknown confounding and mediation and can cause overestimation of the effect of the extra PE lessons in paper II and III.

Natural experiments often suffer from the lack of pre-intervention/baseline data making assessments of changes difficult(104). In our project we partly dealt with that issue by having matched control schools from the same area and by testing the children so early in the intervention period (with in the first months) that intervention would have had little or no effect yet on most outcomes. If this is not the case an eventual early effect of intervention is likely to make results underestimated rather than overestimated. Furthermore schools were tested in a parallel mode with one intervention school and its control being tested at the same time.

Another challenge is the “uncontrolled” intervention. That is: in which way and with how much enthusiasm has the schools actually implementing the extra PE lessons? In the Svendborg Project school leaders and teachers were invited to design the intervention, the research team conducting solely scientific and health professional advices on increasing the amount as much as possible, and more than four lessons, as that was proven not enough in another Danish School-based study(95). This ended up with six weekly PE lessons preferably allocated into two adherent lessons (2x45 minutes) three times every week or one hour every day. Only one school decided to do the latter.

In reality it is difficult to ensure matched controls, ensure standardization of programme implementation in all contexts, and ensure standardization of contexts and control “contamination” of control group (2). The possible threat of a spill over effect on the control group (wanting to be as healthy as the other schools) therefore can potentially reduce the effect of intervention. The effectiveness of "real world" interventions needs to be assessed in the light of that knowledge, meaning that process evaluation (e.g. log books, questionnaires, interviews and mediation analyses), impact evaluation (e.g. change in physical activity behaviours and/or level) and outcome evaluation (e.g. change in body composition) is required to evaluate if it works. These research actions are planned for in the Svendborg Project in the future. This thesis only presents outcome evaluations.

Generalizability of a local and national cohort

The cohort consists of a majority of healthy, normal weight Caucasian children. This may limit the generalizability of our results to other ethnic groups. Furthermore, the high attrition rate in some of the analyses may have further affected the generalizability of our findings, but since associations in imputed and non-imputed samples were fairly similar in all the papers we are confident that the results are not explained by selection bias. Finally, although the two types of schools are well matched, making comparisons between them credible, and although the parental demographic profile of participants resembles that of its target population, some generalizability of the data may not extend beyond that type of population (town with surrounding rural districts in Denmark).

Confounding, mediation and bidirectional association

Confounding means that the effect of exposure of interest is mixed with the effect of another variable – known or unknown. It is important, when seeking to ascertain causality in observed exposure-outcome relationships, to identify potential confounders and to address the issue appropriately. In the experimental design of study II and III, having a control group and adjusting for known confounders available, partly restricts the threat of confounding. Adding potential confounders as explanatory variables in the multivariate analyses used provides an estimate of the effect of the primary exposure variable of interest (in this case school type) that *cannot* be explained by the relationship between the outcome variable (Body Composition or CVD risk level) and the confounding variables included in the model.

Study IV was observational; a design that is even more prone to unknown confounding and not even appropriate adjustment for known confounders can exclude the possibility of unknown confounding. In addition, variables identified as confounders can be imperfectly assessed making residual confounding present even after careful statistical adjustment. The strengths of associations between the included variables in the regression models will be affected by such random errors, which can lead to both under- and over estimation of the association, depending on the accuracy and validity of the measurements chosen. For example our measurement of pubertal status was self-reported using the Tanner Scale, and though this method is validated in adolescents (102) and in a subsample of this cohort (unpublished), we cannot ensure that their pubertal stage is not over- or underestimated, and it was surprising that more boys at baseline reported being pubertal (> Tanner stage 1) than girls. Also our measurement of CRF was indirectly obtained by a field test. Thus, even though we documented high agreement between the field-test and directly measured VO_2-max(65, 106), some measurement error will remain and it is difficult to compare estimates of CRF and adiposity (study IV) as these will be affected by differing degrees of measurement error.

If the association between the primary exposure variable of interest and the outcome variable can be partly or completely attributable to the effect of one of the covariates in analyses mediation is present, and the variable considered a confounder is not a true confounder, but a mediator and part of the causal pathway. In this case, adjustment for the mediator can lead to underestimating the true magnitude of association (107). For example this could be the case when exploring the associations between CRF and adiposity, as both as exposures are putative intermediate variables in the causal pathway to changing levels of CVD (i.e. adiposity is an intermediate variable between CRF and CVD risk). In study IV we tested the independent association of both adiposity and CRF by fitting two different models and by testing for interaction between CRF and adiposity. We found that adiposity was a stronger predictor of future CVD risk levels and low CRF had an additive effect, which was not independent of adiposity level.

Even when carefully assuring the direction of association (i.e. the exposure occurred before the outcome) by stratifying and/or adjusting for baseline values of the outcome, prospective studies might still be vulnerable to reverse causation bias or bidirectional association. That is when the association between outcome and exposure can partly or completely be attributable to the effect of the outcome (e.g. adiposity) on the exposure (physical activity or CRF). This is not likely to be the case in our studies as the outcome in study II and III, adiposity and CVD, is not likely to influence on school type (the exposure). This also applies to study IV as CVD risk levels are not likely to affect adiposity or CRF in a reverse causation.

Other strengths and limitations

In general the strengths of the studies in this thesis are the longitudinal design, large number of children and the detailed collection of covariates, which allows for adjustment for several potential confounders. For paper II and IV the different indicators of adiposity including a direct measure TBF% by DXA is considered a strength. For paper III and IV the relatively high proportion of children accepting the blood sample testing (90%) with 73% of these had both baseline and follow up is a strength. For BP and CRF the follow up attendance was 92%. This high follow up attendance rate is likely to strengthen validity of the estimates, together with thorough sensitivity analyses when attrition rate was lowered due to the inclusion of multiple possible confounders in the statistical models.

In a systemic review assessing school-based interventions introducing extra physical activity to reduce at least one measure of adiposity in children, Connelly *et al* found that compulsory physical activity was more likely to be effective than physical activity offered on a more voluntary basis (108). Wang *et al* assessed the cost effectiveness of the FitKid project (109), an obesity prevention study in 18 elementary schools, and found a significant effect on body fat percentage at a relatively low cost in those attending the program more than 40 % of the time (109). Thus, introducing the extra PE as mandatory at sports schools in our project is considered a strength, as adherence and compliance to intervention is likely to be high compared to afterschool activities introduced as voluntarily, and will eventually contribute to the validity of the exposure (attending a sports school). Another review and meta-analysis on school-based intervention effect on childhood obesity, found that the longer the intervention lasted the larger the effect (92). Therefore the length of 2 years intervention in our analyses is a strength.

The concept of intervention being conducted as a natural experiment means that the researchers had no influence or control of the content and intensity of the PE lessons, other than the expectation, that the teachers followed the age-related concept as taught to them in workshops during the first school year. In this way the observed results are not dependent on researchers or experts set up for intervention, and therefore considered directly transferable into the daily praxis in other school settings. In the perspective of the importance of the diffusion of effective public health strategies, this is considered to be a strength.

Clustering of risk factors occurs in children, and a composite risk score is a better measure of CVD risk than single risk factors (82, 84). Compared to a more vulnerable score of single risks, which is known to be more fluctuating on a day-to-day basis, the use of a composite risk score, as the main outcome in study III and IV, is likely to strengthen the predictive validity of estimates(82, 84). It should be noted, that the single risk factors were not weighted in the calculation of the summed composite risk score. Therefore, the score might not capture the true picture of clinically "at risk". However, little is known as to whether single risk factors should be weighted differently or not.

Main findings in relation to other studies

Effect of intervention (paper II and III)

The main finding in the two studies evaluating the health effect of intervention is that six PE lessons per week at school is significantly effective in preventing OW/OB and reducing levels of CVD risk factors during two school years. Furthermore, the beneficial health effect was larger in the children with the poorest risk score and less healthy weight status at baseline, which is of great importance since this target group can be difficult to reach in other settings without stigmatization.

Body Composition

In study II we found a significant beneficial effect on the prevalence of OW/OB. Moreover, weight status was a significant effect modifier with a larger effect in OW/OB and adipose children. Our findings on change in BMI was insignificant, but in favour of the children in intervention schools.

These results are supported by several recent published school studies on additional PE lessons (93, 95, 110-112). We found no significant effect on BMI although the effect size (β -0.14) on BMI in our study was comparable with those calculated and reaching significance (β -0.15; β - 0.20) in a review and a meta-analysis evaluating the effect of school-based interventions (including PA, dietary and family based programs) on BMI (113, 114). In a cluster randomized school-based study by Jansen et al(115) with a similar intervention volume, a significant change in BMI of β-0.10 was observed for the youngest children (grade

3-5, n=657 intervention versus n=729 controls). These findings could lead to a suggestion of statistical under powering in our study. Post hoc power analysis showed that, given the sample size and the observed effect size (β -0.14) in this study, the power was 0.42. This raises the question whether the effect size is "clinically irrelevant" or the sample size is too small to reach significance for small, but relevant change in BMI. The results of paper IV, linear association with CVD risk factors across the entire BMI distribution, give reason to believe that any change in BMI would be relevant for public health. That any stabilisation of BMI in childhood might be preventive of future co- morbidity in the adult population is supported by a large Danish cohort study(7).

Our findings of a preventive effect on the prevalence of OW/OB is supported by a meta-analysis by Gonzalez-Suarez et al (92). They conclude, that school-based interventions addressing childhood obesity are effective in reducing the prevalence of OW/OB with an OR of 0.74 (95%CI: 0.60 to 0.92) in intervention schools.

It could be put forward that BMI is an inappropriate measure of changing body composition in physical activity intervention studies, as a consequence of the intervention could theoretically be an increase in lean mass. Based on the DXA scans in our study, this was not the case, as we found no significant intervention effect on lean mass (β: -180.2 (g) , p=0.31, 95%CI: -526.19 to 165.76, n=631).

We have not identified any recent school-based intervention studies including DXA scan for the assessment of TBF%. Kriemler et al (the KISS study) reported a significant effect on total body fat measured by a z-score on sum of four skinfolds (β -0.12 95% CI: -0.21—0.03) (116). Their intervention was comparable in volume to the CHAMPS study-DK (5 lessons a week and additional PA homework for 1 year). However prevalence of OW/OB was higher in the KISS population compared to CHAMPS study-DK (26% versus 11%). This higher starting point leaves more room for improvement in both TBF as well as the prevalence of OW/OB. This is in accordance with the higher effect size we found in OW/OB children compared normal weight children. In a meta-analysis (92) including 6 of 19 school-based intervention studies, Gonzalez-Suarez et al also reported significant intervention effects on percentage body fat based on skinfolds. Although sum of skinfolds have been shown to highly correlate with DXA assessment of body composition in children (117)estimations from skinfolds must still be considered as a less direct and precise measurement of adiposity than DXA. Our results might be negatively biased, as the group comprising children with missing data was more OW/OB than those in analysis. Imputing missing data did not change effect estimations significantly, but increased the estimates of effect size on change in TBF% in favour of the intervention schools.

Few school-based physical activity promotion studies report the effect on overweight prevalence or effect modification of overweight. One study by Marcus *et al*(118) measured the effectiveness of a school-based intervention on prevalence of OW/OB, and found in agreement with our results, that the intervention significantly reduced the prevalence at intervention schools. Graf *et al* (112)on the other hand found that neither prevalence nor incidence differed between intervention and control schools after 4 years of intervention. This might be explained by a "levelling off" of intervention compliance during the four years, as reported by the researchers. Also Sollerhed *et al* (93) reported no difference in incidence of OW/OB between intervention and control schools during one school year. Intervention being four PE lessons per week compared to the mandatory one to two PE lessons per week. The study by Sollerhed *et al* might be too small to detect changes in incidence of OW/OB (defined by BMI) in a relatively healthy and lean population (132 children in two schools in Sweden). In our study only 2.5% of the children changed from normal weight to OW/OB defined by BMI. In the study by Sollerhed *et al* that equals approximately two children per intervention group. Results from the meta-analysis by Gonzalez-Suarez *et al* support our findings on effectiveness on prevalence of OW/OB (92).

CVD risk factors

Other school-based interventions aiming at reducing CVD risk factors in childhood have observed divergent effects. Two studies, comparable to the CHAMPS study-DK, used a risk score similar to the one we used in paper III. Bugge *et al* (95) used a composite risk score constructed from sex specific sum of z-scores for SBP, TG, TC:HDL, HOMA-IR and CRF but added the sum of four skinfolds instead of WC. Bugge *et al* found no significant effect on a composite risk score after three years of intervention with four PE lessons per week. This lack of effect could suggest that doubling the amount of PE lessons might not be enough to reduce CVD risk factors. Kriemler et al (116) used, as a secondary outcome, a sum of z scores of TG, inverted HDL cholesterol, glucose, mean blood pressure and WC and found a significant reduction in CVD risk factors after one year of intervention with five PE lessons per week and additional physical activity homework. Results of these two comparable studies support the hypothesis that in order to reach an effect of extra PE in a healthy paediatric population, the magnitude of the intervention must approximate five to six PE lessons per week or one hour per day. This speculation is supported by the study of Resaland *et al*(119) who found significant reductions in single CVD risk factors (blood pressure, VO^2peak, TG, and TC:HDL) after two years of intervention with 60 minutes of PE per day by trained teachers. In contrast to our findings, Resaland and Kriemler observed significantly increased levels of CRF, which is considered a strong predictor of CVD risk (57). The results of three school based intervention studies with no significant effect on CVD risk (120-122) support the idea that a certain volume and intensity is needed in order for an intervention effect to occur, since the intervention program in all of these studies were either of short duration (8 weeks) or small volume (revising existing PE lessons).

The analysis, on the single risk factors included in the composite risk score in our study, suggest that the major contribution to decreased risk was due to considerable changes in SBP, HOMA-IR and TG and less due to changes in TC:HDL, WC and CRF.

The absence of any effect observed on CRF and WC in the present study is somewhat surprising, as these factors are thought to be essential to the underlying cause of the multifactorial development of a poor risk profile. Intervention effect on CRF has substantially been shown in other school-based studies (123). An effect on WC could have been expected in the light of the results on BMI and TBF in paper II. One explanation for the lack of observed effect on these outcomes in our study could be, that the majority of Danish schoolchildren are normal weight and in general have healthy CRF levels, and consequently a change in mean values is not likely to reach significance. Another plausible explanation could be that the primary focus in the intervention program was to ensure joy of moving and playing by enhancing age-related motor skills. Consequently, we speculate that the intensity level in the PE lessons might not have been high enough to improve CRF levels. Furthermore the impact of intervention on change in physical activity levels is not yet evaluated in the project, therefore a compensation for the increased PA at school in leisure time PA, cannot be rejected.

Children, attending intervention schools, decreased their score of HOMA-IR 9% more than children at control schools (borderline significant effect). Two other school-based intervention studies, comparable to the CHAMPS study-DK regarding volume and duration of the intervention program(95, 119), showed no significant effect on HOMA-IR, except for boys in the CoSCIS study (95). In the study by Resaland *et al* (119), introducing 60 min of PE per day, no effect on HOMA-IR was observed despite a significant increase in cardiorespiratory CRF in the intervention group. This is in contrast to our findings where no intervention effect was observed on CRF, even though an effect on HOMA-IR was observed.

Our result on TG is supported by two other school-based studies (116, 119) with similar effect sizes, but not in the CoSCIS (95). The difference in change of the TG equals a 7% fall in serum-TG values in the intervention group compared to the control group (in which TG values actually rose). The clinical relevance of this change is difficult to estimate, but it equals a beneficial change of -0.18 SD compared to the control group.

The observed results on SBP in our study are supported by the studies of Resaland *et al* (119) and McMurray *et al*. (124). Bugge *et al* (95) found only an effect for boys and Kriemler *et al* (116) found no effect on blood pressure despite a significant effect observed on physical activity and CRF levels.

The size of effect on SBP β-1.72 mmHg (equalling β - 0.22 SD) will, if sustained until adulthood, have a considerable impact on future public health, since as little as a 2mmHg downward shift in the blood pressure distribution of the general population could result in an annual reduction in stroke, coronary heart disease, and all-cause mortality of about 6%, 4% and 3%, respectively (125).

Prospective associations of adiposity and CRF with CVD risk

The findings from paper IV are supported by two other recent longitudinal studies in children and adolescents. In The ALSPAC study, a longitudinal study of more than 5000 children from the U.K. aged 9 to 12 at baseline, Lawlor *et al* (51) examined the association of adiposity by the same three indicators of adiposity as used in our study, and reported very similar results on associations of adiposity and CVD risk. In parallel to our findings they found that BMI, WC and TBF% were highly correlated, and were associated with change in CVD risk factor levels with similar magnitudes. Furthermore, Marcus *et al*(126) reported longitudinal results from the HEALTHY trial showing that 2.5-year changes in BMI was strongly associated with changes in single CVD risk factors in U.S. children from 6th to 8th grade (11 to 13 years).

In addition, based on the same cohort, Jago *et al* (127) reported that WC did not add much to the association compared to BMI, except from being better associated to fasting glucose. This is in line with our findings where an increase in WC was only significantly associated to increased HOMA-IR and TG whereas TBF% and BMI was strongly associated to all single risk factors. The HEALTHY trial did not, however, provide direct measures of total body fat by DXA. Hence, our results extend the evidence of the association of adiposity and CVD risk to even younger children than the above-mentioned studies, as our cohort was 2th to 4th grade (7.4 to 11.6 years) followed for two years.

The findings of a linear relationship between CVD risk and adiposity is supported by a report from the ALSPAC cohort (87) and results from the large longitudinal study by Baker *et al*(7). They examined the association of BMI and later coronary heart disease in a large cohort of Danish schoolchildren (276.835 children followed up 25 years later). They concluded that the association of childhood BMI (7 through 13 years of age) to later coronary heart disease was linear across the entire BMI distribution. Our results suggest that this might be valid for the distribution of other adiposity measures as well.

Because CRF level to a large extent is explained by long-term engagement in physical activity, it is likely that adiposity is on the causal pathway between CRF and CVD risk. Our results suggested that the association of CRF with CVD risk was largely explained by adiposity and indicate that this is a key mechanism that explains the inverse association of CRF with CVD risk factors in primary school children. This finding is in line with other longitudinal studies in children (75, 76), but not consistent with cross sectional studies (78, 128) where CRF has been found to be associated with CVD risk independent of adiposity. That the association of CRF tends to attenuate in longitudinal studies might be due to the fact that CRF does fluctuate more rapidly over time than adiposity. The attenuation of the association of CRF to CVD is also not in line with results in the adult population (129). This might suggest that the benefits of CRF are not independent of age, and therefore high fitness becomes more important in youth and adulthood. This speculation is supported by a study of Ondrak *et al* (130). They showed that amongst more than n=1800, 8-16 years old children, association of adiposity to CVD risk declined with age as associations of CRF tended to increase with age.

The finding that greater change in CRF was observed to be more beneficial for boys than girls has to our knowledge not been shown in other studies. Also, results suggest that greater central adiposity has greater effects of raising CVD risk factor levels for boys, although girls increased their WC significantly more than boys. An increase in WC is expected during two years in this age group and a plausible biological explanation to the observed sex-specific associations could be the discordant physiological maturation that occurs between boys and girls.

Conclusions

- Six PE lessons a week can prevent overweight and obesity and reduce the increase in CVD risk factors in school children.
- The effect of intervention is even higher in the children at most need i.e. the most adipose children and the children with the highest CVD risk score.
- The relationship of adiposity to CVD risk factors appears linear across the entire distribution of adiposity regardless which indicator of adiposity is used.
- The relationship of CRF to CVD risk factors appears linear across the entire distribution of CRF.
- The combined effect of CRF and adiposity to CVD risk appeared additive and not multiplicative.
- Any decrease in adiposity and increase in CRF is likely to be beneficial to the future health of the population and no threshold could be established.
- Implementation of six mandatory PE lessons in public schools might be an important contribution to the health of future generations.

Practical implications for future research

In this first part of the CHAMPS study-DK, evaluations have mainly focused on impact (behaviour change regarding physical activity pattern, leisure time PA) and outcome (body composition, CVD risk, CRF, bone health, injuries, back/neck pain, motor skills).

In that perspective the Svendborg Project has been proven effective and implementable in the real world. Given the relatively simple design, primarily increasing the amount of mandatory PE lessons, the concept seems to be achievable for transfer to other Public schools in Denmark. However, more in depth evaluation of the implementation process and the cost effectiveness is necessary before the full advantages and disadvantages of extra PE at school as health promotion can be established and transferred to other settings.

Based on our preliminary results, and the perceived success of implementation of the concept by all stakeholders (politicians, school leaders, teachers, children and parents), the municipality of Svendborg has subsequently sustained the four additional PE lessons and even expanded this to include more grades (pre-school to 7th grade). The number of school children in sports schools receiving extra mandatory PE has now reached more than 3000 compared to the 700 children, which started out in 2008.

In planning future school-based interventions, however, it is important to realize that schools are faced with multiple curriculum mandates and limited financial and staff resources. To encourage politicians, schools and families to give priority to more physical active schools, knowledge transfer concerning the implementation, effectiveness and maintenance of interventions like The Svendborg Project is pivotal.

All sports schools have been asked to provide a kind of "log book" describing how they have dealt with becoming a sports school. Not all sports schools have completed this yet, but they have the potential to provide the municipality with insight of what works, at which level and gain further knowledge on how to run healthy schools. The log books might also be useful as the point of departure for future research on implementation and mediation, exploring the underlying mechanism by which introducing more PE at school influences health and health behaviour.

Transferring effective programs into real world settings, as has been dine in the Svendborg Project, and maintaining them there is a complicated, long term process that requires dealing effectively with the successive, complex phases of program diffusion (131, 132). As the effectiveness of a health promotion program depends on its efficacy, it is important to understand the degree of its reach, adaptation, implementation and maintenance (131, 132). Therefore, understanding the mechanisms for these factors of the intervention is necessary for improving interventions and achieving effectiveness in other settings aiming at making a difference to future public health.

The results in this thesis, showing significant preventive effect on children's health by introducing six PE lessons in public schools, and that any shift in adiposity and CRF is likely to have a public health impact, calls for action by researchers and policymakers engaged in paediatric and public health.

My recommendations are:

- Ensure effective knowledge transferral and diffusion of proven effective interventions.
- Give priority to pragmatic or natural experiments like the Svendborg Project, focusing more on effectiveness than efficacy.
- Replicate effective interventions systematically – the more robust an intervention effect is across different conditions, the greater the confidence that the effect is strong and causal(133)
- Establish a nationwide central registration of effective interventions for Public Health like those seen in the Netherlands(134) and the US (135)
- Act now!

"If we want more evidence-based practice, we need more practice-based evidence"

Larry W Green, 2004, opcit Kessler, 2011(133)

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