Contents

Preface

List of papers

The present Ph.D. thesis is based on the following papers, which are referred to in the text by their roman numerals.

Paper I (Study I): Unit-specific calibration of CSA/MTI accelerometers, model 7164 in a mechanical setup – is it worth the effort?
The effect on random output variation caused by technical inter-instrumental variability in the laboratory and in the field – The European Youth Heart Study

Paper II (Study II): Secular trends in cardio-respiratory fitness and body mass index in Danish children – The European Youth Heart Study
(Scand. J. Med. Sci. Sports., 2007, 17: 331-339)

Paper III (Study III): Secular trends in objectively measured habitual physical activity in Danish children – The European Youth Heart Study

How to read this thesis

In this Ph.D. thesis, Study II & III describe the secular trends in cardio-respiratory fitness, body mass index, and habitual physical activity in two cohorts of Danish third grade school children - the predefined purpose of this thesis. Study I, on the other hand, should be regarded more as a methodological study initiated in a standardized experimental setup in the laboratory since the wish was to improve data quality and study power in Study III. Furthermore, instrument calibration, as performed in Study I, would provide essential information regarding the implementation of potentially important and relevant instrumental precautions needed before assessing habitual physical activity levels by the use of accelerometers within the framework of large scale population studies in general.
The survey text of this thesis will include only the parts of Study I from which results potentially will affect the data analysed in Study III.
The present Ph.D. thesis was initiated and completed at the Institute of Sports Science and Clinical Biomechanics at the University of Southern Denmark, Odense.

Abstract

Introduction: Overweight (OW) and reduced levels of cardio-respiratory fitness (CF) and habitual physical activity (HPA) have been found to be related to cardiovascular disease (CVD) risk factors in children and adolescents. Several risk factors track from childhood into adulthood, and therefore, information about secular changes in body mass index (BMI), CF, and HPA among children could play an important role in the planning of early optimal preventive strategies against CVD. The standardized data investigating the secular trends in CF, BMI, and HPA are rather limited, and we are aware of no other study previously examining these trends in parallel in the same groups of children.

When assessing HPA levels by the use of accelerometry, effective unit-specific calibration seems important in order to minimize the inter-instrumental output variation, which previously has been observed under standardized conditions in mechanical setups. Therefore, unit-specific calibration potentially could improve field data quality and study power (i.e. reduce random measurement error) before performing HPA data analyses – both in this thesis and in general. However, reduced inter-unit variability reached through in-vitro calibration would only be important if random instrumental variability across different units contributes considerably to the total variation in field data.

Aim: The primary aims of the present Ph.D. thesis were:

1) To examine the secular trends in CF, BMI (Study II), and HPA (Study III) in Danish children - all trends will be analysed overall and across socio-economic status (SES).
2) To calculate and apply unit-specific calibration factors in a multiple number of accelerometers in order to examine the impact on random output variation caused by inter-instrumental variability (Study I).

Methods: Two cross sectional studies were conducted in 589 and 458 third grade Danish children in 1997/98 and 2003/04, respectively. CF was determined indirectly by a maximal cycle-ergometer test, and the lowest sex-specific quartile of CF obtained in the study in 1997/98 was used as cut-point for low CF. BMI cut-points according to Cole et al. were used to classify OW, and SES was classified into two different groups (i.e. blue-collar and white-collar) according to parents’ occupation.

Mechanical movements (Study I) and HPA (Study I & III) were assessed objectively by the use of CSA and MTI accelerometers, Model 7164. All accelerometer units were calibrated in the laboratory in four different settings varying in frequencies and/or amplitudes (Study I). The effect of calibration on random output variation caused by inter-unit variability was analysed by comparing raw and calibrated data after multiplying unit-specific calibration factors to a) data obtained during quality checks in a mechanical setup and to b) HPA data collected during free living conditions in the field.
In study III, HPA data was expressed as gender and day type specific (i.e. weekdays vs. weekend days) total counts per registered time in order to yield an estimate of average activity intensity.

Results: Calibration reduced inter-instrumental variability considerably in the mechanical setup, as the SD of the instruments output was reduced from 221 (95% CI=206;239) counts*min^{-1} to 127 (95% CI=118;138) counts*min^{-1} in the group of MTI instruments (CV_{raw}=4.3% vs. $CV_{calibrated}$=2.5%). However, the effect of calibration vanished when applying unit-specific calibration factors to HPA data collected in children, as the SD and CV remained unchanged after calibration (SD_{raw}=219 (95% CI=205;236) counts*min^{-1} vs. $SD_{calibrated}$=220 (95% CI=206;237) counts*min^{-1}, CV_{raw}=30.2% vs. $CV_{calibrated}$=30.4%). In addition, high correlations (r=0.99) were reached between raw and calibrated field data, and the amount of variation introduced by multiplying unit-specific calibration factors to HPA data was estimated to be as low as 1.1% of the total variation. Furthermore, compared to the CSA instruments a significantly increased (9.95%) mean acceleration response was observed post hoc in the batch of MTI instruments.
As the only significant secular trend, this thesis revealed a 2.8% decline in CF in girls overall. Furthermore, significantly increased CF was observed in boys with high SES, significantly increased BMI was observed in boys with low SES, and finally, a significantly increased prevalence of OW was observed in girls with low SES. However, after additional Bonferroni's correction none of the statistical analyses performed across socio-economic gradients reached the level of statistical significance (i.e. $p<0.05$). No significant secular trends were observed in the level of HPA. However, boys where found to be more physically active compared to girls, but especially so during the weekdays.

Conclusion/perspectives: Unit-specific calibration factors, which reduced inter-instrumental variability in the experimental setting in the laboratory, were found to be rather ineffectual when applied to HPA data collected in the field. This inter-instrumental variability was relatively small when compared to the total variation in HPA data, and in all probability the effect of calibration was primarily attenuated in the field by other major sources of variation (e.g. day-to day variation, biomechanical variation, and random variation caused by varying off-vertical axis of measurement between subjects). However, technical assessments should always be conducted in order to determine the acceleration response in any specific batch of instruments being used, thereby avoiding potentially biased results due to batch effects. Furthermore, a simple and less time consuming quality check should be performed in all units immediately after they are returned from the field, since this procedure will prevent decidedly broken instruments from being returned into the field repeatedly.

During the period 1997/98 to 2003/04, CF had declined significantly in girls overall. Although not statistical significant after additional Bonferroni's correction, results in general showed less favourable trends in low SES children. Thus, describing secular trends in CF and BMI overall without any regard to SES might disguise social-caused differences.

The present thesis does not support the idea that Danish children of today are becoming less physically active. However a limited statistical power should be considered, especially when interpreting trends across SES. Further studies should be conducted in subjects of different ages in different countries, in order to verify whether the present results reflect more widespread trends.

Abstract in Danish (Dansk resumé)

Baggrund: Overvægt, lav fysisk form og reduceret fysisk aktivitetsniveau har vist sig at være relateret til hjertekarsygdomsrisikofaktorer hos børn og unge. Flere risikofaktorer har desuden vist sig at "tracke" fra barndommen og ind voksenlivet. Detaljerede informationer i forhold til den sekulære udvikling i "body mass index" (BMI), fysisk form og aktivitetsniveau blandt børn og unge kan derfor vise sig yderst relevant, når man ønsker at planlægge eller evaluere tidlig intervention rettet mod optimale forebyggelsesstrategier. Mængden af standardiserede data der tidligere har beskrevet den sekulære udvikling i fysisk form, BMI og aktivitetsniveau hos børn er relativt begrænset, og intet andet studie har, så vidt det vides, endnu undersøgt den samtidige udvikling indenfor disse tre områder i de samme to grupper af børn.

Når fysisk aktivitet måles ved hjælp af accelerometri, syntes det at være af afgørende betydning at være i stand til at udføre en effektiv enhedsspecifik kalibrering, der kan reducere den betragtelige "inter-instrumentelle" outputvariation, der tidligere er blevet registreret under standardiserede forhold i mekaniske opsætninger. En sådan kalibrering kan derfor potentielt set forbedre kvaliteten af feltdata (reducere den tilfældige målefejl), og dermed øge den statistiske "power" inden der foretages analyser på aktivitetsdata – både i denne Ph.d.-afhandling og generelt set. En eventuel reduceret "inter-instrumentel" variation, opnået ved kalibrering in-vitro, vil i denne sammenhæng dog kun være interessant, hvis målevariationen de enkelte skannere imellem er af væsentlig størrelse i forhold til den totale variation i feltdata.

Formål: De primære formål med denne Ph.d.-afhandling var:

1) At undersøge den sekulære udvikling i fysisk form, BMI (Study II) og fysisk aktivitetsniveau (Study III) hos danske børn, idet udviklingen blev undersøgt både generelt og på tværs af socioøkonomisk status (SØS).
2) At beregne, og efterfølgende anvende, instrumentspecifikke kalibreringsfaktorer i et større antal af accelerometre. Målet var efterfølgende at undersøge kalibreringens indflydelse på den tilfældige målevariation forårsaget af "inter-instrumental" variabilitet (Study I).

Metode: To tværsnitsstudier blev udført på 589 og 458 danske tredjeklasses skolebørn i henholdsvis 1997/98 og 2003/04. Fysisk form blev bestemt ved hjælp af en indirekte maksimal cykeltest. Den laveste kønsspecifikke kvartil i fysisk form opnået i studiet i 1997/98 blev benyttet til bestemmelse af grænseværdien for lav fysisk form. Overvægt blev klassificeret i henhold til BMI-grænseværdier beregnet på baggrund af anbefalinger fra Cole et al. SØS blev klassificeret ud fra oplysninger om forældrenes arbejde, idet der blev skelnet imellem manuelt og ikke manuelt arbejde.

Mekanisk bevægelse (Studie I) og fysisk aktivitet (Studie I & III) blev målt objektivt ved hjælp af CSA og MTI accelerometre, Model 7164. I laboratoriet blev alle accelerometerenhederne kalibreret i fire forskellige opsætninger, som varierede i frekvens og/eller amplitude (Studie I). Effekten af kalibrering på den tilfældige outputvariation forårsaget af "inter-instrumental" variabilitet blev analyseret ved at sammenligne rå data med kalibrerede data, idet de enhedsspecifikke kalibreringsfaktorer blev multipliceret på a) data der var indsamlet under løbende kvalitetstjek i den mekaniske opsætning i laboratoriet, og på b) aktivitetsdata der var indsamlet under naturlige forhold i felten.

For at opnå et gennemsnitligt estimat af børnenes generelle dagligdagsaktivitetsniveau (Studie III) blev det totale antal "counts" bestemt i forhold til den totale registrerede tid på tværs af køn og dagstype (henholdsvis hverdage og weekenddage).

Resultater: Kalibrering reducerede den "inter-instrumentelle" variation betragteligt i den mekaniske opsætning, idet SD på output blev kraftigt reduceret fra 221 (95% CI=206;239) counts/min. til 127 (95% CI=118;138) counts/min i gruppen af MTI skannere ($CV_{rå}$=4.3% vs. $CV_{kalibreret}$=2.5%). Effekten af kalibrering forsvandt dog på data indsamlet på børn i felten, idet SD og CV forblev uændret efter kalibrering ($SD_{rå}$=219 (95% CI=205;236) counts/min. vs. $SD_{kalibreret}$=220 (95% CI=206;237) counts/min., $CV_{rå}$=30.2% vs. $CV_{kalibreret}$=30.4%). Derudover blev der observeret en meget høj korrelation (r=0.99) imellem rå feltdata og kalibrerede feltdata, og mængden af den variation der kunne tilskrives kalibrering blev estimeret til blot at udgøre 1.1% af den totale variation. Desuden blev der sammenlignet med CSA skannerne post hoc konstateret et signifikant højere (9.95%) gennemsnitlig accelerationsrespons i gruppen af MTI skannere.

Som den eneste signifikante sekulære ændring afslørede denne afhandling et gennemsnitligt fald på 2.8% i fysisk form hos pigerne. Sammenlignet med 1997/98 blev der i 2003/04 desuden registeret forøgede værdier i fysisk form hos drenge med høj SØS, forhøjet BMI hos drenge med lav SØS, samt forøget prævalens af overvægt hos de piger, der havde lav SØS. Efter yderligere Bonferroni's korrektion blev der dog ikke opnået statistisk signifikans ($p<0.05$) i de analyser, der blev fortaget på tværs af SØS. Der blev ikke registreret signifikante ændringer i aktivitetsniveauet, hverken hos piger eller drenge. Det blev dog observeret, at den forskel der er i aktivitetsniveau imellem drenge og piger især gør sig gældende i hverdagene.

Konklusion/perspektivering: Enhedsspecifikkalibrering, som reducerede ”inter-instrumentel” variabilitet i den eksperimentelle opsætning i laboratoriet, kunne ikke tilskrives nogen nævneværdig effekt, når den blev anvendt på data, der var indsamlet på børn under helt naturlige forhold i felten. Sammenlignet med andre variationskilder i børns aktivitetsniveau var den ”inter-instrumentelle” variabilitet kun lille, og en mulig effekt af kalibrering i felten blev derfor efter al sandsynlighed reduceret pga. tilstedeværelsen af flere andre store variationskilder.

Det viste sig, at forskellige generationer af den samme type af accelerometer ikke kan forventes at udvise identiske accelerationsresponser. Det er derfor altid vigtigt at foretage tekniske målinger, således at den aktuelle accelerationsrespons kan bestemmes i den gruppe af accelerometre, der benyttes. Sidst men ikke mindst, er det vigtigt at foretage løbende kvalitetstjek af alle enheder når de bruges, idet en sådan procedure vil forhindre, at defekte instrumenter gentagne gange udleveres til nye forsøgspersoner.

I perioden 1997/98 til 2003/04 blev der registeret signifikant nedgang i fysisk form hos danske piger. Selvom der ikke blev opnået statistisk signifikans efter yderligere Bonferroni’s korrektion, så viste resultaterne generelt en mindre gunstig udvikling hos børn med lav SØS. Meget tyder derfor på, at man meget vel kan komme til fejlagtigt at skjule forskelle forårsaget af socioøkonomiske påvirkninger, hvis man blot beskriver den sekulære udvikling i fysisk form og overvægt uden hensyntagen til SØS.

Denne Ph.d.-afhandling støtter ikke opfattelsen af, at nutidens danske børn bliver stadigt mere inaktive. Resultaterne bør ses i lyset af en begrænset statistisk power, specielt når man tolker på analyserne foretaget på tværs af SØS. Vi ved dog ikke i hvor høj grad, vores resultater afspejler en mere generel tendens, førend der iværksættes flere studier af børn i forskellige aldre i flere lande.

List of abbreviations

BF	Body fatness
BMI	Body mass index
CF	Cardio-respiratory fitness
CI	Confidence interval
CV	Coefficient of variation
EYHS	European Youth Heart Study
HPA	Habitual physical activty
OW	Overweight
PA	Physical activity
SD	Standard deviation
SES	Socio-economic status

Introduction

Lifestyle related diseases - a rising problem with indications of establishment early in life

Lifestyle related diseases such as Type II Diabetes, cancer, and cardiovascular diseases (CVD) constitute the most serious threat against public health in the industrialized Western World, and today the burden of non communicable diseases is increasing even in the developing countries [1]. An inappropriate lifestyle recognized in particular by high alcohol intake [2-4], smoking [5,6], sedentary behaviour [7-9], and a high caloric diet rich in fat [10,11], has been found to be associated with the reduced health status. Consequently, changed lifestyle habits, predominantly as a consequence of the wealthy society, are addressed as important issues when trying to identify the elements being responsible for the increased prevalence of non communicable diseases [12,13].

Although the clinical manifestations of lifestyle related diseases typically are absent until adulthood, bad habits picked up in childhood can not be considered benign conditions since there are several pathways through which childhood behavior might influence health status.

For instance, a possible direct relationship between lifestyle during childhood and health status in adulthood is being supported by findings from longitudinal studies, where physical activity (PA) and/or cardio-respiratory fitness (CF) during youth has been found to be related to CVD risk factors in adulthood [14,15]. In addition, CVD and all-cause mortality have been reported to be elevated among adults who were overweight in childhood irrespective of adult weight [16,17]. Furthermore, lifestyle during childhood may be related to health status during childhood, which again is an important predictor for health status in adulthood, or lifestyle in childhood might even be related to lifestyle during adult age, which again is directly related to health status in adulthood [18].

Evidence supporting these theories has been recognized in the literature, where behavioural characteristics in childhood, such as being physically active, has been found to reduce antecedent risk factors for coronary heart diseases [19]. Furthermore, major health risk factors like low PA, low CF, overweight (OW), smoking, and poor food habits have been found to persist from childhood into adolescence [20-22] and from adolescence into the adult years [23,24]. The persistency of an individual's risk factor level is defined as "tracking". The tendency of tracking of risk factors indicates that successful interventions carried out to reduce lifestyle related disease incidences should be initiated as early as in childhood. Therefore, early primary interventions strategies have become a crucial subject of high priority in many countries.

Cardiovascular risk factors and the childhood period

CVD is a disease of major personal and social economic impact, and today it is considered as a major threat to the public health, especially in the Western world [1]. According to the European Heart Network, more than 35% of all deaths in Denmark in 2005 were caused by CVD [25], and the World Health Organization (WHO) has stated that unless current trends are halted or reversed, over a billion people will die from CVD worldwide in the first half of the 21st century [26]. Unfortunately, interventions designed to reduce CVD risk in adults have had only limited effect, but some evidence exists that greater success may be achieved with children [27,28].

Although the established causal links between health status and CVD risk factors not yet have been confirmed in children to the same extent as in adults, both behavioral, physiological, and genetic risk factors for CVD can be identified in children and young people [29,30], and according to autopsy studies evident atherosclerotic lesions are identifiable even in young children [31-33]. Furthermore, besides being recognized as independent risk factors for CVD mortality in men and women [34,35] OW and low CF have been found to be related to CVD risk factors, such as elevated blood pressure and unfavourable blood lipids, at an early stage in children [36-38].

The role of PA in the fight against low CF, excessive body fat, and impaired glucose tolerance in adults is generally believed to be crucial [39-42], and increased PA has been described as an important contributor to reduced CVD and CVD mortality in adults [43-48]. However, CVD and subsequently deaths are typically absent until adulthood. Therefore, when investigating the health benefits of PA in children and young people it is usually performed by focusing on risk factors known to be linked with disease end points in adults. However, when being reviewed, these relationships in children have been reported only to be weak to moderate at best [49]. This could be due to the fact that at early stages of life the beneficial effect of PA can not yet be observed to its fully distinct, as well as due to the use of different and less reliable measurement techniques when trying to assess PA. Rowlands et al. [50] have concluded that the relationship between body fatness (BF) and habitual physical activity (HPA) is dependent on the assessment technique being selected to quantify HPA.

It is biological plausible that PA improves the metabolic health profile - also in children, and objectively measured HPA has previously been found to be inversely related to the risk factors related to the metabolic syndrome in Danish children [51]. Furthermore, a graded negative association has recently been observed between clustering of risk factors and HPA in children from three different European countries [52]. Early indications of reduced health status in children and adolescents indicate that preventions and treatments of CVD risk factors should be thoroughly planned and evaluated if attendant substantial and long-lasting health consequences should be avoided.

Socio-economic status

Several studies have shown that the highest prevalence of CVD risk factors, including obesity, low PA, and smoking, is found in adults with low socio-economic status (SES) [53-55]. In contrast, associations between risk factors and SES have been more inconsistent when examined in children [56-58]. However, unfavourable insulin resistance profile has been reported in Danish children coming from families with low SES [59], and childhood SES has been found to be related to CVD risk factors, CVD, and mortality in adulthood [60-64], indicating that socio-economic characteristics might be a parameter of substantial importance, also when examining early indication of health status during childhood.

Socio-economic status and health status in children – potential links and general considerations

There are several possible pathways, including behavioural, psychosocial, materialistic, economical, and environmental aspects, through which parent's SES might influence children's health status. Environmental aspects, for instance, might be linked with available space and possibilities for outdoor play, safety concerns, etc. [65,66]. Time spend outdoors by children has been reported to be strongly related to the energy expenditure used on physical activities [66], which indicates the significant importance of the environment surrounding the children. Furthermore, poor behavioural and psychosocial parental habits or characteristics might be adopted by children, and thereby parents will serve as an example of how children behave and live their life. Associations between parents being physically active and children's PA have been observed in previous studies [67-69], speaking in the favour of the theory being raised above, although associations between mothers or fathers being active and children's sports participation has been observed to diminish over time when examined during the period 1985 to 1997 [70]. Contributing to the body of evidence indicating that children are influenced by their parents' socio-economic position are results observed in several studies where the food or dietary composition has been examined. Healthy dietary has been found to be more likely in adults with high education compared to those with low educational level [71], indicating a potential link between choice of food and economical resources or reflections towards the importance of healthy lifestyle in general. Therefore, poor dietary intake, another important parameter in relation to health status, recognized in children from lower social groups potentially reflects the influence of the parents as being a role model for their children. Evidences supporting this theory have been observed in studies from Great Britain, where the highest level of fat consumption has been observed in pre-school children coming from households where the head of the family was in manual employment [72], and where 8-11 years old children coming from families with lower parental occupational social class have been reported to be less likely to eat fruit and vegetables than children coming from high social background [73]. Similar findings have been observed in a Swedish study, where adolescents from families with a low educational level of the mother had a higher relatively dietary fat intake than children with highly educated mothers [74].

Demographic influences

Disparities in health status across socio-economic gradients can be observed in both developed and developing countries, but due to the substantial difference between societies in opposite directions [56]. Therefore, in contrast to developed countries, both children and adults with high SES in developing countries have the highest prevalence of OW and obesity, and furthermore they are recognized as being less physically active [56,75]. However, the burden of reduced health status tends to shift towards the groups with lower SES as the country's economic resources increases [76,77]. Therefore, even developing societies are believed to experience an economic growth, which seems to cause a transition throughout the economic strata creating an adoption towards Western lifestyle especially through an increased disposable income, which in return will cause a more sedentary lifestyle and increased prevalence of OW [78].

Different direction of the association between socio-economic position and insulin resistance has been observed between children coming from Denmark and Estonia or Portugal. Lawlor and colleagues [59] also hypothesised that substantial differences in the economical resources across the different countries was the major explanation for these observed disparities.

Commonly used definitions of socio-economic status - strengths and limitations

Studies examining the association between socio-economic position and health have been conducted in many years most often by applying the level of education, income, or occupation as a measure for SES, although there in general is little consensus about conceptualizing and measuring SES [56,79].

When defining SES by educational level, the attempts is to capture the knowledge-related assert of a person, which may affect a person's cognitive function potentially important when receiving health promoting messages, or when trying to communicate with various health organs [80]. Despite holding information on important issues, the classification of SES by focusing on educational level suffers from several limitations, including unequal economic returns for a given level of education across different groups of gender, employments status, and ethnicity. The level of education is usually fixed after young adulthood, and therefore rarely influenced by poor health later in adulthood. This, of course, should be considered as an advantage. On the other hand, the fixed level of education at early stages in life might provide some problems and limitations, since early stability may preclude capturing how changes in economic well being or social standings or networks potentially may affect health status [81,82].

Estimating SES on the basis of income reflects the access to goods and services, which may improve health directly through health services and leisure time activities, or indirectly through an increased level of education [80]. However, problematic is the complex measure of income. Optimally, family income should be adjusted for family size. Family or individual income can be measured, and maybe non-cash benefits should be included, thereby providing a measure of wealth and total assets [79].

Another commonly used proxy of SES is occupational level, which compared to educational level, has been found better to describe social gradients in mortality risk in adults [83]. Occupational level reflects such elements as social networks, stress, control, work tasks, and specific environmental characteristics. Furthermore, occupation may be related to both income and education, and therefore, the association with health status may be both of a direct and indirect relation, characterized by economical and material resources as well as by cognitive perceptions and beliefs [80]. Occupational classification is often reduced to two broad categories of manual and non-manual occupation, which has increased comparability through its rather widespread use in vital statistics and various surveys. A key limitation, however, is the subjectivity of its theoretical or non-theoretical basis. Furthermore, only current occupation is usually considered, which could easily be biased by the impact of health or disease [84]. In addition, occupational classification will suffer from recent changes in the occupational structure, which might complicate comparison across time or across different countries [80].

Optimally, childhood socio-economic position should be based on the household class in which the child resides, rather than using only individual-level measures of socio-economic position of either the woman or the man. Two different approaches commonly used to capture household class are recognized by a) registration of the most dominant and powerful individual class position in the household, regardless of gender, and by b) classification of the actual, and at time discordant, class and gender composition of the relevant heads of household [82].

The European Youth Hearth Study

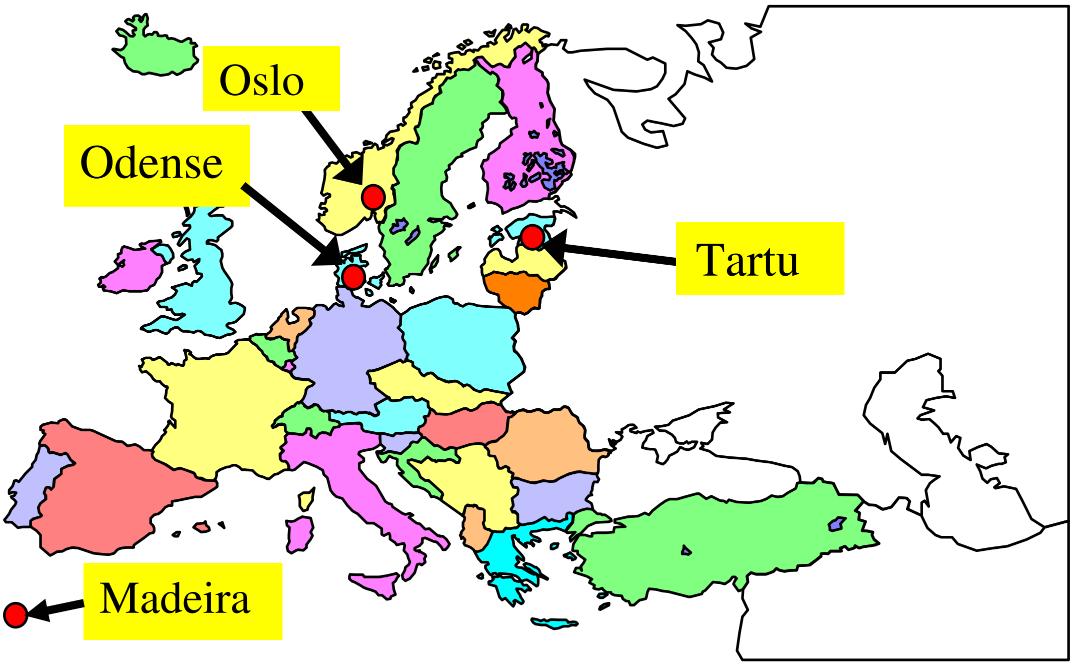
Effective interventions towards increased health status in early life can only be performed when detailed knowledge in terms of multiple risk factors and their possible pre-disposers is possessed. In order to oblige this demand, several large scale epidemiological studies have been designed and initiated (e.g. The Amsterdam growth and Health Study, The Bogalusa Health Study, The National Health and Nutrition Examination Survey, The Northern Ireland Young Hearts Project, The Danish Youth and Sport Study, and The European Youth Hearth Study).

The present Ph.D. thesis relies on cross sectional data from the Danish part I & II of the European Youth Heart Study (EYHS), a study which first was contemplated in the mid nineties when a group of European exercise scientists met and decided to investigate possible multiple CVD risk factors in children and young people.

The EYHS is an international multi-center-study (see Figure 1), designed to study and focus on a wide range of possible CVD risk factors and their potential pre-disposers.

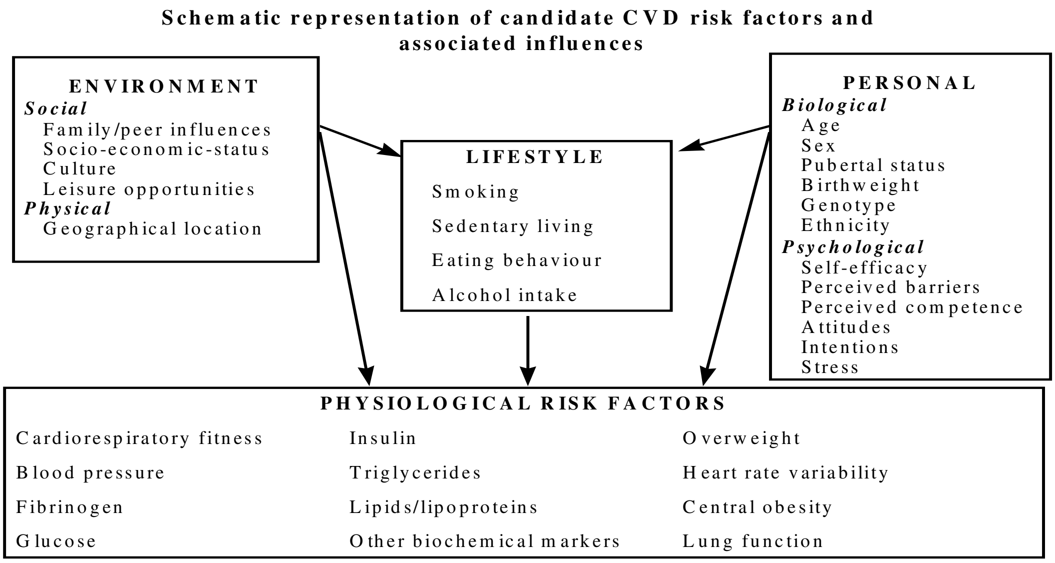
Figure 1

Study locations in the European Youth Heart Study



The overall aim of the study is to study the nature, strengths, and interaction between personal, environmental, and lifestyle influences on CVD risk factors in children and young people of different age, sex, culture, and ethnicity. The risk factors and their possible interrelated associations are schematically illustrated in Figure 2.

Figure 2



The Danish part of the European Youth Heart Study

In 1997/98, the first part of EYHS was carried out in Denmark. The target group was third grade children (aged 8-10 years) and ninth grade adolescents (aged 14-16 years), and the following parameters were assessed in a sample of 1020 children and adolescents:

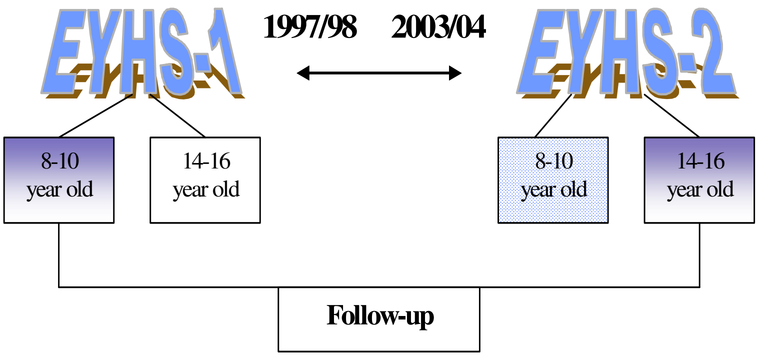
- Biological factors: s-lipid profile, blood pressure, insulin sensitivity, obesity, and CF.
- Lifestyle factors: HPA level, dietary habits, and smoking habits.
- Personal and environmental factors: Self-perception, socioeconomic issues.
- Genetic factors: Familiar predisposition of CVD.
- Furthermore, the children and adolescents were examined for any back problems (MR-scanning).

Six years after in 2003, the second part of the EYHS was carried out in Denmark. The implementation of EYHS-II has extended the design into a mixed longitudinal design, and today the study is one of few large cohort studies in Denmark capable of isolating possible age, cohort, and time of measurement effects. Therefore, the EYHS has the potential to provide valuable information in the area of concern, and thereby play an important role especially in the fight against lifestyle related diseases (e.g. CVD and Type II diabetes).

Prior main results from the Danish part of the EYHS-I revealed that clustering of CVD risk factors was evident in children and adolescents with low CF [85], and in addition a secular trend with declining CF and increasing BF was observed in children during the period 1985/86 to 1997/98 [86]. Therefore, the predefined purpose of this Ph.D. thesis was to provide valuable information to the question as to whether the previously observed unfavourable trends had continued into the first years of the 21st century or not, and whether secular trends in HPA during the same period of time would yield comparable results.

The overall mixed longitudinal design in the Danish part of the EYHS has been out-lined in Figure 3.

Figure 3:



Briefly presentation of basic health related terms examined in the present thesis

Cardio-respiratory fitness

Physical fitness is a complex phenomenon that has been defined in various terms. However, it is widely accepted as a number of characteristics that people have or achieve, which relate to the ability to perform PA in one form or the other [87]. Although several dimensions of physical fitness have been identified and applied in research studies, the component that correlates most strongly to health status is cardiovascular endurance. The term has been intensively studied in both children and adults and is also often referred to as aerobic- or cardio-respiratory fitness [88]. Low CF has been found to be an important independent predictor for atherosclerosis in middle aged men [89]. Furthermore, low CF is strongly associated with CVD, and causes increased mortality independent of other risk factors, including body weight [35,90].

Maximal oxygen uptake (VO_2 max) is widely recognised as the best single indicator of CF since it represents the highest rate to which an individual can consume oxygen during exercise and, therefore, limits the capacity to perform aerobic exercises [91]. Evaluating the level of VO_2 max is primarily performed in the laboratory due to the impractical aspects when carried out in the field. Although the activities characterized by a marked aerobic component are numerous, especially in children, practical considerations typically have limited testing in the laboratory to a treadmill- or cycle-ergometer setting. In these two settings, VO_2 max can be measured objectively and precisely by respiratory gas analyses during an all-out performance [92]. Alternatively, cardio-respiratory capacity can be estimated as VO_2 max obtained from theoretical extraction of data obtained during a submaximal performance (e.g. a cycle- or step test) [93,94],

or more straightforward estimated according to maximal performance attained in the field, typically by the use of various maximal running tests [95]. However, directly measured VO_2 max is considered as the "Golden Standard" in the evaluation of aerobic fitness [92].
When assessing VO_2 max, the conventional method assumes that oxygen uptake rises with increasing exercise intensity up to a certain point, usual referred to as a VO_2-plateau, beyond which no further increase in oxygen uptake takes place, even though the subject is still able to increase her/his exercise intensity [96]. In children and adolescents, on the other hand, a definite plateau does not always occur. However, a study by Armstrong et al. [97] failed to prove any difference in the level of oxygen uptake between children who did and did not reach a VO_2-plateau. Therefore, in the paediatric work physiology, it has become common to use the maximal level of oxygen uptake attained with increasing exercise intensity as the definition of VO_2 max, even though the VO_2-plateau might not be reached. However, the term peak VO_2 has been suggested as an appropriate term to use in children and adolescents rather than the term VO_2 max, which conventionally implies a plateau in the oxygen uptake [92].
Since no plateau in the VO_2 uptake can be expected to occur in children or adolescents, alternative methods must be applied in combination with the registration of a possible levelling off when trying to decide whether or not a child actually have reached the maximal level of cardio-respiratory capacity. Parameters commonly applied in order to determine this typically include maximal heart rate, blood lactate, respiratory exchange ratio, as well as a subjective judgement by the test-observer.
Unfortunately, directly measurement of the maximal oxygen uptake in children has limited applicability in epidemiological research where the wish is to evaluate the maximal aerobic capacity in a large number of subjects, although Hansen and colleagues [98] recently proved that it is possible, even in 6-7-year-old children. The best alternative to the direct assessment technique is an indirect maximal VO_2 test known to correlate highly with directly measured VO_2 max. Estimating maximal oxygen uptake indirectly by applying algorithms or equations has been successfully validated in children on several occasions [99-101].

Body fatness

Obesity and OW is a widely formed definition that relates to excessive fat tissue emerging as a result of a long-term positive energy balance, and several methods have been applied in order to describe body composition or give an estimate of the amount of body fat. Methods range from indirect calculations (e.g. BMI, Waist/hip ratio, and Skinfold Measurements) primarily based on rather simple anthropometrics to more sophisticated techniques introducing directly measurements of the amount of body fat (e.g. Electrical Impedance, Isotopic Dilution, Hydrostatic Weighing, Computerized tomography (CT), and Dual-energy x-ray absorptiometry (DEXA)). Hydrostatic Weighing is problematic in children, however, due to a changing relationship between density and skinfold thickness caused by changing maturity of the chemical component of the fat free mass during growth and maturity [102,103].

Furthermore, in general direct measurement techniques are recognized by high costs and reduced practicability in large scale population studies in particular. Therefore, skindfolds measurements or BMI calculations have been predominantly used as an indirect indicator of body fat in epidemiological studies [36,104,105]. Although skinfolds measurements represent a direct measure of the subcutaneous fat tissue at the specific locations being measured, the technique must be regarded as indirectly responsive to biological maturity, gender, and ethnicity when applied in order to estimate of the total amount of body fat. Therefore, Slaughter et al. [106] developed a number of equations, which relate sum of skinfolds to the percentage of body fat in children and adolescents taking potential confounders, including chemical immaturity of the fat free mass, into account. Deurenberg et al. [107] also proved that fairly good validity of skinfolds measurements can be obtained when using underwater weighing as reference, and when dividing girls and boys into pre pubertal, pubertal, and post pubertal groups. However, the skinfold measurement technique displays substantial sensitivity to inter-user variability [108], and in general limited consensus has been reached when trying to convert skinfold thickness in children into body fat percentage [109].

Although BMI ignores the possibility that muscle tissue rather than fat tissue may contribute to an excessive body weight relative to height, and although BMI is proportional with height, especially causing reservations in growing children, BMI has been reported to be a useful measure of BF in children as long as age, gender, and biological maturity is controlled for [110,111]. BMI is very easy to measure and highly reproducible, and therefore highly feasible in large scale population studies. BMI is the most often used measure of BF in epidemiological studies, and BMI is used as a standard definition of OW by the International Obesity Task Force [112].

Physical activity

Average daily energy expenditure consists of three main components: basal metabolic rate, the thermic effect of food, and PA [113,114]. The term PA is typically operationalized in two different modes, which refer to the context in which they typically take place. The two different modes are: 1) occupational PA, and 2) leisure time PA [115].

The first mode takes place at the working location and is constituted by different task requiring any body movement, whereas the latter mode refers to any activity that an individual volunteer to engage in, which will lead to an increased energy expenditure [116]. Leisure time PA comprises different elements such as household chores, gardening, and transportation serving other purposes than PA itself, as well as more structured and planned exercise performed in order to maintain or enhance physical fitness and health. However, a more general and overall definition of PA is described as a complex set of behavioural patterns, which in one way or the other involve body movement on the condition of energy expenditure [87].

Due to the typical everyday life of children the traditional classification of PA into the two modes of occupational- and leisure time activities are less meaningful, however. Children have been reported typically to be involved in a wide range of short burst activities (e.g. different games, jumping, dancing, running, climbing, and biking), which to a high degree are based on spontaneity [117,118]. In the research literature, the term HPA refers to the energy expenditure reflecting the total amount of all physical activities. HPA is generally considered as an important dimension of childhood activity, especially since the high spontaneity of children's movement characteristics will complicate activity specific assessments and comparisons [119]. Therefore, especially in the paediatric population there is an urgent need for an accurate and precise measuring technique capable of registering the various and complex aspects of PA in children, which in total will constitute HPA. However, severe methodological difficulties and inconsistencies combined with substantial biological variation have characterized the major problem regarding reduced validity and reliability when trying to assess the level of the complex phenomenon PA - especially among children.

Over the years, more than 30 different instruments have been utilized in the attempt trying to develop a reliable and practical measurement technique. Methods have ranged from subjective measures such as questionnaires, interviews, and diaries to more or less objective measures such as behavioural observations, heart rate monitors, doubly labelled water, and calorimetry. However, none of these methods have proven sufficient practical, valid, and reliable to emerge as a standard method [120,121]. The common problem is, that the easier and more practical a method of measurement is the less precise and valid it becomes. Considerably reduced reliability and validity have been reported in particular when applying self-report measures in children, and self-report methods based on recalls has been dissuaded if assessments among children younger than 10 years of age is the goal [122]. Even more critical, PA estimates by the use of self-reports have severe limitations with people of all ages [88,123,124]. This is highly regrettable, considering the fact that self-reporting has been the most frequently used method when assessing PA in epidemiological studies, primarily due to the low costs, the high degree of feasibility, and the potential availability of detailed information (e.g. type, frequency, duration, and intensity of PA).

The doubly labelled water- and indirect calorimetry techniques are the most accurate methods when trying to assess PA in the field, and doubly labelled water is considered to be the "Golden Standard" for the quantification of total energy expenditure in free living [125]. However, the major draw backs of this technique are the high costs and time consume, as well as the limited available information with no frequency, intensity, or duration of activity reported, thus not applicable for large scale population studies [120,126].

PA can also be measured using heart rate monitors. Heart rate does not measure PA directly, but instead provides an adequate information of the relative stress placed upon the cardiopulmonary system as a consequence of sustained PA [127]. Problematic, however, is that PA is not expected to be the only factor responsible for an changed heart rate [128], and instead monitoring of heart rate is influenced by several factors, including body size, age, temperature, genetic, and fitness [129]. The use of heart rate within low PA intensities is associated with reduced validity in particular, due to the fact that monitoring of heart rate in the lower zones is affected substantially by transient emotional caused factors with no relation to physiological stress. In general, heart rates below 120 bpm. are not considered to be a valid estimate of PA [130]. Therefore, using heart rate monitoring as an estimate of daily PA in children must be considered inappropriate since between 60% and 70% of a day typically is spend with heart rates below 120 bpm., when examined in children aged 3-5 year old and 6-7 year old, respectively [131,132].

Furthermore, to be able to account for inter-individual heart rate differences, individual calibration curves reflecting the association between heart rate and oxygen uptake should be established. This procedure will limit the feasibility in large scale population studies, however, and few research studies have performed such calibration [133].

In time with recent technological advances and developments much emphasis has been placed on accelerometers or integrated instruments combining both accelerometry and heart rate monitoring. Accelerometers are devises that measure the mechanical aspects of PA, and a number of different instruments have been developed, including different pedometers [134,135] with a limited purpose range suitable for only walking and running [136,137]. Early pedometers were known as rather primitive mechanical motion sensors, but advantages within electronic technology during recent decades have lead to the introduction of modern electronic motion sensors. Several devises have been developed, and are most easily characterized by the number of available measuring axis. Available instruments contain one (uniaxial) [138] [139] or three (triaxial) [140-142] bender elements, measuring one dimension (longitudinal) or all three dimensions (longitudinal, anterior-posterior, and medio-lateral) of movement. Since accelerometers is capable of practically to register important aspects of PA (e.g. duration, frequency, and intensity) the instrument will provide many of the objective requirements, which seems essential within the assessment of PA in children, and recently an increasingly use of accelerometers has emerged when monitoring PA levels in children [143-145]. Lately, increased attention has been paid to integrated instruments combining both accelerometry and heart rate monitoring, and in general improved measurement precision have been achieved when compared to single-measure estimates derived from accelerometry alone [146-148].

The large number and diversity of methods, which have been applied throughout the years in order to assess PA witness not only of the difficulty in comparing PA data between different studies and the complexity of the parameter itself, but it also witness of the huge challenge left for future research in order to combine accuracy and practicability, especially when monitoring children's HPA in large scale population studies.

Rationale for carrying out the studies described in the present thesis

Why examine the effect of unit-specific calibration of accelerometers?

Information on instrumental validity and reliability is important not only within the framework of the EYHS, but also in general since the use of accelerometers has become more and more common in recent research. The raw accelerometer output (usually referred to as accelerometer counts) is somehow difficult to relate to a more meaningful and understandable indicator of PA, and therefore, several studies have been initiated in order to validate how these accelerometer counts is related to different types of activities and/or intensity thresholds [149-154], as well as to overall or activity specific energy expenditure [155-159].

Most companies perform a calibration check before shipping in order to ensure that different units provide similar acceleration responses. However, this type of technical calibration has rarely been described by the manufactures, and therefore, as a consequence some research groups began to conduct their own calibration [160]. On the other hand, most research teams do not incorporate unit-specific calibration into their study protocol, but instead extensive calibration is often performed only when broken instruments are returned to the manufactures for reparation.

Rather few studies have been conducted in which technical instrument reliability has not only been assessed but also separated from biological variability [161-163], and only on few occasions the need for unit-specific calibration to ensure that different units provide similar acceleration responses has been described in the literature. However, effective unit-specific calibration seems highly relevant in order to minimize the substantial inter-instrumental output differences, which have been observed under standardized conditions in mechanical setups [164,165]. Therefore, unit-specific calibration potentially could improve field data quality and study power (i.e. reduce random measurement error) before performing HPA data analyses – both in this thesis (Study III) and in general. Furthermore, unit-specific calibration potentially would provide important and relevant information regarding the implementation of essential instrumental precautions when monitoring HPA within the framework of the EYHS or other research studies where multiple accelerometer units are being applied.

On the other hand, a possible reduced inter-unit variability reached through in-vitro calibration would only be important if random instrumental variability across different units contributes considerable to the total variation in field data. Otherwise, the primary focus can shift to other sources of variation (e.g. variation over time, or position worn on the body including compliance with the instructions given how to mount and wear the accelerometer).

To the best of our knowledge, no other study has examined and compared the effect of calibration on inter-instrumental reliability after applying unit-specific calibration factors to data obtained both in the laboratory and in the field during free-living conditions in a large scale population study.

Why describe Secular trends in cardio-respiratory fitness, body mass index, and habitual physical activity?

Even though it is generally believed that children and young people in the Western society are experiencing a continuous decline in their PA level, the standardized data investigating such a trend are limited and currently it is unclear whether or not children's PA is falling [166]. Typically, the few studies which have been designed and conducted in order to examine the secular trends in the level of PA in children or young people have assessed PA levels by the use of questionnaires [167,168]. However, children tend to overestimate the time spend on physical activities [169,170], and unrealistic statements caused by fantasy, misunderstandings, and selective or bad memory have been reported [171], leaving the reliability and validity of secular trends based on self reports, or information gained by interviews, rather questionable.

Suspicions that children are getting more inactive are often build on indirect evidence suggesting that children are getting fatter [172,173] or less fit [174]. However, the etiology of obesity does not include PA alone but is multidimensional in its nature. The simple principle of energy balance suggests that when energy intake exceeds the energy expenditure, weight gain is the inevitable result. Energy intake depends solely on dietary composition and portion size, whereas energy expenditure is dependent on several components with PA being the primary modifiable mechanism.

High levels of CF may partly be genetically based, meaning that high-fit individuals automatically would be blessed with improved health status. An alternative, but necessarily not mutually exclusive, explanation might be that high levels of fitness act as a marker for high levels of activity. However, in relation to that it is worth noticing that the concept of PA necessarily not only has the potential to improve cardiovascular capacity, since it also might encourage parameters linked more closely to biochemical and haemostatic changes (e.g. lower blood pressure, more favourable cholesterol composition, lower triglyceride, improved glucose tolerance, etc.) known to be associated with health status [166]. Therefore, OW and low CF should not be used uncritical as synonyms for an insufficient level of PA, but instead PA should be regarded and investigated as an independent parameter potentially highly associated with OW and CF, however.

There is an urgent need for studies repeatedly performed, and properly designed, in order to assess secular trends in CF, BF and PA in children and young people, since the implementation of such studies potentially will provide important information regarding social, environmental, and lifestyle caused influences on a range of important risk factors among children.

Simultaneously, and equally important, the first warning signals of unfavourable levels of health related parameters might be revealed in different groups of the coming adult generation.
The need for repeated studies examining these secular trends is stressed even more if one bears in mind the substantial and rapidly occurring changes in lifestyle, especially in the modern Western society, which potentially will modify the risk factors of interest. As an example, when looking at PA in a historical perspective, occupational PA used to be an important source of exercise for many individuals [175]. However, today leisure-time PA serves as the major source of PA because contemporary occupations for many industrialized societies no longer involve substantial PA [176].
The present U.S. society has been described as a toxic environment for the development of obesity in young people, recognized in particular by sedentary behaviour caused by motorized transportation [177], time spend on TV or other electronic and screen based entertainment [178-180], and unhealthy food habits characterized by unstructured and fatty- or sweet meals [181-183]. Although European and other modern Western societies differ in many ways compared to the U.S., similar findings have been reported outside the U.S. [29,184-190].
In conclusion, in order to be able develop effective early primary preventive strategies against CVD, or to evaluate initiatives, which have already been employed, secular trends in major risk factors need to be well understood. However, comparing secular trends in lifestyle related risk factors across different countries, or even within a single country, is difficult and not unproblematic due to the different research protocol often being used (e.g. different definitions, cut-off criteria, and sampling procedures). Therefore, there is a need for several large scale population studies carried out in children of different ages in different countries where identical protocols are applied. As described previously in this thesis, the EYHS is designed to oblige this demand, and potentially the study will provide valuable information to the question as to whether children of today are experiencing a declined health status when compared to previously generations. Thereby, the study has the potential to play an important role, especially in the fight against future CVD.

Why study secular trends across socio-economic status?

Special attention towards secular trends in CVD risk factors across SES in children is relevant in order to verify whether parts of society are experiencing less favourable trends than others. Such graded trends would be disturbing since the logic consequence of this would be expected to be an even more marked discrepancy in the health status across socio-economic gradients in future generations of adults. Therefore, possible adverse secular trends in children coming from families with low socio-economic background would stress the importance of considering a graded intervention in the fight against lifestyle related diseases. Examining secular trends in CF, BMI, and HPA across socio-economic gradients will help us answering the question whether part of the children is changing more than expected compared to the total group of children. A more pronounced secular change, or even polarization, could occur when distinguishing between specific socio-economic subgroups, maybe leaving the overall mean unchanged.

Aims

1) The predefined primary aim of this thesis is to examine the secular trends in CF and BF between 1997/98 and 2003/04 in Danish third grade children (Study II).
2) Additionally, the aim is to analyse the secular trends in the prevalence of low CF and OW (Study II) in order to pay special attention to the children with the most unfavourable levels of CF and BF.
3) Furthermore, the aim is to examine whether the level of HPA has changed during the same period of time, when assessed objectively by the use of accelerometers. Special attention is paid to specific day types in order to determine if secular trends occurred in parallel across weekdays and weekend days (Study III).
4) In addition, the aim is to analyse all secular trends (Study II & III) across socio-economic gradients.
5) Finally, the aim is to examine the impact of unit-specific calibration on random output variation caused by inter-instrumental variability when assessing HPA objectively by accelerometers (Study I).
6) Based on observations observed when performing calibration in the laboratory, a post hoc analysis was applied in order to examine whether the acceleration response differed between different generations of instruments (i.e. CSA and MTI instruments, respectively). This post hoc analysis was performed in order to prevent potentially biased results in study III, due to a possible diverse acceleration response characterized in the two generations of instruments being applied.

Materials and methods

Design

Two cross sectional studies were conducted in 8-10-year-old children in the Danish municipality of Odense in 1997/98 and 2003/04, respectively. Both studies are part of the "European Youth Heart Study" and they follow the EYHS-protocol, which has been extensively described elsewhere [191].

Sampling

The sampling frame was a complete list of public schools in the municipality of Odense. Schools were stratified according to the location (urban, suburban, and rural) and the socio-economic character (high, middle, and low) of its uptake area. From each stratum, a proportional, two-stage cluster sample of children was selected. The primary units were the schools. Schools were selected using probability proportional to school size. Each school on the sampling list was allocated a weighting equivalent to the number of children in the schools who were eligible to be selected for the study. The secondary units were the children in the schools. Equal numbers of children were sampled from each school. Children were allocated code numbers and randomly selected using random number tables. In 1997/98, 28 out of 35 schools were sampled to participate in the study and 25 agreed to participate. In 2003/04, 25 schools were sampled. Replacement schools were sampled within each stratum to ensure a final sample size of 25 schools. One school did not wish to participate and was replaced. A more detailed description of the sampling procedure has been given elsewhere [191].

Subjects

In the first study in 1997/98, 771 children were sampled and invited to participate. In the end 589 children (310 girls and 279 boys, in all 76 % of the invited children) volunteered. In the second study in 2003/04, 709 children were sampled, and of these 458 children (259 girls and 199 boys, in all 65 % of the invited children) participated in the study.

Representativeness

1997/98

In each case of a child not participating in 1997/98, the form master was asked whether the child differed from the rest of the class in any way. All the children not participating were described as being normal with respect to body weight and level of PA in comparison with their class peers, except one child who was autistic.

2003/04

After completing the data collection in the study in 2003/04, body weight and body height was measured in all third grade children invited to participate in the study by the physician at the schools the children usually attended. Post hoc analyses revealed no differences in height, weight, or BMI between the non-participants and the children participating in the study.

Measurements

Anthropometry and pubertal status

Body height to the nearest 0.1 cm and body weight to the nearest 100g was determined by standard anthropometric methods, using a SV-Seca model 710 stadiometer and sliding weight with the children wearing only underwear and no shoes. Subsequently, BMI was calculated as weight*height^{-2} (kg*m^{-2}) and used as a measure for body fat. OW was classified using BMI cut-points published by Cole et al. [192]. Children were categorized as being OW if they exceeded BMI cut-points for OW or obesity.

Socio-economic status

A measure for SES was divided into two different groups, a white-collar group and a blue-collar group, based on information collected from a parent's questionnaire. The International Standard Classification of Occupation schema [193], which was used to define the two SES groups holds information on nine major categories. White-collar was defined according to the categories 1-4 in the schema, and blue-collar according to the categories 5-9. The parent or guardian in the household with the highest level of occupation was used to define the family's SES.

Cardio-respiratory fitness

CF was determined by a maximal cycle ergometer test, using the computerised Monark 839 Ergomedic cycle ergometer. The workload was automatically increased every third minute until exhaustion.

Children with a body mass less than 30 kg started at 20 W, and children with a body mass of 30 kg or more started at 25 W. The increment in workload every third minute was equal to the start load. Criteria for exhaustion were:

- Heart rate above or equal to 185 beats per minute
- That the child could not keep a pedalling frequency of at least 30 revolutions per minute
- Subjective judgement by the observer

The cycle ergometer was electronically calibrated every day and mechanically calibrated after being moved. The maximal power out put (watt-max) was calculated as the watts in the last fully completed workload (W_1), plus the increment in watts of the last step (W_i) multiplied by the number of seconds completed in the last step (t_{ls}) divided by 180 seconds. The equation was:

Watt-max = $W_1 + (W_i * t_{ls}/180)$.

The maximal watt output relative to body weight (watt*kg^{-1}) was used as an estimate for maximal CF. Two researchers, one in each study, supervised the CF-tests, and heart rate was registered using a Polar Vantage NV heart rate monitor. The CF-test has been validated with a high association (r=0.89) against directly measured maximal oxygen uptake [191].

The lowest sex-specific quartile of CF obtained in the study in 1997/98 was used to classify the cut-point for low CF, since no standard definition exists for low CF in children. Cut-points were 2.47 watt*kg^{-1} for girls and 2.81 watt*kg^{-1} for boys.

Physical activity

Instrumentation

Mechanical movement (Study I) and HPA (Study III) was assessed objectively by the CSA/MTI accelerometer, Model 7164, which has been described in details by Tryon & Williams [139].

The CSA/MTI is a single channel uniaxial piezo-electric accelerometer designed to capture and record accelerations along the vertical axis of the body ranging in magnitude from 0.05 to 2.13g. The acceleration signal is filtered by a frequency dependent bandpass that can be regarded as a mathematical weighting function [164]. The CSA/MTI samples and converts the acceleration signal by an 8-bit analog to digital (A/D) converter at 10 HZ. Each A/D value is summed over a user specific epoch. The CSA instrument has been found to exhibit good intra-instrument reliability, but less comforting inter-instrumental differences have been reported under standardized conditions in a mechanical setting in the laboratory [164].

Experimental laboratory calibration

Before performing the data analyses describing the secular trends in HPA in Study III, the CSA/MTI accelerometers were applied in a calibration study performed in the laboratory (Study I), which is methodologically explained below.

Acceleration responses were examined under standardized conditions in a mechanical setup in the laboratory in a total number of 78 MTI/CSA accelerometers, Model 7164, before and during the data collection period in the Danish part of EYHS-II. Subsequently, individual calibration factors were calculated in all units as instrumental outputs were checked and compared.

Mechanic laboratorial setup

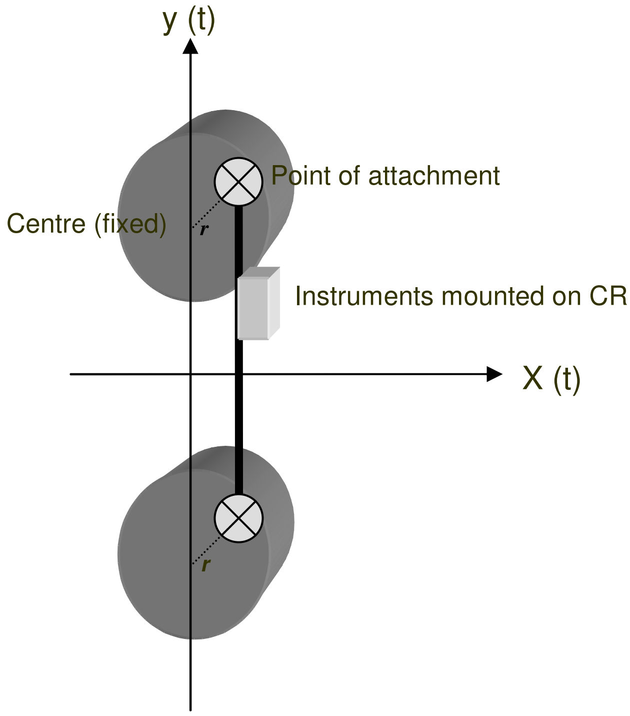
The calibration machine used in the experimental setup in the laboratory consists of two swinging wheels, both rotating with the same constant angular velocity (ω). The wheels are connected by a rod (CR) and driven by a powerful electric motor. The accelerometer units are attached to a plate at the rod when performing calibration. When attachments of the rod is placed away from the centre of the swinging wheels the instruments will experience accelerations and decelerations with a vertical displacement equal to two length of the radius (r) from the centre to the point of attachment.

ω (radians/sec) is directly related to the movement frequency f in Hertz by the equation: $\omega=2*\pi*f$

The radius, or half the amplitude of movement, is restricted to three different settings (22.0, 35.5, and 49.0 mm) in the mechanical setup, which together with the adjustable movement frequency will regulate acceleration values according to the following equation: $A(t) = 8*r*\pi*f^2$

The mechanical setup, which previously has been used and described by Brage et al. [164], is illustrated in Figure 5.

Figure 5: Calibration machine used in the laboratory with abscissa, X(t) and ordinate, Y(t). CR is the vertical connecting rod and *r* is the radius



Full laboratorial calibration protocol

The calibration settings in the mechanical setup were established to produce a range of physiologically relevant accelerometer count outputs usually observed during free-living activity. All units were calibrated in four different settings (see below) varying in frequencies and/or amplitudes, which in average produced accelerometer outputs roughly equal to 3000, 5000, 3000, and 8000 counts*min^{-1}, respectively. Compared with validation in children [194], these outputs range in locomotive field speed from approximately 4.0 to 8.0 km*h^{-1} (e.g. the range from walking to running).

Calibration was performed at three different time points (November 2003, January 2004, and Marts 2004). When performing calibrations in the mechanical setup, the epoch was set at 60 s comprising an integral of 600 measurements of each data point. In order to ensure full epochs, unit-specific CSA/MTI output was expressed as the mean of the minutes 2-8 in each trial, which in total lasted 10 minutes.

To begin with, ten instruments were randomly selected to identify appropriate frequencies and amplitudes producing accelerometer output equal to 3000, 5000, 3000, and 8000 counts*min^{-1}, respectively. The mean frequencies from the ten different units derived at the different amplitudes, where the desired accelerometer outputs were produced, were used as “the golden standard” frequencies for the whole population of instruments.

Since evidence of different acceleration responses had been observed across different generations of instruments (i.e. a group of CSA and MTI instruments, respectively), when acceleration responses were checked initially before the data collecting period to make sure that all units were functional, “golden standard” frequencies were calculated and used separately for the two generations of instruments. Subsequently, unit-specific calibration was performed in all units as individual acceleration responses were analysed when exposed to the “golden standard” frequencies. The unit-specific calibration factors were estimated as individual instrument acceleration responses were related to the mean acceleration responses within the whole generation of instruments of interest. A mean of the unit specific calibration factors reached in the 4 different settings at the 3 different time points was used as the final unit-specific calibration factor, used for further analyses in Study I.

In order to reduce the random variation in the experimental setup in the laboratory, frequencies were adjusted within 0.01 Hz, and each individual instrument was allocated one specific position of attachment to the plate at the calibration machine, which was retained during the entire study.

Calibration settings for CSA accelerometers in the mechanical setup

Setting #1 ≈ 3000 counts/min.: (Radius=22.0 mm. and frequency=1.705 HZ) =1.61 $m*S^{-2}$

Setting #2 ≈ 5000 counts/min.: (Radius=35.5 mm. and frequency=1.578 HZ) =2.22 $m*S^{-2}$

Setting #3 ≈ 3000 counts/min.: (Radius=49.0 mm. and frequency=0.970 HZ) =1.16 $m*S^{-2}$

Setting #4 ≈ 8000 counts/min.: (Radius=49.0 mm. and frequency=1.717 HZ) =3.63 $m*S^{-2}$

Calibration settings for MTI accelerometers in the mechanical setup

Setting #1 ≈ 3000 counts/min.: (Radius=22.0 mm. and frequency=1.657 HZ) =1.52 $m*S^{-2}$

Setting #2 ≈ 5000 counts/min.: (Radius=35.5 mm. and frequency=1.537 HZ) =2.08 $m*S^{-2}$

Setting #3 ≈ 3000 counts/min.: (Radius=49.0 mm. and frequency=0.950 HZ) =1.11 $m*S^{-2}$

Setting #4 ≈ 8000 counts/min.: (Radius=49.0 mm. and frequency=1.657 HZ) =3.38 $m*S^{-2}$

Effect of applying calibration factors

Unit-specific calibration factors were applied to a) instrument output derived in the mechanical setup in the laboratory as all units were checked in setting #2 each time they were returned from the field during the field data collecting period in order to ensure that the instruments maintained properly function, and to b) instrument output obtained during free-living conditions in the field (see the paragraph *"Field measurement protocol and data reduction"*). The effect of calibration on random output variation caused by inter-instrument variability was analysed by comparing characteristics of raw and calibrated data. Technical assessments in the total number of 78 accelerometers was examined separately in two subgroups of instruments consisting of 53 instruments purchased new in 1997, in the following referred to as CSA instruments, and 25 instruments purchased new in 2003, in the following referred to as MTI instruments. In contrast to Study I, this survey text will only include the effect of unit-specific MTI calibration.

Field measurement protocol and data reduction

The registration of HPA (Study III) was performed during the academic third grade school year. Due to a limited number of accelerometers available in the study in 1997/98, only 550 children had their HPA level recorded, after being randomly selected. Sixty-four CSA units, purchased new in 1997, were used in the study in 1997/98. In the study in 2003/04, 25 MTI units, purchased new in 2003, were distributed to a total number of 425 children. Furthermore, 24 CSA units, which were purchased new in 1997, were distributed to a total number of 33 children.

Children were asked to wear the accelerometer for at least five consecutive days, including both weekdays and weekend days. The accelerometer was returned by the participant, and data downloaded at the day of the child's physical examination. In order to distinguish true zeroes from zeroes recorded where the accelerometer had been taken off, all data files were screened for periods of zero activity. In cases of zero activity of 10 min or longer, it was assumed that the accelerometer had been taken of, and these periods did not contribute to the required minimum minutes of valid registered activity.

All children were asked to take off the activity monitor while they were asleep. However, "Activity" between 00.00 and 06.00 h was cut away in all data files in order to avoid biased data caused by the fact that some children forgot to take off the accelerometer.

Given these criteria, activity data were included for further analyses if the child had accumulated minimum 10 hours of activity data per day, for at least 3 days. Finally, suspicious recordings

highlighted by the data-reduction program developed within the framework of the EYHS, as well as extreme records highly questionable of being produced by normal human locomotion were checked manually and excluded for further analyses if generated by broken instruments.
In order to yield a measure of average activity intensity, data were expressed as total counts per registered time of total minutes (counts*min^{-1}), which previously has been shown to correlate strongly (r=0.58) with free-living PA energy expenditure measured by doubly labelled water [157]. In the study in 1997/98 the epoch was set at 60 s, and in 2003/04 the HPA counts were reintegrated from three 20 s epochs, thereby comprising an integral of 600 measurements of each data point in both studies.

Day types

The data reduction program was set up to analyse activity data on a daily basis, which revealed significantly different HPA levels between weekdays and weekend days. Other studies have supported the idea of different levels of PA when distinguishing between weekdays and weekend days [195-197]. However, according to our findings Friday apparently is to be regarded as a weekend day in this context in third grade children, meaning that the lowest activity estimates in general were found on Fridays, Saturdays, and Sundays [198]. Primarily, the secular trends in HPA were analysed separately across weekdays and weekend days, respectively. However, the distribution of day types differed significantly ($p<0.001$) between the two samples of children examined in 1997/98 and 2003/04, respectively. Therefore, when describing the secular trends in HPA across socio-economic gradients, children's activities achieved during weekdays were weighted by 4/7 and weekend activity was weighted by 3/7, thereby estimating one variable taking the different levels of HPA across different day types into account. This procedure was performed in order to a) achieve valid mean levels of HPA, b) prevent biased secular trends, c) reduce the number of subgroups being compared, thereby minimizing the issues related to multiple testing. The weighting according to day types was also applied when describing the effect of applying unit-specific calibration factors to HPA data (Study III).

Data quality and calibration of habitual physical activity data

Acceleration responses were examined in all units before the data collection period to make sure that all instruments were functional, and in 2003/04 an additional initiative was undertaken to ensure the quality of data. Each time an accelerometer was returned from the field during the data collecting period, the instrument was checked under standardized conditions in a mechanical setup in the laboratory in order to ensure that it maintained properly function.

Part of the purpose in Study I was to improve data quality and study power before analysing secular trends in the level of HPA between 1997/98 and 2003/04 in Study III. In order to be able to describe the methodological aspects in Study III linked to the calibration performed in Study I, the main results observed in Study I must be revealed here. Analyses performed in Study I showed that unit-specific calibration factors, estimated on the basis of acceleration responses in a mechanical setup where simple sinusoidal movements are produced, does not reduce random output variation caused by inter-instrumental variability in the field, despite the fact that a considerable effect was observed when applying calibration factors to data derived under standardized conditions in the laboratory. Therefore, in the end no unit-specific calibration factors were applied to the field data analysed in study III. However, compared to the CSA instruments, which were purchased new in 1997, a 9.95% increased mean acceleration response was observed post hoc in study I in the MTI instruments, which were purchased new in 2003. Therefore, before performing the data analyses in Study III raw field data captured with MTI units in the study in 2003/04 were calibrated according to the average acceleration response revealed in the CSA units, which predominantly were used in the study in 1997/98.

Statistical analyses

Study I

One-way analysis of variance estimating the standard deviation (SD) between and within the instruments was used to describe the potential for increasing inter-instrumental reliability through unit-specific calibration. The effect of calibration on random output variation caused by inter-unit variability was performed by comparing the SD and the coefficient of variation (CV) of the raw and calibrated accelerometer output derived in the mechanical setup in the laboratory and in the field during free-living conditions, respectively.

The amount of variation introduced to field data when performing calibration was estimated by dividing the variance of the delta instrument output between raw and calibrated field data by the total variation in field data. Pearson's correlation coefficients were calculated in order to describe the association between raw and calibrated accelerometer output in the field, and a

Bland-Altman plot was additionally applied in order to assess the individual agreement between raw and calibrated field data.

Multiple regressions with robust standard errors were used post hoc to test if mean acceleration responses differed between the groups of MTI and CSA instruments, when adjusting for calibration setting and time of calibration.

Study II & III

Primarily, the secular trends in CF and BF between 1997/98 and 2003/04 were analysed as changes in the continuous variables watt-max (watt*kg^{-1}) and BMI (kg*m^{-2}), and secondarily as changes in the dichotomised variables low CF and OW (Study II).

The third grade children examined in 2003/04 were systematically older and possessed a systematic slightly higher maximal heart rate when compared to the third grade children examined in 1997/98. Therefore, multiple linear regressions with robust standard errors were applied to adjust for potential modifying variables (i.e. age, maximal heart rate, and pubertal status) when analysing the secular trends in the continuous variables CF and BMI. Multiple logistic regressions with robust standard errors were applied to calculate the odds ratios used to describe the secular trends in the dichotomised variables low CF and OW between 1997/98 and 2003/04, after adjusting for potential modifying variables. To get a better numerical impression of the odds ratios, the unadjusted raw prevalence of low CF and OW for the two samples was also presented (Study II).

The secular trends in the level of HPA were analysed as gender and day type specific (i.e. weekdays vs. weekend days) changes in the level of total mean counts*min^{-1} (Study III). Multiple linear regressions with robust standard errors were used to describe all secular trends in the level of HPA. Reliability is related to the accumulated number of valid days when monitoring HPA at an individual level [195]. Therefore, all secular trends were presented both as crude estimates and as adjusted estimates weighted according to the accumulated number of valid days (study III).

After paying no attention to SES to begin with, all secular trends (Study II & III) were analysed across socio-economic gradients. An interaction term between year of study and SES was applied to the different regression models describing the secular trends in order to test for a polarized change in CF, low CF, BMI, OW, and HPA across socio-economic gradients between 1997/98 and 2003/04 (Study II & III). Furthermore, an interaction term between gender and day

type was applied in both samples of examined children in study III, in order to examine whether gender differences differed between weekdays and weekend days.
Nominal p-values were presented to begin with when describing the secular trends. However, in order to allow for multiple testing Bonferroni's correction was applied post hoc to the analyses not involving the primarily aim before drawing final conclusions by interpreting the levels of significance.

Ethics

All parents gave written informed consent for their child to participate, and all children gave verbal consent. The study was approved by the local scientific ethics committee for the counties of Vejle and Funen, and follows the rules stipulated in the Helsinki declaration.

Results

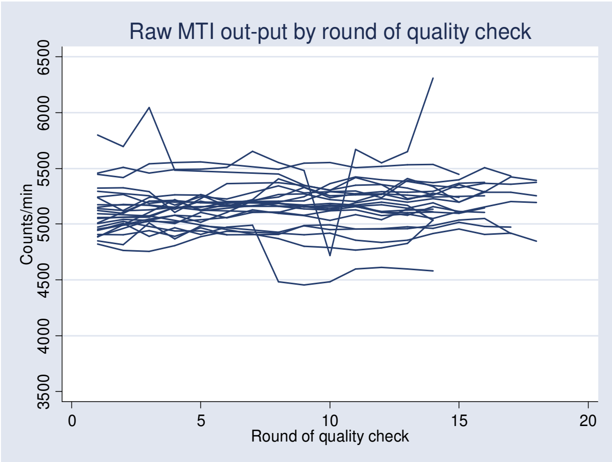
The paragraph "*Results*" is described in two parts: 1) The effect on random output variation caused by accelerometers' inter-instrumental variability in the laboratory and in the field, and 2) Secular trends in cardio-respiratory fitness, body mass index, and habitual physical activity.

The effect on random output variation caused by accelerometers' inter-instrumental variability in the laboratory and in the field

Potential for increasing inter-instrumental reliability

Examining the effect of unit-specific calibration on the random variation in HPA would be relevant only if calibration can be expected to improve inter-instrumental reliability. The potential for increasing inter-instrumental reliability through unit-specific calibration has been outlined in Figure 6 where the raw unit-specific accelerometer output is plotted against the different rounds of quality checks performed in setting #2 in the mechanical setup in the laboratory. In average, the different MTI units were undergoing a quality check 13.6 times (ranging from 4 to 18). Substantial variation between the different units, but also a considerable fluctuation within each single instrument (intra-instrumental variation), can be observed over time in Figure 6. However, subsequent analyses showed that the SD representing the variation between MTI units was about twice the size of the SD representing the variation within MTI units. This indicates some potential for increasing inter-instrumental reliability through the performance of an unit-specific calibration.

Figure 6



Within units variation: SD=112 counts*min^{-1}
Between units variation: SD=195 counts*min^{-1}

The Effect of applying unit-specific calibration factors to data derived during quality checks in the mechanical setup in the laboratory

The impact on between units variation observed in the laboratory after applying unit-specific calibration factors to data obtained in setting #2 in the mechanical setup can be seen in Table 1. The SD was reduced considerably from 221 (95% CI 206;239) counts*min^{-1} to 127 (95% CI 118;138) counts*min^{-1}, and the CV was reduced from 4.3% to 2.5% across the population of MTI instruments after being calibrated.

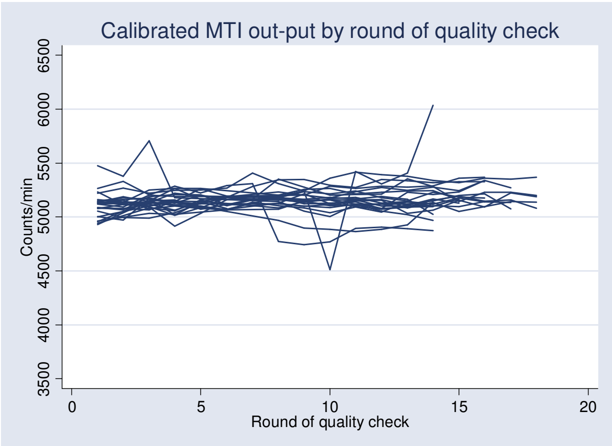
Table 1. Raw and calibrated accelerometer output derived through repeated measurements in setting #2 in the mechanical setup. Data are means and standard deviations with 95% CI, and coefficients of variation

	N	Average output (counts*min^{-1})	SD	CV
Raw MTI output	340	5160 (5137;5184)	221 (206;239)	4.3%
Calibrated MTI output	340	5155 (5142;5169)	127 (118;138)	2.5%

(N equals the total number of repeated quality checks performed in setting #2)

When examining the impact on between units variation in the laboratory after applying unit-specific calibration factors to data obtained in setting #2 in the mechanical setup by comparing the SD of the instrument effect and within the instruments, results showed that the SD representing the variation between units was approximately one half of the SD representing the variation within units after performing calibration (Figure 7). Therefore, based on information observed in the mechanical setup in the laboratory it seems reasonable to perform unit-specific calibration in the laboratory. On the other hand, considerable inter-instrumental variability still exists across the two batches of instruments ($p<0.0001$), even after performing unit-specific calibration.

Figure 7



Within units variation: SD=110 counts*min^{-1}
Between units variation: SD=65 counts*min^{-1}

Valid activity derived with MTI instruments during free living conditions in the field

Valid HPA data was reached in 389 third grade children (227 girls and 162 boys) when measured with MTI accelerometers - the type of instrument that in the next paragraph will achieve further attention when describing the effect of applying unit-specific calibration factors to field data.

Effect of applying unit-specific calibration factors to field data

The effect of calibration on random variation caused by inter-instrumental variability observed in field data recorded during free living conditions with MTI actigraph accelerometers in third grade children can be seen in Table 2. After calibration, average HPA intensity was maintained at 724 counts*min^{-1}, and the SD and CV also remained unchanged (SD_{raw}=219 (95% CI=205;236) counts*min^{-1} vs. $SD_{calibrated}$=220 (95% CI=206;237) counts*min^{-1}, CV_{raw}=30.2% vs. $CV_{calibrated}$=30.4%). The amount of variation introduced to field data after performing calibration was estimated to account for only 1.1% of the total amount of variation observed in children's field data.

Table 2. Raw and calibrated accelerometer output recorded during free-living conditions in third grade children. Data are means and standard deviations with 95% CI, and coefficients of variation.

	N	Average activity (Counts*min^{-1})	SD	CV
Raw MTI output	389	724 (702;745)	219 (205;236)	30.2%
Calibrated MTI output	389	724 (702;746)	220 (206;237)	30.4%

(N equals the number of children achieving valid MTI activity files)

The correlation, including the line of equality, and the relative 95% limits of agreement between raw and calibrated accelerometer output can be seen in Figure 8 & 9, respectively. As it can be observed, calibrated accelerometer output was highly correlated to raw accelerometer output (r=0.99), and the relative 95% limits of agreement between raw and calibrated instrument output in the field was approximately ±5.5%.

Figure 8

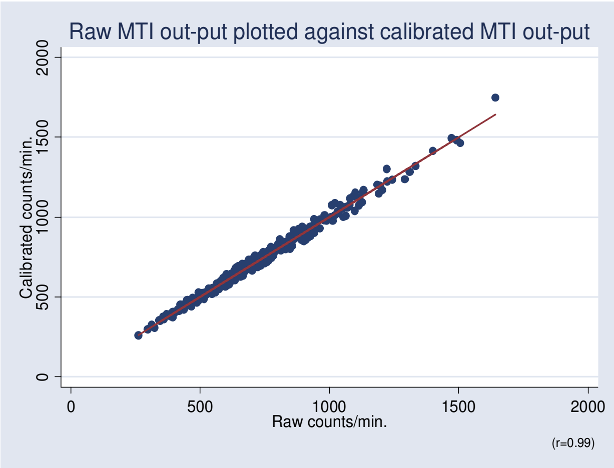
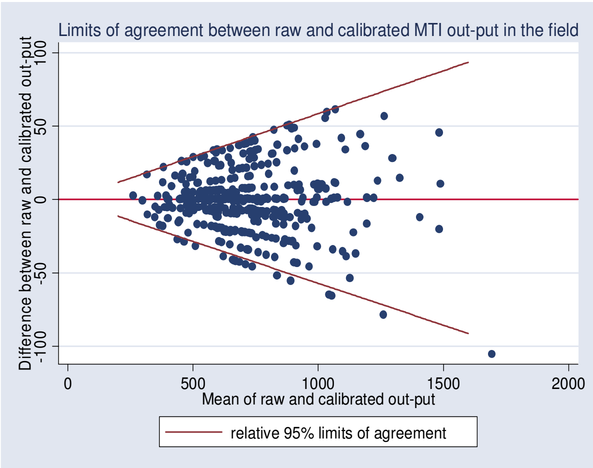


Figure 9



Post hoc examinations of the MTI & CSA instruments

MTI and CSA instrument outputs when exposed to "golden standard" frequencies in the laboratory

Table 3 and Table 4 show the mean accelerometer output obtained by the MTI and CSA instruments, when exposed to golden standard frequencies during calibration performed at three different time points in the mechanical setup.

Table 3. MTI actigraph instrument outputs derived when exposed to "golden standard" accelerations in the mechanical setup. Data are means and standard deviations with 95% CI, and coefficients of variation

November 2003	n	Measured acceleration (Counts*min^{-1})	SD	CV
Setting #1 (1.52 m*s^{-2})	25	3023 (2991;3054)	76 (59;106)	2.5%
Setting #2 (2.08 m*s^{-2})	25	5015 (4960;5071)	134 (105;187)	2.7%
Setting #3 (1.11 m*s^{-2})	25	2986 (2958;3013)	67 (53;94)	2.2%
Setting #4 (3.38 m*s^{-2})	25	7967 (7876;8058)	219 (171;305)	2.7%
January 2004				
Setting #1 (1.52 m*s^{-2})	25	3083 (3029;3137)	132 (103;183)	4.2%
Setting #2 (2.08 m*s^{-2})	25	5133 (5046;5219)	210 (164;292)	4.1%
Setting #3 (1.11 m*s^{-2})	25	3070 (3012;3128)	141 (110;196)	4.6%
Setting #4 (3.38 m*s^{-2})	25	8175 (8035;8315)	339 (265;472)	4.1%
Marts 2004				
Setting #1 (1.52 m*s^{-2})	23	3100 (3034;3167)	153 (119;217)	5.0%
Setting #2 (2.08 m*s^{-2})	23	5142 (5042;5241)	230 (178;325)	4.5%
Setting #3 (1.11 m*s^{-2})	23	3048 (2988;3108)	139 (108;197)	4.6%
Setting #4 (3.38 m*s^{-2})	23	8160 (8013;8308)	341 (264;483)	4.2%

Table 4. CSA instrument outputs derived when exposed to "golden standard" accelerations in the mechanical setup. Data are means and standard deviations with 95% CI, and coefficients of variation

November 2003	n	Measured acceleration (Counts*min^{-1})	SD	CV
Setting #1 (1.61 m*s^{-2})	50	2952 (2883;3021)	243 (203;303)	8.2%
Setting #2 (2.22 m*s^{-2})	50	4861 (4757;4965)	366 (306;456)	7.5%
Setting #3 (1.16 m*s^{-2})	50	2952 (2887;3017)	229 (191;285)	7.8%
Setting #4 (3.63 m*s^{-2})	50	7831 (7672;7990)	560 (468;698)	7.2%
January 2004				
Setting #1 (1.61 m*s^{-2})	53	2970 (2900;3040)	253 (212;312)	8.5%
Setting #2 (2.22 m*s^{-2})	53	4883 (4775;4992)	393 (330;486)	8.0%
Setting #3 (1.16 m*s^{-2})	53	2981 (2916;3045)	234 (197;290)	7.8%
Setting #4 (3.63 m*s^{-2})	53	7857 (7689;8026)	611 (512;756)	7.8%
Marts 2004				
Setting #1 (1.61 m*s^{-2})	53	3021 (2945;3097)	275 (231;341)	9.1%
Setting #2 (2.22 m*s^{-2})	53	4966 (4852;5080)	414 (347;512)	8.3%
Setting #3 (1.16 m*s^{-2})	53	3014 (2944;3084)	253 (212;313)	8.4%
Setting #4 (3.63 m*s^{-2})	53	7967 (7796;8138)	620 (521;768)	7.8%

In order to estimate the mean magnitude of the systematic different acceleration response (acceleration/accelerometer output) recognized in the group of MTI and CSA instruments, respectively, the accelerations needed to produce one thousand accelerometer counts (1000 counts*min^{-1}) were estimated for all units. Comparison between the two groups of instruments was performed in all four settings at the three different time points in the mechanical setup, and results can be seen in Table 5.

When comparing acceleration responses between the MTI and CSA instruments across all settings and time points, the MTI instruments in average experienced a significantly increased (9.95%) mean acceleration response.

Table 5 Accelerations (m*s^{-2}) needed to produce 1000 accelerometer count*min^{-1}, calculated in four different settings at three different time points in the mechanical setting. Data are means and SD

	Setting #1	**Setting #2**	**Setting #3**	**Setting#4**
MTI (November)	0.5026 (0.0124)	0.4144 (0.0110)	0.3724 (0.0084)	0.4245 (0.0117)
CSA (November)	0.5483 (0.0476)	0.4599 (0.0365)	0.3950 (0.0329)	0.4658 (0.0345)
MTI (January)	0.4932 (0.0199)	0.4053 (0.0157)	0.3627 (0.0157)	0.4141 (0.0162)
CSA (January)	0.5453 (0.0492)	0.4582 (0.0388)	0.3913 (0.0332)	0.4647 (0.0374)
MTI (Marts)	0.4908 (0.0245)	0.4048 (0.0187)	0.3654 (0.0172)	0.4149 (0.0175)
CSA (Marts)	0.5368 (0.0534)	0.4509 (0.0408)	0.3874 (0.0360)	0.4583 (0.0374)

Compared to the group of CSA instruments, a significantly ($p<0.001$) increased mean acceleration response was observed in the group of MTI units

Secular trends in cardio-respiratory fitness, body mass index, and habitual physical activity

Valid measurements/information

Cardio-respiratory fitness, body mass index, and habitual physical activity

In 1997/98, the number of children meeting the criteria for exhaustion in the CF-test was 281 girls (91%) and 258 boys (92%). Valid HPA during weekdays was obtained in 381 children (203 girls and 178 boys), and in the weekend 349 children (183 girls and 166 boys) had valid HPA data recorded, as determined by the criteria described in the paragraph *"Field measurement* *protocol and data reduction"*. The mean number of valid days accumulated in weekdays and weekend days were 2.25 ± 1.02 and 2.11 ± 0.77, respectively.

In 2003/04, 243 girls (94%) and 185 boys (93%) met the criteria for exhaustion in the CF test. Valid HPA during weekdays was provided in 416 children (238 girls and 178 boys), and 416 children (240 girls and 176 boys) also met the inclusion criteria set to define valid HPA in the weekend days. The mean numbers of valid days accumulated in weekdays and weekend days were 2.10 ± 0.57 and 2.39 ± 0.68, respectively.

Valid BMI estimates were reached in all children, both in the study conducted in 1997/98 and in the study in 2003/04.

Socio-economic status and pubertal status

Information on SES was available in 559 and 423 children in 1997/98 and 2003/04, respectively. The number of children not only provided with socio-economic information but also with valid HPA data in weekdays and weekend days was 333 (171 girls & 162 boys) in the study in 1997/98, and 391 (224 girls & 167 boys) in the study conducted in 2003/04.

In 1997/98, pubertal status was assessed in 306 girls and 275 boys, and in 2003/04 pubertal status was categorized in all 256 girls and 199 boys.

Participant characteristics are shown in Table 6 & 7. Please note that due to systematic age and maximal heart rate differences between the studies conducted in 1997/98 and 2003/04, respectively, descriptive data on CF and BMI should serve solely as a presentation of data, and not as a numeric comparison describing any secular trends.

Table 6. Characteristics (mean and standard deviation) for girls.

Girls	Overall	n	Blue-collar	n	White-collar	n
1997-98						
Age (years)	9.6 (0.4)	310	9.6 (0.4)	110	9.6 (0.4)	183
Height (cm)	138.5 (6.3)	310	137.4 (5.5)	110	139.1 (6.5)	183
Weight (kg)	33.2 (6.3)	310	32.8 (6.2)	110	33.3 (6.4)	183
Max. heart rate (bpm)	199.4 (7.0)	282	199.3 (7.3)	96	199.4 (6.9)	172
Tanner stage (1/2/3/4)	216/85/5/0	306	76/27/4/0	107	129/52/1/0	182
BMI ($kg*m^{-2}$)	17.2 (2.5)	310	17.3 (2.5)	110	17.1 (2.5)	183
CF ($watt*kg^{-1}$)	2.82 (0.5)	282	2.75 (0.5)	96	2.87 (0.5)	172
2003-04						
Age (years)	9.8^{*} (0.4)	259	$9.8^{‡}$ (0.4)	91	9.7 (0.3)	146
Height (cm)	139.5 (6.5)	259	140.0 (6.7)	91	139.8 (6.3)	146
Weight (kg)	34.2 (7.0)	259	35.0 (7.3)	91	33.6 (6.1)	146
Max. heart rate (bpm)	$201.6^{†}$ (8.0)	243	$202.3^{§}$ (8.4)	84	$201.8^{††}$ (7.7)	139
Tanner stage (1/2/3/4)	199/52/4/1	256	64/22/2/1	89	121/22/2/0	145
BMI ($kg*m^{-2}$)	17.4 (2.4)	259	17.8 (2.9)	91	17.1 (2.3)	146
CF ($watt*kg^{-1}$)	2.81 (0.4)	243	2.78 (0.5)	84	2.87 (0.4)	139

*: p<0.0002, †: p<0.002, ‡: p<0.007, §: p<0.02, ††: p<0.007, all different from 1997/98

Table 7. Characteristics (mean and standard deviation) for boys.

Boys	Overall	n	Blue-collar	N	White-collar	n
1997-98						
Age (years)	9.7 (0.4)	279	9.7 (0.4)	125	9.7 (0.5)	141
Height (cm)	139.5 (6.3)	279	139.5 (7.1)	125	139.5 (5.8)	141
Weight (kg)	34.0 (6.4)	279	34.0 (7.2)	125	34.0 (5.8)	141
Max. heart rate (bpm)	198.0 (7.2)	258	198.0 (7.4)	114	199.3 (7.0)	131
Tanner stage (1/2/3/4)	275/0/0/0	275	122/0/0/0	122	140/0/0/0	140
BMI ($kg*m^{-2}$)	17.4 (2.4)	279	17.3 (2.6)	125	17.4 (2.4)	141
CF ($watt*kg^{-1}$)	3.18 (0.6)	258	3.16 (0.6)	114	3.21(0.5)	131
2003-04						
Age (years)	9.9^{*} (0.4)	199	$10.0^{‡}$ (0.5)	65	9.7 (0.3)	121
Height (cm)	140.5 (6.9)	199	140.5 (7.9)	65	140.6 (6.2)	121
Weight (kg)	34.5 (6.8)	199	36.0 (8.0)	65	33.5 (5.2)	121
Max. heart rate (bpm)	$200.4^{†}$ (7.6)	185	200.0 (7.3)	64	200.6 (7.8)	110
Tanner stage (1/2/3/4)	199/0/0/0	199	65/0/0/0	65	121/0/0/0	121
BMI ($kg*m^{-2}$)	17.3 (2.4)	199	18.0 (2.8)	65	16.9 (2.0)	121
CF ($watt*kg^{-1}$)	3.30 (0.5)	185	3.21 (0.5)	64	3.37 (0.5)	110

*: p<0.0002, †: p<0.04, ‡: p<0.0005, all different from 1997/98

Overall secular trends

Cardio-respiratory fitness and body mass index

When looking at the two samples overall without paying attention to SES a significant secular trend in CF, corresponding to a 2.8% decline, was observed in girls in 2003/04 compared to 1997/98 (β= -0.08 & p=0.03).

No overall significant secular trends were found in CF in boys, and no significant trends had occurred in low CF in boys or girls overall. Finally, no overall significant secular trends in BMI or OW were observed in any gender (Table 8 & 9).

Table 8. Regression coefficients (β) describing the overall changes in cardio-respiratory fitness and BMI between 1997/98 and 2003/04. Regression coefficients are adjusted for age, pubertal status, and maximal heart rate in the analyses involving cardio-respiratory fitness

	Cardio-respiratory fitness			**BMI**		
	β	**95% CI**	**P**	**β**	**95% CI**	**P**
Girls	-0.08	-0.16;-0.01	0.031	0.33	-0.08;0.74	0.117
Boys	0.07	-0.03;0.17	0.162	0.006	-0.42;0.43	0.979

Table 9. Odds ratios for having low cardio-respiratory fitness/being overweight in 2003/04 compared to 1997/98. Odds ratios are adjusted for age, pubertal status, and maximal heart rate in the analyses involving low cardio-respiratory fitness

	Low cardio-respiratory fitness			**Overweight**		
	Odds ratio	**95% CI**	**P**	**Odds ratio**	**95% CI**	**P**
Girls	1.27 (0.29)	0.81;1.98	0.293	1.46 (0.36)	0.90;2.36	0.126
Boys	0.66 (0.16)	0.41;1.06	0.086‡	0.99 (0.27)	0.59;1.68	0.977

‡: p not borderline significant after additional post hoc Bonferroni's correction

Habitual physical activity

Figure 10 shows the crude gender and day type distributions of HPA by year of study. In girls, no significant secular trends were observed in the level of HPA during weekdays or weekend days between 1997/98 and 2003/04, neither when performing crude analyses nor when applying adjusted weights according to the accumulated number of valid days. When performing the crude analysis, a borderline significant (p=0.082) interactive effect was observed between year of study and day type in girls, indicating a tendency towards polarization in the changes of HPA level across different day types between 1997/98 and 2003/04. However, the tendency towards polarization decreased (p=0.141) when performing the adjusted analysis weighted according to the accumulated number of valid days (Figure 10 & Table 10).

In boys, no significant secular trends were observed in the level of HPA during weekdays or weekend days, neither when performing crude analyses, nor when applying adjusted weights according to the accumulated number of valid days (Figure 10 & Table 10).

Day type and gender variations

Significantly gender and day type variations have previously been described in the level of HPA in the two samples of children being examined in the present thesis [198]. In Study III, an interaction was furthermore observed between gender and day types in both samples of children (p=0.01 & p=0.04, respectively). Post hoc analyses revealed that gender differences were found to be more distinct during weekdays than during weekend days. During weekdays in 1997/98, boys were found to be 28% more active compared to girls (β=170.9, p<0.001), whereas boys only reached 11% more activity than girls in the weekends (β=66.8, p=0.027). During the weekdays in 2003/04, boys were 25% more active than girls (β=154.3, p<0.001). In the weekends, however, boys were only found to be 16% more active compared to the girls (β=90.8, p<0.001) (Figure 10).

Figure 10. Columns are crude means with 95% CI

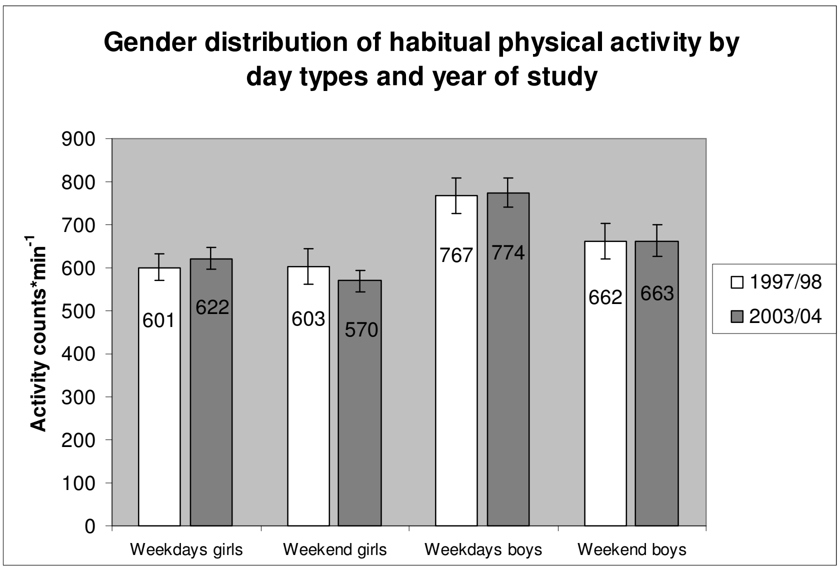


Table 10. Regression coefficients (β) describing the overall changes in the level of habitual physical activity between 1997/98 and 2003/04. Regression coefficients are presented both as crude estimates and as adjusted estimates weighted according to the accumulated number of valid days.

	Crude model			Adjusted model according to the accumulated number of valid days		
	β	**95% CI**	**P**	**β**	**95% CI**	**P**
Girls						
Weekdays	19.8	-19.3;58.9	0.319	21.3	-18.2;60.8	0.290
Weekend days	-34.8	-82.4;12.9	0.152	-24.3	-70.6;22.0	0.304
Test for polarization between weekdays and weekend days between 1997/98 and 2003/04			0.082			0.141
Boys						
Weekdays	7.75	-46.5;62.0	0.779	4.6	-52.5;61.7	0.873
Weekend days	1.76	-53.2;56.7	0.950	-0.2	-57.5;57.1	0.994
Test for polarization between weekdays and weekend days between 1997/98 and 2003/04			0.879			0.906

Secular trends across socio-economic status

When looking at specific socio-economic subgroups of the two samples changed levels of CF/low CF or BMI/OW were observed in both genders, with results in general showing more unfavourable trends in the blue-collar group. However, these results could not be considered statistical significant after additional Bonferroni's correction (Table 11 & 12).

Cardio-respiratory fitness (continuous variable)

No significant secular trends, or polarization over time, were found in CF in girls when distinguishing between SES.

In blue-collar boys, no significant secular trends were observed in CF. However, CF had improved 4.4% in the white-collar boys in 2003/04, compared to 1997/98 (β=0.14 & p=0.03). A tendency (p=0.104) towards polarization in CF over time was observed across SES in boys. However, the p-values did not reach the levels of statistical significance and borderline significance after post hoc Bonferroni's correction (Table 11).

Body mass index (continuous variable)

In girls, no significant secular trends, or polarization, in BMI had occurred.

In the blue-collar boys, a 4.7% increase in BMI was observed in 2003/04 compared to 1997/98 (β=0.82 & p=0.049), whereas a 2.8% decrease in BMI was found in the white-collar boys (β= -0.48 & p=0.069). A polarization in BMI was observed in boys (p=0.008), meaning that the difference in BMI between the blue- and white-collar boys had increased between 1997/98 and 2003/04. However, after post hoc Bonferroni's correction no significant secular trends, or polarization or time, were observed across socio-economic gradients (Table 11).

Table 11. Regression coefficients (β) describing changes in cardio-respiratory fitness and BMI across socio-economic gradients between 1997/98 and 2003/04. Regression coefficients are adjusted for age, pubertal status, and maximal heart rate in the analyses involving cardio-respiratory fitness

	Cardio-respiratory fitness			**BMI**		
	β	**95% CI**	**P**	**β**	**95% CI**	**P**
Girls						
blue-collar	-0.03	-0.16;0.11	0.683	0.46	-0.24;1.17	0.197
white-collar	-0.09	-0.19;0.01	0.081‡	0.29	-0.22;0.79	0.265
Test for polarization between blue- and white-collar between 1997/98 and 2003/04			0.882			0.571
Boys						
blue-collar	-0.05	-0.23;0.12	0.561	0.82	0.004;1.64	0.049†
white-collar	0.14	0.02;0.27	0.026†	-0.48	-0.99;0.04	0.069‡
Test for polarization between blue- and white-collar between 1997/98 and 2003/04			0.104‡			0.008†

†: p>0.05 after additional post hoc Bonferroni's correction
‡: p not borderline significant after additional post hoc Bonferroni's correction

Low cardio-respiratory fitness (dichotomized variable)

When compared to 1997/98, no significant different odds for having low CF were observed in any gender or group of SES in 2003/04. But a tendency (p=0.065) towards lower odds for having low CF in 2003/04 compared to 1997/98 was observed in white-collar boys. This tendency, however, was absent after additionally Bonferroni's correction.

No significant polarization in the prevalence of low CF was observed across SES in boys or girls between 1997/98 and 2003/04 (Table 12).

Overweight (dichotomized variable)

Higher odds for being OW in 2003/04 compared to 1997/98 were found in blue-collar girls, the odds ratio was 2.30 (p=0.040). Opposite, but non-significant, secular trends observed in OW in blue- and white-collar boys between 1997/98 and 2003/04 were leading to a tendency (p=0.055) towards a polarization in OW between boys coming from blue- and white-collar families, respectively. However, after Bonferroni's correction no significant secular trends, or tendency towards polarization over time, were observed across socio-economic gradients.

No significant polarization in OW had occurred across SES in girls (Table 12).

The unadjusted raw prevalences of low CF and OW are shown in Table 13.

Table 12. Odds ratios for having low cardio-respiratory fitness/being overweight in 2003/04 compared to 1997/98. Odds ratios describes changes across socio-economic gradients and are adjusted for age, pubertal status, and maximal heart rate in the analyses involving low cardio-respiratory fitness

	Low cardio-respiratory fitness			**Overweight**		
	Odds ratio	**95% CI**	**P**	**Odds ratio**	**95% CI**	**P**
Girls						
blue-collar	1.59 (0.58)	0.78;3.25	0.204	2.30 (0.93)	1.04;5.08	0.040†
White-collar	0.90 (0.30)	0.47;1.71	0.749	1.15 (0.39)	0.59;2.21	0.686
Test for polarization between blue- and white-collar between 1997/98 and 2003/04			0.116			0.155
Boys						
blue-collar	1.03 (0.37)	0.51;2.07	0.936	1.61 (0.68)	0.71;3.70	0.257
white-collar	0.50 (0.19)	0.24;1.04	0.065‡	0.59 (0.24)	0.27;1.29	0.185
Test for polarization between blue- and white-collar between 1997/98 and 2003/04			0.175			0.055‡

†: p>0.05 after additional post hoc Bonferroni's correction

‡: p not borderline significant after additional post hoc Bonferroni's correction

Table 13. Unadjusted prevalence of low cardio-respiratory fitness and overweight in the two samples, presented overall and by socio-economic status

	Low cardio-respiratory fitness			**Overweight**		
	Total group size (n)	Prevalence of **low CF** (n)	% of total group size	Total group size (n)	Prevalence of **OW** (n)	% of total group size
1997-98						
Girls overall	282	70	24.82%	310	45	14.51%
Girls blue-collar	96	27	28.13%	110	15	13.64%
Girls white-collar	172	37	21.51%	183	27	14.75%
Boys overall	258	64	24.81%	279	37	13.26%
Boys blue-collar	114	31	27.19%	125	17	13.60%
Boys white-collar	131	27	20.61%	141	20	14.18%
2003-04						
Girls overall	243	58	23.87%	259	45	17.37%
Girls blue-collar	84	27	32.14%	91	21	23.08%
Girls white-collar	139	21	15.11%	146	20	13.70%
Boys overall	185	30	16.22%	199	26	13.07%
Boys blue-collar	64	15	23.44%	65	13	20.00%
Boys white-collar	110	12	10.91%	121	10	8.26%

Habitual physical activity

Figure 11 shows the crude gender and socio-economic distribution of HPA by year of study. During the period 1997/98 to 2003/004, no significant secular trends had occurred in the level of HPA across socio-economic gradients in girls or boys, neither when performing crude analyses nor when applying adjusted weights according to the accumulated number of valid days. Furthermore, no significant interactive effect was observed between year of study and SES in girls or boys, thereby indicating no polarized change in the level of HPA across socio-economic gradients between 1997/98 and 2003/04 (Table 14).

Figure 11. Columns are crude means with 95% CI

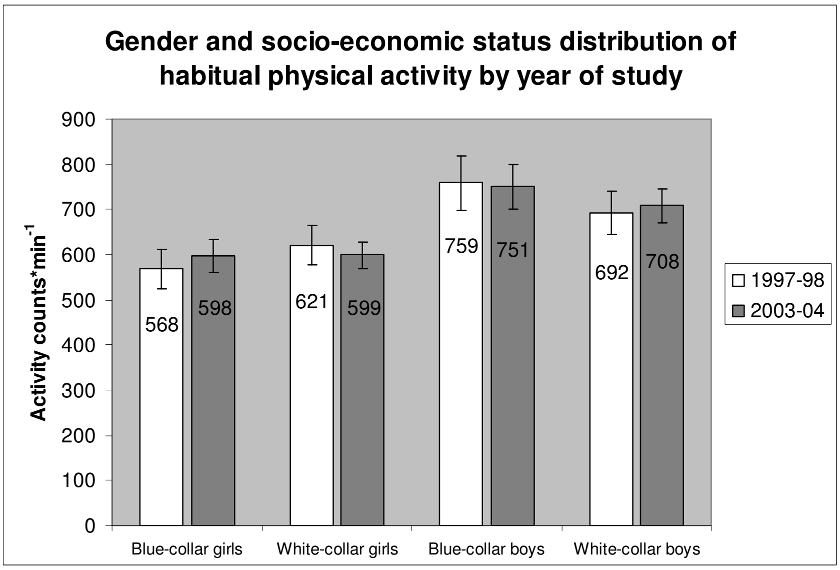


Table 14. Regression coefficients (β) describing changes in the level of habitual physical activity across socio-economic gradients between 1997/98 and 2003/04. Regression coefficients are presented both as crude estimates and as adjusted estimates weighted according to the accumulated number of valid days.

	Crude model			Adjusted model according to the accumulated number of valid days		
	β	**95% CI**	**P**	**β**	**95% CI**	**P**
Girls						
Blue-collar	29.5	-28.3;87.3	0.314	34.3	-21.0;89.6	0.222
White-collar	-22.9	-75.1;29.3	0.389	-20.3	-72.2;31.5	0.441
Test for polarization between blue- and white-collar between 1997/98 and 2003/04			0.185			0.155
Boys						
Blue-collar	-8.2	-86.6;70.2	0.837	-23.9	-108.0;60.3	0.576
White-collar	15.5	-46.3;77.3	0.622	27.8	-37.5;93.1	0.403
Test for polarization between blue- and white-collar between 1997/98 and 2003/04			0.640			0.338

Discussion

The paragraph "*Discussion*" is described in two parts: 1) The effect on random output variation caused by accelerometers' inter-instrumental accelerometer variability in the laboratory and in the field, and 2) Secular trends in cardio-respiratory fitness, body mass index, and habitual physical activity.

The effect on random output variation caused by accelerometers' inter-instrumental accelerometer variability in the laboratory and in the field

As the primarily finding, this Ph.D. thesis (Study I) revealed that unit-specific calibration factors, shown to reduce inter-instrumental variability considerably in the experimental setup in the laboratory should be addressed as rather ineffectual when applied to field data reflecting more complex and heterogeneous movements of the human body. Furthermore, compared to older generations of the uniaxial accelerometer Model 7164 (i.e. CSA instruments) new instrument generations (i.e. MTI instruments) were found to display a significantly increased (9.95%) mean acceleration response, when acceleration responses were compared between the two groups of instruments under standardized conditions in the mechanical setup. This, of course, will add to further speculations regarding the reliability and validity of accelerometers.

The present findings should be interpreted in the light of several considerations, which will be discussed in the following.

General strengths and limitations

The strengths in Study I include the large number of accelerometer units examined in a mechanical setup producing a highly standardized reference of acceleration, in order to try to estimate and subsequently apply appropriate unit-specific calibration factors to field data. On the other hand, serving as a limitation, the calibration machine in the laboratory solely offers an isolated and standardized sinusoidal way of movement, which potentially will affect the comparability between the inter-instrumental variability estimated according to mechanical movements in the laboratory and the inter-instrumental variability, which actually occurs in the field when instruments are exposed to more complex human locomotion. Therefore, in order to improve the variation and complexity of movement in the mechanical setup, all units were calibrated in three different radius settings using four different frequencies, which produced a total of four different acceleration magnitudes.

Even though intra-instrumental reliability has been reported to be fairly good [163,164] the registrations of unit-specific acceleration responses were performed at three different time points in all instruments during the period November 2003 to Marts 2004 in order to minimize the effect of intra-instrumental variation.

The fact that laboratory data was collected in parallel with field data will increase the comparability between results observed in the mechanical setup and during free living conditions, respectively.

Acceleration magnitudes

The target was to examine acceleration responses in the laboratory under standardized conditions where accelerometer outputs (counts*min^{-1}) were comparable in size to the experienced free-living activities of walking (≈3000 counts*min^{-1}), fast walking/light running (≈5000 counts*min^{-1}), and running (≈8000 counts*min^{-1}), since the hypothesis was that most children would be involved in such activities quite often when monitored during free-living conditions. On the other hand, children's PA have been reported typically to include short burst activities with rather high intensities [117]. When taking a short look at the raw free-living activity files post hoc in Study I, it was confirmed that children actually experience rather sporadic activity patterns including numerous periods of reduced body movement where activity output is considerably below the sizes comparable to walking or running. Therefore, the absence of any acceleration responses examined individually in each unit where only very limited instrument output was produced must be included as a limitation in the present thesis. This is stressed further by findings observed by Brage et al. [164] who previously showed that the CSA accelerometer displays particularly poor inter-instrument reliability at very low accelerations. However, Brage and colleagues explained the poor reliability at the lower accelerations by the dead band of the CSA instrument (approx. 0.3 m*S^{-2}), meaning that different units have different lower thresholds at which they begin to register movement. However, normal human locomotion is expected clearly to exceed accelerations near in size to the dead band, and therefore, in relation to issues linked to proper calibration of field data the clinical significance of poor reliability at the very low accelerations caused by the dead band is probably very limited.

Furthermore, it should be noted, that the rather limited amount of time where children might experience activity output comparable in size to walking, and especially running, when examined during free-living conditions, probably partly is a result of epochs influenced in size by periods were children are experiencing no activity at all. Therefore, it can be argued that the time where children apparently are involved in activities where the accelerometer output is considerably reduced compared to the output range where calibration in the laboratory was performed falsely will be overestimated due to periods of zero activity. This, of course, would leave the absence of any calibration performed at very low accelerations less problematic.
It might be speculated that numerous periods of zero activity to some degree will attenuate a potential impact of unit-specific calibration when applied to field data due to the fact that when exposed to no acceleration at all, all instruments will produce the exact same output - namely zero. Therefore, the effect of applying unit-specific calibration factors to field data representing the percentage of total registered time spend in high or vigorous activity levels, defined according to Trost et al. [153] was analysed post hoc when applying unit-specific calibration factors separately for each epoch being downloaded (data not shown). However, under these circumstances, where periods of zero activity are greatly eliminated form field data, the exact same result was observed – calibration did not reduce random variation caused by inter-instrumental variability across the examined group of children.

Movement characteristics

The mechanical setup solely offers isolated and standardized sinusoid movements, as already mentioned in the paragraph *"General strengths and limitations"*. However, children have been reported typically to be involved in numerous numbers of different activities, including different games, jumping, dancing, running, climbing, and biking [118], introducing a wide range of frequencies and moving directions through more complex movements of the body. Potentially, these dissimilarities between types of movements, as well as biomechanical variation between subjects, even when involved in the same type of activity, might affect the comparability between inter-instrumental variability characterized in the mechanical setup and in the field when assessing the complex and heterogeneous behaviour of human locomotion in children. Relevant biomechanical issues especially involve individual step frequency since the MTI/CSA outputs are filtered more at higher movement frequencies. In relation to that, differences in step frequencies have been reported to explain 11% and 40% of the speed-adjusted variance in CSA output in walking and running, respectively [199]. Therefore, the effect of calibration on increased inter-instrumental reliability might be reduced due to an increased between-subject variation caused by individual step frequencies preferred during free living conditions in the field.

Furthermore, technical inter-instrumental variability might be related to the frequency and/or acceleration magnitudes. This theory is indirectly supported by a previous study by Brage et al. [164] who found inter-instrumental differences to be heteroschedastic in response to the acceleration magnitude, meaning that the measurement errors depend on the acceleration magnitude. Similar findings have been reported by Jakicic et al. [142] who found that inter-instrument reliability in the TriTrac-R3D accelerometer appeared to depend on the specific type of PA being assessed.

Optimal measuring axis of movement

Very relevant when trying to achieve successful calibration is the thoroughness shown in order to optimize the parallelism between the measuring axis of the instrument and the axis of movement actually experienced. A standardized attachment of the instruments to the plate at the calibration machine was secured when calibration and quality checks were performed in the laboratory in order to ensure that the registration of the acceleration along the vertical axis was optimized. Ideally, every child would wear the monitor at the exact same angle in the field. However, even though subjects were instructed how to mount and wear the accelerometer, rather individual attachments of the devise to the body must be expected, and furthermore, instrument positions might change as a result of consistently body movements. This will cause a varying off-vertical instrument position between subjects, which in the end will contribute to an increased random output variation in the field. The scope of this problem is illustrated by previous findings showing reduced accelerometer outputs of 6%, 16%, and 29% when reducing the optimal angle at the axis of measurement by 15^o, 30^o, and 45^o, respectively, during standardized conditions in the laboratory [163].

Intra-instrumental variation

Unit-specific acceleration responses varies over time (intra-instrumental variation), and inevitable the calibration factors estimated and applied in Study I will include a rest of the so called standardized unit-specific test- retest variation. However intra-instrumental variation should be present both in the laboratory and in the field, but maybe not to the same extend? Mean intra-instrumental reliability has been reported to be 4.4% and 3.5% in a mechanical setup in the laboratory and during treadmill and over ground walking/running, respectively [200], indicating approximately similar variations. However, Metcalf et al. [163] found mean intra-instrumental variability in CSA instruments to be only 0.81% and 1.83% at fast and medium speeds, respectively, when calibrated in a turntable in the laboratory within the first 8 weeks of the children carrying them in the field. This might indicate some controversies regarding the size of intra-instrumental variability when examined in different settings.

In order to compensate for intra-instrumental variations over time, it might not be sufficient to perform calibration at three different time points in the period from November 2003 to Marts 2004, as it was performed in Study I. However, examining acceleration responses more frequently, maybe even within a wider rage of accelerations, becomes rather problematic if calibration should be feasible in large scale population studies, since this procedure would require huge demands with regard to manpower and time consume.

Agreement between raw and calibrated field data

The amount of variation introduced to field data when performing calibration was estimated to be only 1.1% when compared to the total amount of variation in HPA in children. This amount of variation must be considered as rather limited, especially considering the size of the reproducibility coefficient (R) of a 4-day period, which previously has been found to be 0.66 in the children examined in the present thesis [201].

As described in the paragraph *"Effect of applying unit-specific calibration factors to field data"* very high correlation coefficients were observed between raw and calibrated MTI output in children in the field. Furthermore, the Bland-Altman plot showed that relative 95% limit of agreement between raw and calibrated instrument output in the field was approximately ±5.5%. The high agreement between raw and calibrated field data is probably explained by a combination of improved data quality due to the repeated quality checks performed in the mechanical setup and the presence of other major sources of variation (e.g. biological variation, day to day variation, seasonal variation, and poor compliance with correct mounting of the devise to the body). As indicated in this thesis (Study I), the inter-instrumental variability must be considered as relatively small when compared to the total variation in field data.

Theoretically, ideal calibration factors applied to instruments with zero intra-instrumental variation would cause inter-instrumental variability to disappear when examined under standardized conditions in the mechanical setup in the laboratory. However, as indicated in Figure 7, even though inter-instrumental variability was substantially reduced after performing calibration considerably inter-instrumental variations were still observed when examined under standardized conditions in the laboratory. Therefore, even though we must assume that the subjects who moved considerable after calibration actually achieved a PA level closer to their "true" level if monitored with no measurement error at all, the calibration factors estimated and applied in Study I inevitable will include a rest of the so called standardized unit-specific test- re-test variation, and therefore, eventually they will also add to the amount of random variation.
Furthermore, it might be speculated that unit-specific calibration factors estimated in the laboratory only to a certain degree reflect inter-instrumental variations in the field, depending on the actual comparability between inter-instrumental variability characterized in the mechanical setup and during free living conditions in the field. This in combination with the presence of other major sources of variation indicates that the Bland-Altman plot only to a certain degree will capture the "true" individual diversity between raw field data and field data if monitored without measurement error at all. Nevertheless, continuously performed instrumental quality checks will in all probability add to an increased inter-instrumental reliability (i.e. avoid instruments recognized by rather extreme acceleration responses defined according to an a priori limit of variability).

Comparing acceleration responses between the group of MTI and CSA instruments

The CSA/MTI count output has previously been found to increase as frequency decreases at a given acceleration [164,165]. Therefore, the fact that the group of CSA instruments were exposed to slightly higher frequencies compared to the groups of MTI instruments, when comparing the mean acceleration responses across the two different generations of instruments in the mechanical setup in the present thesis (Study I), potentially will challenge the validity of our results. However, when identical frequencies and acceleration magnitudes were applied post hoc in at the time being 53 functional CSA- and 18 functional MTI instruments in June 2004, the MTI instruments in average displayed a significantly ($p<0.001$) increased acceleration response of 9.50% when compared to the group of CSA instruments. This indicates comparable diversities as being observed when the two generations of instruments were exposed to accelerations produced within slightly different frequencies ranges.

Indications of batch/lot effects have also previously been revealed by Esliger et al. [165] who compared mean accelerometer output in six testing conditions in a mechanical setup.

Secular trends in cardio-respiratory fitness, body mass index, and habitual physical activity.

This Ph.D. thesis is the first to describe simultaneously examined secular trends in CF, BMI, and HPA in a large scale population study when using an objective reliable maximal fitness test to exhaustion as well as objectively registrations of HPA by accelerometers. The thesis (Study II & III) has three main findings. Firstly, a significant secular decline was revealed in CF in girls overall during the period from 1997/98 to 2003/04. Secondly, although not statistical significant after additional Bonferroni's correction, results describing secular trends in CF and BF in general showed less favourable trends in children with low SES. Thirdly, no significant secular trends in the level of HPA had occurred between 1997/98 and 2003/04, neither when distinguishing between different day types, nor when looking at the two samples across socio-economic gradients.

General strengths and limitations

The major strengths in Study II & III imply the use of identical protocols and sampling frames in two large cohort studies, the use of an objective reliable maximal fitness test to exhaustion, as well as objectively assessment of HPA in a large number of randomly selected children. In both studies the sampling frame was a complete list of public schools in the Municipality of Odense (Odense is the third largest city in Denmark). By comparison with the National Statistic Registry, the distribution of both educational level and income of the parents in 1997/98 was representative of Denmark.

Limitations include the relatively large number of non-participants, especially in the study conducted in 2003/04, as well as the dependence on subjective statement given by the form master when considering the characteristics of participants vs. non-participants in the study in 1997/98. However, although questionable if only one non-participating child differed with respect to body weight and PA level compared with the class peers, there is little reason to believe that the study in 1997/98 suffered from severe selection bias, considering the thorough sampling procedure introduced to secure a representative sample of third grade children and the acceptable participation rate, which was achieved.

Extraordinary information was available in the study in 2003/04 where access to anthropromefric measurements (i.e. height, weight, and BMI), highly relevant in relation to the variables being examined in the present thesis, was offered in all third grade children invited to participate in the study. Subsequent analyses did not reveal any differences between participants and non-participants, as already described in the paragraph *"Representativeness"* in the opening of this thesis. These findings, combined with the use of identical sampling frame used in the study in 1997/98, also indicate that the sample in 1997/98 did not suffer from bias caused by non-participants, especially considering the higher participation rate when compared to the study conducted in 2003/04.

Although not without limitations and assumptions, BMI was used as a measure of BF in the present study. Other studies have indicated, however, that BMI is a useful measure of BF in children as long as age, gender, and biological maturity is controlled for [110,111].

Despite the thorough precautions shown in order to secure representative data, the validity of the results describing secular trends in HPA potentially is challenged by the reduced number of children in the end confirming with the criteria set to define valid activity files. Finally, valid activity files representing weekdays were reached in 70% and 91% of the total number of children being monitored in 1997/98 and 2003/04, respectively. During weekend days, valid activity files were achieved in 63% and 91% of the children registered in 1997/98 and 2003/04, respectively. This indicates a substantial number of children, especially in the study in 1997/98, not meeting the criteria set to define valid data.

The reduced sample sizes available when paying special attention to the socio-economic background, primarily explained by a combination of a limited number of children both complying with socio-economic information and the inclusion criteria set to define valid activity files, and the large variation within the PA data caused that only poor statistical power was obtained when comparing secular trends in HPA across socio-economic gradients. In fact, a post hoc analysis confirmed that with the statistical power set at 0.8, it was only possible for us to detect mean differences between 73 and 110 counts*min^{-1}, when comparing secular trends in HPA across socio-economic gradients in boys and girls, respectively.

Present results and previous findings

Cardio-respiratory fitness and low cardio-respiratory fitness

Overall

A secular trend with a decline in CF has previously been demonstrated in Danish boys between the mid eighties and late nineties, whereas no overall difference in CF was found in girls during that period of time [202]. The findings in the present thesis of a negative trend of approximately 2.8% in CF in girls overall, but no significant change in CF in boys overall, suggest that CF has decreased in girls and apparently stabilised in boys during the examined period of six years.

Rather limited data exist where secular trends in CF have been explored in children or adolescents, and even less data are available from studies originally designed to examine such trends. Therefore, statements on children's and young people's level of fitness have typically relied on findings from meta-analysis or reviews [174,203,204], which potentially suffer from methodological variations, including different protocols and sampling frames. Other surveys, which have been designed to examine secular trends in CF have relied on less reliable field tests such as running distance covered in 9 minutes, the 1 mile run/walk test, and the 50m sprint test as the fitness variables when measuring CF [205,206]. However, disadvantages of aerobic field tests, which potentially could complicate secular comparisons, include parameters like practice, motivation, strategy, and environmental conditions [204].

Based on analyses of data over almost six decades, Armstrong & Van Mechelen [128] reported that there is no scientific evidence to support that young people's aerobic fitness has declined over the past 50 years. When examining secular changes in directly measured peak VO_2 in US boys and girls, by comparing available data from the 20^{th} century (1930s-1990s), Eisenmann et al. [203] found no changes in absolute or weight-relative peak VO_2 in boys aged 6-18 years since 1938, and no changes in young girls since 1960. However, a decline in CF was observed in adolescent girls since the 1980s.

Across socio-economic status

The absence of any significant secular trends in the continuous variable of CF in both blue-collar- and white-collar girls, as observed in Study II, seems contradictory considering the observed significant decline in CF in girls overall. We assume that our finding is not a result of a type-1 error; therefore, the insignificant secular trends in both socio-economic subgroups probably represent a true trend in at least one group, which would have turned out significantly given a higher statistical power.

The increased level of CF in boys in the white-collar socio-economic group in 2003/04 compared to 1997/98, but no changes in CF in boys in the blue-collar group indicates a heterogeneous development in CF between 1997/98 and 2003/04 in different socio-economic subgroups of the boys. Heterogeneity was also suggested by the tendency towards a decline in the prevalence of low CF in white-collar boys but no signs of evidence of a change in the prevalence of low CF in the blue-collar boys. Despite the observed dissimilarities in the secular trends in CF and low CF when distinguishing between the two groups of SES, a tendency towards polarization across SES was only observed in CF in Danish boys between 1997/98 and 2003/04. It should be realized, however, that after additional post hoc Bonferroni's correction, needed due to multiple testing procedures caused by a numerous number of statistical analyses, none of the statistical analyses performed across socio-economic gradients no longer reached significant or borderline significant p-values. Therefore, theoretically we can not exclude that results regarded as significant or borderline significant to begin might have been reached by chance. Therefore, further studies designed to deal with secular trends in CF across socio-economic gradients and/or polarization in CF over time between socio-economic subgroups are needed in order to elaborate definite conclusions regarding the future socio-economic caused impact on secular trends in CF in children. On the other hand, in general it should be emphasized that our findings of several results or tendencies pointing in the same directions when distinguishing between secular trends across SES certainly will reduce the probability of results solely reached by chance.

A polarization in CF over time has previously been observed in both Danish boys and girls overall by Wedderkopp et al. [202] who revealed an increased difference between the least fit and the most fit children between 1985/86 and 1997/98.

According to our knowledge, no other study has previously examined the secular trends in CF across SES in a large number of randomly selected children. Therefore, no direct comparison between the present results and the literature is possible. However, if different development patterns in CF should be expected across socio-economic groups over time it seams logical also to expect a certain socio-economic gradient in CF when examined in single cross sectional studies. Studies of the association between socio-economic position and CF in children and youth have produced inconsistent results, showing both associations [74,207,208] and no associations between social class and performance [57,209]. The inconsistent results could be due to differences in the ages, different assessment techniques of CF, as well as due to different classifications used to describe SES of the populations under study.

Body mass index and overweight

Overall

In Study II, no significant overall secular trends were observed in BMI or OW in Danish boys or girls between 1997/98 and 2003/04, neither before nor after performing Bonferroni's correction. This indicates that BMI levels and the prevalence of OW have stabilised in Danish children during the examined period of six years.

Secular trends in BMI, Skinfold thickness, and waist hip circumferences have previously been described in children in other large scale population studies [205,206,210-212] and a general picture showing adverse prevalence of OW and obesity in the paediatric population has emerged both in the U.S. and in European countries, especially during the last decades of the 20'th century [172,173]. A secular increase in skinfold thickness and obesity has also previously been described in Danish boys overall between 1985/86 and 1997/98, and during the same period of time a polarization between the fat and lean had occurred in both boys and girls[202]. However, we are aware of only few studies, which have examined similar secular trends in large samples of Danish children outside the framework of the EYHS. A secular increase in OW was revealed in Danish boys born in the period 1930-1975 when examined in a study conducted by Thomsen et al. [213]. In another study conducted by Petersen et al. [214], a secular increase in BMI in both boys and girls was observed when comparing data collected from 1986/87 to 1996/97 with data collected in 1971/72.

Across socio-economic status

The increased BMI in boys in the blue-collar socio-economic group, the tendency towards decreased BMI in boys in the white-collar group, as well as the higher odds for being OW in 2003/04 compared to 1997/98 among blue-collar girls, as observed in Study II, indicates that secular changes in BMI and OW can be present in specific socio-economic sub groups, even though no overall secular changes can be observed. This theory was supported by the observed polarization in BMI across SES in boys between 1997/98 and 2003/04, as well as by the tendency towards polarization in OW between blue- and white-collar boys, indicating that the difference in BMI and prevalence of OW between the blue- and white-collar boys had increased during the examined period of six years. However, after additional Bonferroni's correction none of the analyses describing secular trends in BMI and OW across socio-economic gradients no longer reached significant or borderline significant p-values. Therefore, results from Study II call for the need for further examinations dealing with secular trends in BMI/OW across socio-economic gradients in children, before final statements can be provided.

In general the literature has failed to prove any consistent association between adiposity and SES in children [56,58]. However, the indication of less favourable trends in BMI and OW in children from low SES families, as observed in the present thesis, is being supported by previous findings where increased risk for OW over time has been reported in young Swedish men with low educated mothers compared with those whose mothers had university education [215]. Furthermore, the rate of increase in skinfold thickness among Australian school children between 1997 and 2002 has been found to be related to socio-demographic milieu in girls, but not in boys [216]. However, in a previous Danish study the association between BMI and SES could only be confirmed in adolescent boys [214]. The limited number of studies reporting contradictory results illustrates the need for further studies dealing with secular trends in body composition across socio-economic gradients in children.

Habitual physical activity

Overall

According to the best of our knowledge, this thesis (Study III) is the first to report secular trends in the level of HPA in a large cohort study, using objective assessments by the use of accelerometers. The study proved no evidence of an overall secular change in the level of HPA between 1997/98 and 2003/04.

Rather few studies have been designed and conducted in order to examine the secular trends in the level of PA in children or young people, and they have typically assessed PA by the use of questionnaires. A study by Eisenmann et al. [167] proved evidence of increased leisure time PA in Canadian adolescents between 1981 and 1988, but there after activity remained relatively stable until the end of examination in 1998. Evidence supporting stable conditions was observed in a study by Pratt et al. [168] who observed no decline in self reported vigorous PA between 1993 and 1997. In a previous Danish study, Holstein et al. [217] found no evidence of a change in the proportion of 11 years old children who reported to be vigorously physical active or physically inactive in their leisure-time when examined in the period 1988 to 2002. However, the proportion of 15 years old adolescents who reported to be physically inactive increased during the same period of time, whereas the proportion of adolescents being vigorously physical active was observed to decrease.

Other studies, which previously have examined the secular trends in PA in children or adolescents, have typically focussed on PA data in a range of more specific contexts due to the absence of suitable baseline data representing a more copious spectrum of PA. No evidence of an overall decline in the enrolment in physical education classes and activity during these classes was observed among adolescents when examined in 1991 and 2003, respectively [218,219]. In another study from Sweden, more adolescents were found to participate in leisure-time sports activity in 1995 than in 1974 [220], whereas a considerable decrease in the level of leisure-time PA was observed in Scottish adolescents between 1987 and 1991 [221].

However, these surveys all used questionnaires or interviews when quantifying the level of PA. Subjectively statements on PA (e.g. intensity and duration) may be biased by decreased levels of fitness or reduced exposure of specific activities, which falsely might cause an increased level of perceived exertion, and generally self reports will challenge the validity of the observed results as previously described in the paragraph *"Introduction"*.

Inconsistent results published in the research literature might at least partly be due to various definitions used in order to capture the concept PA, as well as due to different measurement techniques being applied when assessing PA. However, a recent review performed by Dollman et al. [78] concluded that there is no evidence supporting that PA is declining in children and young people when no particular contexts of PA are specified. However, PA in clearly defined contexts such as transportation, organised sport, and physical education in school settings was reported to be declining in several countries.

Across socio-economic status

This thesis (Study III) revealed no significant secular trends in the level of HPA across socio-economic gradients between 1997/98 and 2003/04. According to the best of our knowledge, no other study has previously been designed in order to examine if secular trends in PA differed across socio-economic gradients in a large scale population study of children. However, Inchley et al. [222] found persistently increased levels of leisure time vigorous PA in children with high SES during the years 1990 to 2002. In general, studies conducted in younger people have reported rather inconsistent findings when examining the association between SES and PA in single cross sectional studies [66]. However, in adults, low education has been found to have an adverse effect on the level of PA, both when examined during follow up in a longitudinal study [223] and when examined in a single cross sectional study [224]. A positive association between SES and PA in adults has also been observed by Galobardes et al. [225]. However, the disparity remained stable through the examined period from 1993 to 2000, thereby failing to prove evidence of any further adverse secular trends across socio-economic gradients.

Quality and validity of cardio-respiratory fitness data

Maximal heart rate and chronological age

Compared to the study conducted in 1997/98, systematic higher maximal heart rates were achieved in the study in 2003/04 when performing the watt-max test. This indicates that the two groups of children examined in the studies in 1997/98 and 2003/04, respectively, did not reach identical levels of exhaustion. Furthermore, the heavy logistic work load in 2003/04 caused that by the time of examination the children were approximately 2 months older when compared to the children examined in 1997/98. Data describing the association between chronological age and maximal aerobic capacity in children and young people is rather inconsistent across several longitudinal studies; however, little speaks for no change in aerobic fitness across different ages [226]. Furthermore, age-associated variation in fat free mass and body fat percentage can be observed, also during the childhood period [227], thereby allowing for a possible changed chemical composition of fat free mass – also during early growth.

The association between chronological age and physiological characteristics is at least to some degree influenced by pubertal status. Chronological age is not the optimal indicator of biological maturity, and at any given age the stages in progression toward adult physiological characteristics can be expected to vary widely from one person to another. However, the classification of pubertal status as performed in the present thesis by rating breast development in girls and pubic hair status in boys, as proposed by Tanner [228], has been questioned by Rowland due to the potential inter-individual differences regarding the amount of time needed to pass a certain developmental stage, as well as due to the question being raised as to whether all subjects follow the prescribed order of stages [229]. Therefore, controlling for pubertal status and chronological age was performed simultaneously in Study II, in order to minimize the chance of biased results due to the different relationship between physiological developments and chronological age/pubertal status across the group of individuals. As another phenomenon speaking in the favour of controlling for both chronological age and pubertal status, at least in the analyses describing secular trends in maximal CF estimated on the basis of the maximal watt performed in the cycle-ergometer test, is the improved movement economic observed with increasing chronological age. This improvement, however, has been suggested to be more closely associated to neurological control used to synchronize inter-related action between muscle groups rather than to hormone status and somatic growth tightly linked with pubertal development [226].

Identical maximal heart rates and age distributions would have been preferable in the two cross sectional studies in the present thesis, although being able to avoid biased results by controlling for maximal heart rate and age at the time of examination in all statistical analyses describing the secular trends in CF and/or BF.

Quality and validity of habitual physical activity data

The potential recall bias, which is an unavoidable component of HPA, especially in children, was not an issue in the present thesis due to the objectively assessment of HPA with accelerometers. However, this method also has limitations, and therefore several issues should be considered when interpreting the present results. Some of them are discussed in the following.

Valid activity files

The increased number of children not meeting the criteria of inclusion in 1997/98 is explained in particular by an increased number of instrument breakages in the study in 1997/98, since the rather thorough quality check performed in 2003/04 in order to ensure that instruments were functional prevented broken devises from being returned into the field repeatedly. Furthermore, due to an excessive number of instruments available in the study in 2003/04, it was possible to ask a child to wear the accelerometer again if forgotten to begin with. However, the increased exclusion of children in the study in 1997/98, due to the reason listed above, is expected to be random across the range of HPA level (i.e. non-differential selection). Therefore, although valid HPA files were reached more frequently in children in the study in 2003/04 than in the study in 1997/98, there is little reason to believe that the figures describing the secular trends in the level of HPA was biased by the increased number of subjects being excluded due to instrument breakages in the study in 1997/98.

After complying with all inclusion criteria, the final number of children achieving valid activity files might not seem impressing. However, especially after having discovered the substantial amount of broken instruments, missing data, and limited number of valid days we believe that the thorough precautions carried out in order to increase data quality should not be compromised in order to increase the participant rate, since it indeed will reduce the data quality and validity.

Minimum number of registered valid days

Four days of HPA registration have previously been shown to be representative of a whole week [195]. However, since the interest in HPA was placed on a group level, the child was included if she or he accumulated valid activity in minimum three days, in order to reduce the number of children failing to reach the required minimum number of valid days. Therefore, on a group level we believe that our figures represent a realistic estimation of the level of HPA. However, in order to try to compensate for any possible reduced validity especially in subjects encumbered with a more limited number of valid days, all analyses describing the secular trends were performed both as crude estimates and as adjusted estimates weighted according to the accumulated number of valid days (i.e. more valid days equal higher weights). However, adjustments performed according to the accumulated number of valid days did not introduce any appreciable effects when analysing secular trends in HPA (Study III) - although not identical with crude results. The explanation for the observed limited effect probably lies in the fact, that the majority of all children actually accumulate the same number, or very close to the same number, of valid days.

Reactive modification of the habitual physical activity level

When accelerometry is applied in order to register the level of HPA in children, it might introduce an undesirable reactive behaviour as a result of the awareness and interest of having the personal level of PA registered. This will possibly modify the level of HPA in the children being examined. Such a problem is hard to avoid, unless repeated measurements are performed. However, in large scale population studies the procedure of repeated measurements is less feasible. Observations indicating that the level of PA might be modified through the excitement of being selected for participating in studies where the monitoring of the daily PA level is introduced have been reported in adults [230]. However, another study conducted in children revealed no indication of reactivity when assessing the PA level with pedometers [231].

It should be emphasized, that in Study III the primary purpose was to analyse the secular trends in HPA during a period of six years. Therefore, in worst case only the absolute level of HPA would be biased even if a reactive behaviour occurred among the children being monitored, unless the modifying effect could be expected to differ across the two periods of registration. Fortunately, however, such an adverse modifying effect seems very unrealistic.

Seasonal variations

The fact that registrations were performed during the academic third grade school year means that the monitoring of HPA was performed at different seasonal time points, which in the end will contribute to an increased validity when trying to assess the mean level of HPA in a group of children. Seasonal variations are likely to be highly associated with outside temperature and other weather conditions, and it seems obvious to expect children to be more involved in physical activities and out-door games during spring and summer than during wintertime. Other studies have supported the idea of increased PA levels during spring and summertime in children [232-235]. Due to a comprehensive logistic work load in the study in 2003/04, assessments could not be initiated until the beginning of November, and therefore, the study in 2003/04 did not include registrations in the months of August, September, and October. However, only ten children had their PA level monitored in August in the study in 1997/98, and a post hoc analysis controlling for the effect of months revealed no indications of biased secular trends due to the effect of seasonal variations.

Generally, it might be hard to predict the change of biased results when comparing different studies due to the effect of months if you do not have any detailed retrospective information on the weather condition across the different months, even if assessments are distributed identically across the different months.

Biking and swimming

The validity of mean total output per registered time (counts*min^{-1}) is challenged in particular in the field, if the subjects being examined are frequently involved in biking activities, since assessment with the uni-axial accelerometer underestimates bicycling intensity with up to 90% when mounted to the hip [200], due to a limited biomechanical vibration of the abdominal region during bicycling [236]. Furthermore, as another instrumental limitation, the CSA/MTI instrument is being restricted only to a dry environment.

The Danish population is one of the most frequently user of the bicycle for transportation in the world [237]. On average every adult Dane bicycle 400 km per year [238], and Danish children have been reported to be even more frequently involved in biking activities than adults [239]. Therefore, in Study III, the absolute level of HPA is likely to be underestimated due to the poor registration of activity obtained from swimming and biking in particular.

Potentially, substantial underestimation of these two specific activities will not only increase random variation in the HPA level across the examined group of subjects, but in worst case and more problematic in relation to Study III any possible general shifts in biking and swimming activities between 1997/98 and 2003/04 would, of course, strongly challenge the validity of the results describing the secular trends in the HPA level. In the present thesis, the only empirical information available when trying to examine the issue of a possible shift in biking was obtained through a questionnaire where all children were asked how they usually travel to school. A post hoc analysis revealed that both in 1997/98 as well as in 2003/04, 38% of all children used their bike for transportation when travelling to school. This indicates no biased secular trends due to the poor registration of bicycling, although no information regarding general biking was available.

Studies in both adolescents and adults indicates that cycling habits vary by socioeconomic characteristics [65,240], which in combination with the frequently biking of Danish children potentially will introduce a bias when trying to compare absolute levels of accelerometer-assessed HPA in children with diverse socio-economic backgrounds.

Although not being a question of research in the present thesis, our figures indicates that boys with high SES are less physically active in comparison with boys coming from families with low SES - both in study in conducted in 1997/98 and in the study in 2003/04. This is rather surprisingly and unexpected, however. Studies conducted in younger people have reported rather inconsistent findings regarding the association between socio-economic background and PA. However, the controversy has usually been related to whether SES and PA are positively associated or not related at all [57,241,242], and only very few studies have reported a negative association, as indicated in a review study provided by Sallis et al. [66].

In girls, no indication of increased HPA level was observed in the group of low SES in the present study, on the contrary. Theoretically, this could be explained by more homogeneous biking patterns across the socio-economic gradients in girls than in boys. However, a post hoc analysis revealed no difference across SES in the frequency of biking to school, neither in boys (p=1.0) nor in girls (p=0.84). Apparently, this indicates that bicycling did not cause biased levels of HPA across SES. It should be realized, however, that no information regarding general biking, duration, or intensity was available.

Differential measurement error potentially might be an important source of bias if diverse biking habits can be expected across socio-economic gradients, as indicated from the literature [65,240]. Therefore, possible problems related to the registration of biking activities within specific socio-economic subgroups stresses the importance of that additional analyses are performed across SES, when describing secular trends in the level of HPA assessed by the use of accelerometers.

Instrumental differences between MTI and CSA units

Compared to the group of CSA instruments, a significantly increased (9.95%) mean acceleration was revealed in the group of MTI instruments, when comparing acceleration responses separately in the two groups of instruments under standardized conditions in a mechanical setting before and during the data collecting period in the study in 2003/04 (Study I). Therefore, raw field data recorded with MTI instruments in the study in 2003/04 were calibrated according to the systematic magnitude of deviation observed between the two generations of instruments before performing the data analyses describing the secular trends in the level of HPA (Study III). Although being able to calibrate MTI readings according to findings in the laboratory in order to prevent biased data caused by systematic instrumental differences between the two batch of instruments, it might be speculated whether the mean acceleration response observed in the batch of CSA instruments in 2003/04 did reflect the mean acceleration response which originally characterized the group of CSA instruments back in 1997/98. However, the mean acceleration response was compared post hoc in the mechanical setup in 2006 in the two groups of instruments immediately after all CSA units were calibrated according to the procedure recommended by the factory [139]. At that time, results revealed a significantly (p=0.002) increased mean acceleration response of 10.7% in the group of MTI instrument, indicating that the diversity previously observed three years earlier not only remained unchanged over time, but apparently also mirrored a more universal disparity between the two generations of instruments.

Parallels between secular trends in cardio-respiratory fitness, body mass index, and habitual physical activity

Physical activity has been found to play a key role in the aetiology of low CF and excessive body fat in the adult population [40,41,243-245]. In children, however, the evidence supporting this relationship is generally less clear and obvious, and only a modest positive effect have been observed at best [49,50,246-248]. Intuitively, however, the secular trends in the level of HPA observed between 1997/98 and 2003/04 (Study III) would be expected to some degree to be accompanied by comparable trends in CF and BF during the same period of time (Study II), unless energy intake changed considerably during the examined period of time.

The substantial overall stability in the level of HPA, CF, and BF, simultaneously observed in the two samples of children being examined in the present thesis indirectly increases the validity of the observed results. Only in girls overall, a limited decline in CF was observed. Contradictory to the general picture emerging when examining secular trends across SES in the present thesis, our results indicated that this negative trend in CF could be a result of reduced CF in the girls with high SES in particular - although not quite statistical significant. Regarding the secular trends in HPA observed across socio-economic gradients in the present thesis, only very modest and non-significant tendencies could be observed. However, although not reaching the level of statistical significance, the trends in HPA tended to be less favourable in the group of girls coming from families with high socio-economic status. Apparently, this speaks in the favour of comparable trends in girls' level of CF and HPA, respectively.

The similarity between trends in HPA and OW across socio-economic gradients in girls seems less convincing, however, due to the less favourable trends reported in OW in the group of girls with low SES. On the other hand, OW is expected to be highly modifiable not only through PA but also through energy intake (e.g. food composition and portion size). Furthermore, it is worth noticing, that the blue-collar girls examined in the present study in 1997/98 possessed a significantly reduced body height in comparison with all other groups of examined girls. Short persons are characterized by higher step frequencies compared to taller persons, and therefore it might be speculated that the reduced level of HPA, apparently observed in the group of blue-collar girls in 1997/98, partly is a result of biomechanical characteristics leading to higher step frequencies, which in the end will cause a reduced instrumental output due to the frequency dependent filter weight [194]. The implication of this might be that a tendency reflecting unfavourable secular trends in the level of HPA in both groups of socio-economic subgroups between 1997/98 and 2003/04 actually would mirror the true trend.

In boys, results from the present thesis in general indicates consistent secular trends across the different parameters being examined, since less favourable trends in general were observed in the groups of low socio-economic children - although no statistical significance could be reached. However, the limited combined compliance with information on SES and valid activity files should be considered when interpreting the secular trends in HPA across socio-economic gradients. Furthermore, in general it should be realized that minor secular trends in the level of HPA might not be captured due to a reduced measurement precisions and accuracy. Such declines, however, might have the potential to cause a changed level BF when experienced over a long period of time. For instance, based on results in a longitudinal study over four years [249], Goran et al. [250] showed that as little as a continual daily energy imbalance of 105 kJ/day, or approximately 2% of total daily flux, has the potential to lead to obesity. From a methodological standpoint this finding illustrates the importance of improving the accuracy of future measurement techniques feasible of assessing HPA levels and energy intake in large scale population studies. Otherwise, extremely large sample sizes will remain required in order to prove minor but potentially relevant effects of, and/or changes in, PA levels.

Adequate socio-economic definition

In the present thesis (Study II & III), the parent or guardian in the household with the highest level of occupation was used to define the family's SES since we hypothesized was that the presence of a well occupied person (woman or man) in general not only would reflect psychosocial characteristics but at the same time also capture aspects related to income and/or educational level, which in combination potentially would mark health related family decisions.

Typically, most studies have used only one measure of SES. However, focusing on occupational level alone might not capture a person's true SES completely [251,252]. Therefore, by using only one single measure of SES in the present thesis probably will underestimate the complete health related burden of the socio-economic disparities. Furthermore, measurement procedures, sample characteristics, and analysis strategies chosen in any study potentially may determine or influence the level of significance and strength of the SES-health related association [66]. Despite the change of an underestimated effect of SES on the secular trends in the health related parameters in the present thesis, our results indicate that socio-economic characteristics might be a parameter of substantial importance also when examining early indication of health status during childhood.

Conclusion

Unit-specific calibration of accelerometers

In conclusion, this thesis (Study I) indicates that unit-specific calibration factors, estimated on the basis of acceleration responses in a mechanical setup producing standardized sinusoidal movements, should be addressed as rather ineffectual when applied to data collected during free living conditions in children. The inter-instrumental variability was relatively small when compared to the total amount of variation in field data, and in all probability, the effect of calibration was attenuated in the field by other major sources of variation (e.g. day-to day variation, seasonal variation, biomechanical variation, and random variation caused by varying off-vertical axis of measurement between subjects).

Compared to the group of CSA instruments a significantly increased mean acceleration response was observed post hoc in the group of MTI instruments (Study I), indicating different acceleration responses across different generations of instruments. Therefore, investigators are advised to be precautious when interpreting and comparing PA data/studies where identical instruments have not been applied.

Secular trends in cardio-respiratory fitness, body mass index, and habitual physical activity

This thesis (Study II) revealed a significant secular decline (2.8%) in CF in girls overall in the period from 1997/98 to 2003/04. Indications of a secular increase in CF together with a secular decrease in the prevalence of low CF in white-collar boys, a secular increase in BMI in blue-collar boys, a secular increase in the prevalence of OW among blue-collar girls, as well as a polarization in BMI an OW across SES over time in boys, was observed during the same period of time. This indicates less favourable trends in low SES children, although statistical significance could not be reached after additional Bonferroni's correction, which was needed in order to account for multiple testing.

The present thesis (Study III) indicates that the level of HPA apparently has stabilized in Danish third grade children during the examined period from 1997/98 to 2003/04. No significantly secular trends were observed in the level of HPA between 1997/98 and 2003/04, neither when paying special attention to specific day types (i.e. weekdays vs. weekend days) nor when looking at the two samples across socio-economic gradients. However, a poor statistical power caused by a combination of the large variation in HPA and the limited combined compliance with information on SES and valid activity files should be considered when interpreting the secular trends in HPA across socio-economic gradients.

In both samples gender differences in the level of HPA were found to be more distinct during the weekdays than during the weekend days.

In conclusion, results from this thesis indicate that SES should be considered when describing secular trends in CF, BF, and PA in children, although secular trends in HPA seemed to be less influenced by SES. However, potentially this could partly be due to issues linked with reduced measurement precision Therefore, ignoring SES by only looking overall at the children being under examination might potentially lead to non-adequate conclusions.

Perspectives

Although the present thesis (Study I) indicates that the inter-instrumental variability is relatively small when compared to other sources of variation, information still seems to be lacking in key areas. More work is needed to understand 1: to which extend proper calibration is damped down during free living conditions in the field by other major sources of variation and 2: to which extend technical inter-instrumental reliability in the laboratory reflects technical inter-instrumental reliability during free living conditions. A study examining the parallel between inter-instrumental output variation in the laboratory and during free living conditions in the field is practically possible by asking a group of children to wear two or three accelerometers at the same time immediately after being examined in the mechanical setup. Potentially this design will help us answer the questions of interest, and currently we are planning such a study. However, at present time previous findings from the Danish part of the European Youth Heart Study, describing temporal sources of variation in the level of HPA in children and adolescents, indicates that the attention should be drawn to the importance of that HPA is monitored using multiple units in large groups of children/adolescents during many days, taking day types, day to day variation within subjects, and seasonal variation of the year into account when the aim is to reduce variation in field data [198].

Based on the diverse acceleration response observed between the different generations of instruments we strongly recommend for all research units to determine the acceleration response characterized in the specific population of instruments being used (i.e. avoid biased results due to batch effects). Furthermore, the point is not to discourage instrumental evaluation in the laboratory, as we strongly suggest that a simple and less time consuming quality check should be performed in all units each time they are returned from the field since this procedure will prevent broken instruments, defined according to an a priori limit of variability, from being returned into the field repeatedly.

A secular decline observed in CF in third grade girls (Study II) indicates the need for early interventions designed to prevent undesirable developments of CF in children and youth. Furthermore, the indication of adverse secular changes in CF/low CF and BMI/OW among children from blue-collar families suggests that the low educated part of society in particular might be in danger of experiencing a future generation with more unfavourable CVD risk status, meaning that preventive efforts against CVD aimed to improve CF and body composition especially should be optimized in, and targeted at, lower socio-economic subgroups. However, there is a need for future studies performed in order to increase the knowledge regarding the potential link between SES and health status, especially among the youngest members of the family. In particular, more studies supporting the present findings are needed before predictions about future CVD risk status and suggestions for intervention should be regarded as final statements.

This thesis (Study III) does not support the idea that Danish children of today are getting less physically active. However, further studies should be implemented in subjects of different ages in different countries in order to verify whether the present results reflect more widespread trends. Furthermore, the important issue of whether or not children and young people are active enough to reduce lifestyle related risk factors, still needs to be further clarified. In relation to that, substantial efforts should be undertaken in order to estimate appropriate universal accelerometer intensity cut-points where potential confounders like gender, age, body size, and biomechanical characteristics are taken into account. Finally, this thesis indicates that gender and day type variations are important and interesting aspects if interventions aimed to increase the level of PA in children or young people are planed, carried out, and evaluated.

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