Contents

List of Papers

This Ph.D. thesis is based on the following three papers that will be referred to in the text by their roman numerals.

Paper I

Kristensen PL, Korsholm L, Moller NC, Wedderkopp N, Andersen LB, Froberg K: **Sources of variation in habitual physical activity of children and adolescents. The European Youth Heart Study.** *Scand J Med Sci Sports (in review)* 2006.

Paper II

Kristensen PL, Møller NC, Korsholm L, Wedderkopp N, Andersen LB, Froberg K: **Tracking of objectively measured physical activity from childhood to adolescence. The European Youth Heart Study.** *Scand J Med Sci Sports (Accepted)* 2006.

Paper III

Kristensen PL, Wedderkopp N, Moller NC, Andersen LB, Bai CN, Froberg K: **Tracking and prevalence of cardiovascular disease risk factors across socio-economic classes: a longitudinal substudy of the European Youth Heart Study.** *BMC Public Health* 2006, **6:** 20.

Summary in English

Introduction

Cardiovascular diseases are among the leading causes of death in modern western society. The diseases are usually observed in adults only, however childhood health status and health behaviour are thought to play an important role in the development of these diseases. Earlier studies have shown that important cardiovascular disease risk factors, as for instance low physical fitness and obesity, tend to persist from childhood and into adult life. The phenomenon is termed tracking and has been widely studied in the literature. However, the present knowledge in this field is by no means exhaustive and can be questioned at certain points. Earlier studies of tracking of physical activity have almost exclusively been based on self-report instruments (e.g. questionnaires), and validation studies performed in children less than 10 years have revealed insignificant validation coefficients, indicating that most self-report instruments do not measure what they are intended to measure among young children. In addition, tracking of cardiovascular disease risk factors have predominantly been studied in representative samples of total populations and, apart from gender differences, no previous study has tested whether tracking varies significantly between subgroups of the population.

The aim of the present Ph.D. thesis was to study tracking of physical activity over an age range of approximately 9-15 years using accelerometry and to study potential sources of variation in physical activity that could be introduced by the shift in methods from self-report instruments to objective assessments of physical activity. Futhermore, the aim was to study tracking of physical fitness and body fatness over the same age range, and to examine whether tracking of these risk factors are related to socio-economic status.

Material and methods

The thesis is based on data from the Danish part of the international multi-centre study *The European Youth Heart Study (EYHS)*. At present, two measurement series have been conducted within the framework of EYHS in Denmark. In 1997-98 a baseline examination *EYHS-I* was conducted and a total of 589 8-10-year-old children and 429 14-16-year-old adolescents participated. Six years later the youngest of the EYHS-I cohorts were re-examined and a new cohort of 8-10-years-old were included in the study. In all, 458 8-10-year-olds and 444 14-16-year-olds participated in EYHS-II.

Habitual physical activity was measured objectively by use of the uniaxial MTI Actigraph. Physical fitness was determined by a progressive maximal cycle ergometer test (Watt Max Test) and body mass index was used as an indicator of body fatness.

Results

The results showed that the habitual physical activity level of children and adolescents is significantly influenced by temporal factors, including the type of measurement day and measurement month. Specific estimates of the effect of days and months were calculated and the analyses revealed two clusters of days – weekend days and weekdays. Both the 8-10-year-old children and the 14-16-year-old adolescents were significantly less physically active in weekends compared to weekdays. Moreover, the results showed that 8-10-year-old children are more physically active in the months of spring compared to the months of winter and autumn, with a range of 318 counts per minute between the most active and the most inactive month. Finally, the results revealed a significant day-to-day variation in physical activity with reproducibility coefficients of ≈0.45, after controlling for the significant day-type differences in physical activity.

Unadjusted stability coefficients for physical activity were estimated to be 0.18 and 0.19 for boys and girls, respectively. However, adjusted for temporal sources of variation in physical activity (i.e. day-to-day variation, within-week variation and seasonal variation) moderate tracking was observed with stability coefficients of approximately 0.50 for both genders.

Physical fitness and body mass index showed moderate and moderately high tracking, respectively, with stability coefficients of 0.50 and 0.73, respectively. Socio-economic status was not significantly related to tracking of physical fitness and body mass index, however when studying separate aspects of the term tracking (i.e. maintenance and development of risk) social gradients were observed. The odds of developing and maintaining overweight/low physical fitness were estimated to be approximately twice as high in the group of low socio-economic status compared to the group of high socio-economic status. Moreover, an inverse association between the prevalence of low physical fitness and socio-economic status was observed as early as at an age of 8-10-years, whereas a significant socio-economic gradient in the prevalence of overweight developed during the age range from 9-15-years.

Conclusion

It was concluded that temporal sources of variation in physical activity could introduce significant error into individual comparisons of physical activity and in analyses of association in which physical activity is included. Suggestions for dealing with these sources of variation were given. Furthermore, it was concluded that inactivity, low physical fitness, and overweight should be recognized as possible targets for intervention strategies - not only to improve health in childhood but also in order to lower the prevalence of these risk factors in later stages of life. Finally, it was concluded that children of low socio-economic status should be considered as a particularly important target for preventive strategies, due to the significant socio-economic differences observed in the present study. In this context, it was suggested, as a potentially important intervention strategy, to carry out initiatives aiming at reducing the occurrence of new cases of overweight and low physical fitness in the group of low SES during childhood or early adolescence.

Summary in Danish

Introduktion

Kardiovaskulære sygdomme er blandt de væsentligste dødsårsager i det moderne vestlige samfund. Sygdommene observeres almindeligvis kun hos voksne, men den almene sundhedstilstand og sundhedsadfærd i barndommen anses for at spille en væsentlig rolle i udviklingen af disse sygdomme. Tidligere studier har påvist, at væsentlige kardiovaskulære sygdomsrisikofaktorer, som f.eks. lav fysisk form og fedme, har en tendens til at fastholdes fra barndom og ind i voksenlivet. Fænomenet kaldes *tracking* og er ofte blevet studeret i litteraturen. Dog er der oplagte mangler og problemer i den viden, der foreligger på dette område. Tracking af fysisk aktivitet er i altovervejende grad undersøgt på baggrund af selvrapporterede oplysninger, såsom spørgeskemadata, og valideringsundersøgelser har vist, at subjektive metoder til måling af fysisk aktivitet på børn under 10 år ikke er anbefalelsesværdige. Endvidere gælder det generelt, at tracking af kardiovaskulære sygdomsrisikofaktorer er blevet studeret i repræsentative udsnit af totalpopulationer, og tidligere studier har kun i meget begrænset omfang undersøgt, hvorvidt tracking varierer mellem veldefinerede undergrupper af befolkningen. Formålet med indeværende Ph.D. afhandling var, at analysere tracking af fysisk aktivitet over et aldersspænd fra ca. 9 til 15 år vha. accelerometri, samt at studere potentielle variationskilder i fysisk aktivitet, som kan introduceres ved metodeskiftet fra subjektiv til objektiv bestemmelse af fysisk aktivitet. Endvidere var formålet at studere tracking af fysisk form og kropsmasseindeks over samme aldersperiode, samt at undersøge om tracking er relateret til socioøkonomisk status.

Materiale og metoder

Afhandlingen bygger på data fra den danske del af det internationale multicenter studie *The European Youth Heart Study (EYHS)*. Der er på nuværende tidspunkt gennemført to målerunder i EYHS regi i Danmark. I 1997-98 blev baselineundersøgelsen *EYHS-I* foretaget, hvor 589 8-10-årige og 429 14-16-årige deltog. Seks år senere blev den yngste af EYHS-I kohorterne genundersøgt, og en ny kohorte af 8-10-årige blev inkluderet i studiet. I alt deltog 458 8-10-årige og 444 14-16-årige i EYHS-II.

Fysisk aktivitet blev målt objektivt vha. accelerometri (The uniaxial MTI Actigraph). Fysisk form blev estimeret indirekte gennem en progressiv maksimal cykelergometertest (Watt Max Test) og kropsmasseindeks blev benyttet som et udtryk for kropsfedme.

Resultater

Resultaterne viste, at børn og unges fysiske aktivitetsniveau er betydeligt influeret af temporale faktorer, herunder typen af måledag og måned. Konkrete dags- og månedsestimater blev beregnet og analyserne afslørede to grundlæggende typer af måledage – hverdage og weekenddage. Både 8-10-årige børn og 14-16-årige unge var signifikant mere aktive i hverdagene end i weekenden. Ligeledes viste resultaterne at 8-10-årige børn er signifikant mere aktive i forårsmåneder sammenlignet med vinter- og efterårsmåneder, med et spænd på 318 counts per minut mellem den mest aktive og mest inaktive måned. Endelig viste resultaterne en betydelig dag-til-dag variation i fysisk aktivitet med reproducerbarhedskoefficienter på ≈0.45, efter at analyserne blev korrigeret for den signifikante variation i fysisk aktivitet mellem dagstyper.

Ujusterede stabilitetskoefficienter for fysisk aktivitet blev estimeret til 0.18 og 0.19 for henholdsvis drenge og piger. Justeret for temporale variationskilder i fysisk aktivitet herunder dag-til-dag variation, variation mellem dagstyper og målemåneder blev der observeret moderat tracking for fysisk aktivitet med stabilitetskoefficienter på ≈0.50 for både drenge og piger.

Fysisk form og kropsmasseindeks viste henholdsvis moderat og høj tracking med stabilitetskoefficienter på henholdsvis 0.50 og 0.73 uafhængigt af køn. Socioøkonomisk status var ikke signifikant relateret til tracking af fysisk form og kropsmasseindeks, men resultaterne viste, at sandsynligheden for henholdsvis at udvikle og fastholde overvægt/lav fysisk form gennem måleperioden, hvilket kan betragtes som underaspekter af begrebet tracking, var relateret til socioøkonomisk status. Oddsene for at udvikle og fastholde overvægt/lav fysisk form blev estimeret til at være ca. dobbelt så store i gruppen af lav socioøkonomisk status i forhold til gruppen af høj socioøkonomisk status. Endvidere blev der observeret en invers sammenhæng mellem prævalensen af lav fysisk form og socioøkonomisk status allerede i 8-10 års alderen, mens en signifikant socioøkonomisk forskel i prævalensen af overvægt først udviklede sig i aldersspændet fra 9-15 år.

Konklusion

Det blev konkluderet, at temporale variationskilder i fysisk aktivitet kan give anledning til betydelig fejlvariation eller ligefrem bias i individuelle sammenligninger af fysisk aktivitet og i sammenhængsanalyser, hvor fysisk aktivitet optræder som enten den uafhængige eller afhængige variabel. Konkrete forslag til statistisk håndtering af variationskilderne blev givet. Endvidere blev det konkluderet, at både inaktivitet, lav fysisk form og fedme i barndommen skal betragtes som potentielle mål for interventionsstrategier, der sigter mod at forebygge disse tilstande i voksenlivet. Endelig blev det konkluderet, at børn af lav socioøkonomisk status bør tildeles særlig opmærksomhed i forebyggelsesøjemed, grundet de socioøkonomiske forskelle, som blev observeret i dette studie. I denne forbindelse blev det fremhævet, som en potentiel vigtig strategisk mulighed, at iværksætte initiativer der har til hensigt at sænke antallet af nye tilfælde af overvægt og lav fysisk form i barndommen eller de tidlige teenageår blandt børn af lav socioøkonomisk status.

Abbreviations

AEE	Activity energy expenditure
BMI	Body mass index
BMR	Basal metabolic rate
CPM	Counts per minute
CVD	Cardiovascular disease
EYHS	The European Youth Heart Study
PAL	Daily physical activity level
SES	Socio-economic status
TEE	Total energy expenditure
TS	Tanner stage
Wmax	Maximal power output

1 Background

1.1 General perspective

A dramatic change has happened in the common causes of death during the 20th century. At the beginning of that century, the main causes of death were diseases of the respiratory and digestive systems and by other infectious and parasitic diseases [1]. Today, in western society, lifestyle related diseases are responsible for the majority of premature deaths [1]. The leading lifestyle diseases are cardiovascular diseases (CVD), cancer, and diabetes mellitus II [2]. According to the National Health Service of Denmark, heart diseases and tumours caused approximately half of all deaths in Denmark in 2005, whereas only 1.3 percent died from infectious diseases [3].

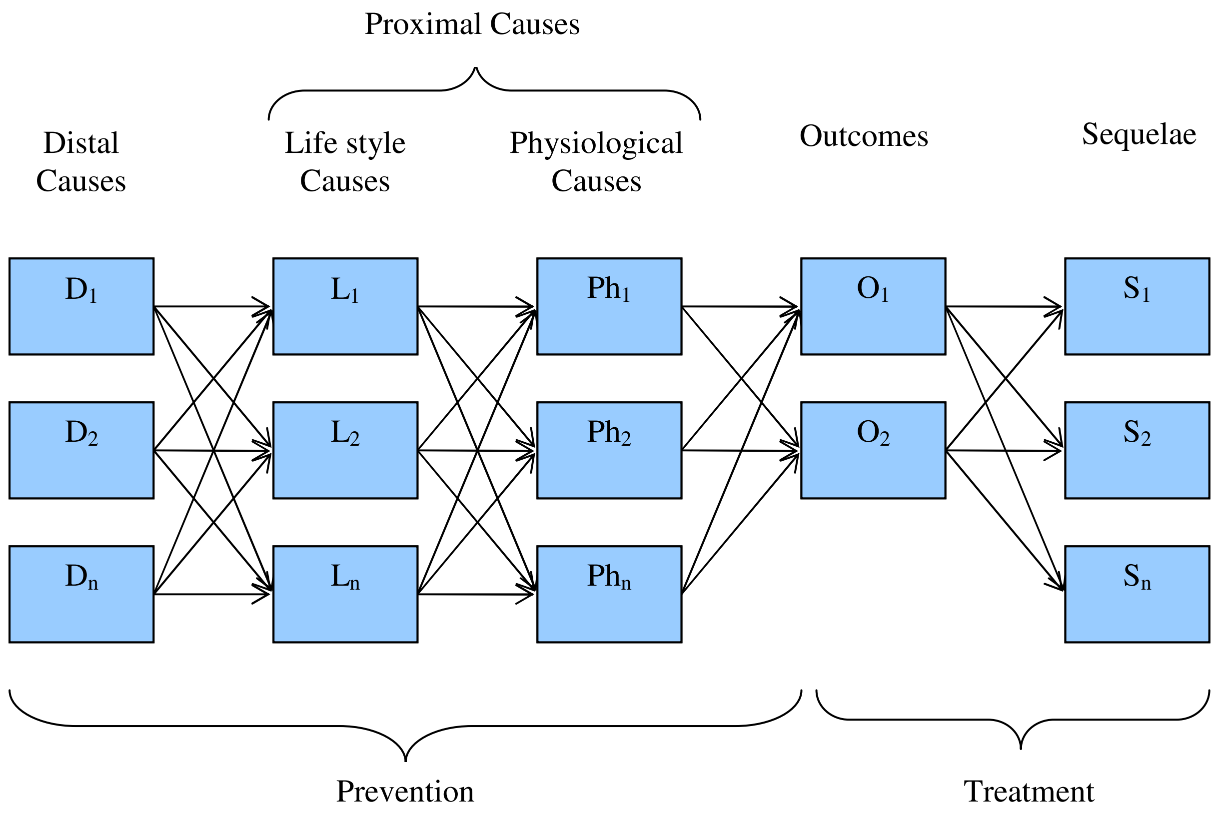
As evident by the name, lifestyle related diseases originate from the individual's way of living. The aspects of lifestyle particularly relevant are diet, exercise, alcohol consumption, and smoking habits. In the World Health Report 2002, the World Health Organization estimated that physical inactivity is the cause of 10-16 % of all global cases of breast cancer, colon/rectal cancers, and diabetes mellitus II [4]. Furthermore, 22 % of all cases of ischemic heart disease worldwide are caused by physical inactivity, and physical inactivity is the cause of 1.9 million deaths each year [4]. Smoking is estimated to cause approximately 70-90% of all cases of lung cancer among industrialized countries and is the cause of 22% of all cases of CVD [4]. As regard dieting habits, several factors can cause disease. Undernourishment, excess food intake, and malnutrition are all potential sources of disease. For instance, the World Health Organization estimated that a low intake of fruit and vegetables causes about 19% of all cases of gastrointestinal cancer and about 31% of the cases of ischemic heart disease worldwide. Overall, 2.7 million deaths each year is attributable to low fruit and vegetable intake [4].

The causal pathway from lifestyle to disease is very complex and the physiological mechanisms involved vary depending on which aspect of lifestyle is being considered (e.g. smoking, diet or physical inactivity). However, figure 1 provides a basic overview of the causal pathway from exposure to disease. Except from minor adjustments, the figure is identical to figure 2.2 in the world health report from 2002 [4]. As illustrated in the figure, some disease risk factors can be regarded as distal in the sense that they exert their effect through several intermediating factors. Other risk factors are proximal, since they exert their effect through very few intermediate factors or directly cause the illness [4]. An example of a distal risk factor for the development of lifestyle related diseases is socio-economic status (SES). In adults, low SES, usually defined according to education/occupation or income, is positively associated to more proximal risk factors for lifestyle related diseases, as for instance physical inactivity [5-7] and a diet rich in fat [5]. These latter risk factors are themselves associated to risk factors even further along the causal chain leading to disease such as high blood pressure [8,9] and obesity [10,11]. The graduation in distal and proximal causes is important to keep in mind, particularly, when studying the effect of single risk factors, since attention is directed towards the fact that risk factors interact and exert their effect on different levels, thus caution is needed. The full impact of a distal risk factor may not be captured in a traditional regression analysis in which both proximal and distal variables are included [4].

Lifestyle related diseases usually do not develop until the adult stage; however, the stages of childhood may still play an important role in the development of these particular diseases. Several pathways through which health behaviour and health status during childhood can affect health outcomes at adult age have been proposed [12]. One of the most obvious possibilities is that unhealthy habits developed in childhood, as well as the immediate consequences such as obesity and low physical fitness, carry over into later stages of life. The phenomenon is termed "tracking"[13], and to the extent risk factors for lifestyle related diseases track from childhood to adulthood, health behaviour and health status during childhood become part of the causal chain leading to lifestyle related diseases in adult life. Thus, studies designed to evaluate tracking of risk factors for lifestyle related diseases have the potential of contributing significantly to our understanding of the development of these diseases and, equally important, could have important implications for the design of preventive intervention strategies. In case that important risk factors of lifestyle related diseases show a high degree of tracking from childhood to adulthood, it implicates that adults with an unfavourable risk profile, in all probability, could have been identified as early as in childhood and specific preventive interventions could have been initiated at an early stage in life. A low tracking effect, on the other hand, indicates that childhood risk status holds no predictive value of adult risk status and, consequently, any preventive actions carried out in childhood to lower future risk should be aimed at children in general independent of health status.

Figure 1

A basic overview of the causal pathway from exposure to disease.



1.2 The European Youth Heart Study

Tracking of risk factors for lifestyle related diseases is a primary research objective in many large-scale epidemiological studies, as for instance *The Amsterdam Growth and Health Study* [14], *The Cardiovascular Risk in Young Finns Study* [15], *The Bogalusa Heart Study* [16], *The Leuven Longtudinal Study on Lifestyle, Fitness and Health* [17] and *The Danish Youth and Sport Study* [18]. The present thesis is based on data from the Danish part of the European Youth Heart Study (EYHS) - an international multi-centre study aiming at evaluating *the nature, strength and interactions between personal, environmental and lifestyle influences on cardiovascular disease risk factors in children of differing age, sex, culture and ethnicity* [19]. The target population of the study is 8-10 year-old-children and 14-16-year-old adolescents. The study was initiated in 1997 and designed so that a follow-up study could be conducted every six years. The EYHS provides unique opportunities to study both longitudinal and cross sectional research questions and a predefined research interest is tracking of CVD risk factors. CVD risk factors of central interest to the study are physical activity, physical fitness, and body fatness. Each of these factors is thought to be an independent and important predictor of CVD [20-22] and the methods used in EYHS to assess these risk factors are widely accepted within the field of epidemiology.

The overall objective of the present Ph.D. thesis is, as intended by the original developers of the EYHS, to contribute to the present knowledge of tracking of physical activity, physical fitness, and body fatness from childhood to adolescence using data from EYHS.

The design and methodology of the EYHS will be extensively described in the method section.

1.3 Tracking of physical activity, physical fitness and body fatness

A number of previous studies have investigated tracking of physical activity, physical fitness, and body fatness based on a variety of different research methodologies and designs. In summing up the results of these studies, it is important to keep in mind that factors such as the length of the follow-up period used and the actual age range covered by the studies influences the results significantly. In general, the longer the interval between baseline and follow-up measurements, the lower the probability of observing high tracking effects [13]. Keeping this in mind, earlier studies have generally shown that physical activity tracks at low to moderate levels during childhood/adolescence and from childhood/adolescence into adulthood [14,15,18,23-27]. In contrast, body fatness show moderate to moderately high tracking across the various stages of life [23,28-34], however, it should be noted that the odds of predicting obesity increases after the adiposity rebound occurs by the age of approximately six. Before this age, most individual body mass index (BMI) curves run across BMI ranges, but after this age, obesity has a better predictive value of the adiposity status in later childhood and adulthood [35-37]. As regards physical fitness, a general tendency towards moderate tracking across the various stages of life has been observed [14,18,29,33,38-43].

The results summarized originate from years of thorough research of tracking of physical activity, physical fitness, and body fatness, still the present knowledge in this field is by no means exhaustive and can be questioned at certain points. Especially the results concerning physical activity can be called into question. The most commonly used methods of assessing physical activity are self-report instruments as for instance questionnaires [44]. Self-report instruments can be both useful and valid methods of gaining insight into habitual physical activity habits [45], however self report instruments are not equally useful in all research settings. For instance, children, in particular, find it difficult to provide reliable information on physical activity. In fact, validation studies of self-report instruments performed on children less than 10 years have revealed insignificant validation coefficients, indicating that most self-report instruments do not measure what they are intended to measure among young children [44,46-48]. In recent times it has become increasingly common to use objective methods to assess physical activity, but to the best of our knowledge only very few studies of tracking of physical activity are based on objective methods [49,50] – perhaps due to the fact that studies of tracking run over long periods of time and only few research units have, at the present time, been able to collect follow-up data on large representative sections of the population using an objective method of assessment. Thus, earlier studies of tracking of physical activity, which in general have shown low to moderate effects, could have underestimated the real tracking effect, and there is a need for studies examining tracking of physical activity from childhood to adolescence/adulthood using an objective method of assessment.

A shift in methods from self-report instruments to objective assessments of physical activity, however, will introduce new methodological issues requiring attention. An important point in which objective methods of assessing physical activity differ from self-report instruments relates to the time range considered by the methods. Usually when studying physical activity, it is the subject's general physical activity pattern that is of interest and self-report instruments, as for instance questionnaires, can ask questions directly about this topic. In contrast, objective measurements of physical activity have to be conducted within a limited time interval; usually, a measurement period of a week or less has been used [49,51-57]. Thus, the extent to which objective estimates of physical activity represents average levels of physical activity will depend on the length-scale over which physical activity participation is time-dependent. Significant temporal variations in habitual physical activity could bias the results of studies investigating tracking of objectively measured physical activity, unless the measurement period is standardized.

Temporal sources of variation in physical activity have been studied earlier in the literature in children and adolescents. A limited number of studies have addressed seasonal variation in physical activity and reported a general tendency towards higher physical activity levels in the months of spring and summer compared to the months of winter and fall [58-62]. Furthermore, a number of studies have compared physical activity levels between weekdays and weekend days and reported significant differences [63-66]. Finally, a significant day-to-day variation in physical activity has been reported with coefficients of reproducibility of approximately 0.45 for a single day of measurement [63,67].

These results have led to the recommendation that a measurement period of a whole week should be used when assessing physical activity to account for both the large day-to-day variation in physical activity and the significant influence of the type of measurement day on physical activity [63]. However, these recommendations do not offer a complete solution to the issues of temporal sources of variation in physical activity. A measurement period of a whole week would, in all probability, not result in valid physical activity registration on both weekend days and weekdays for all subjects, since data have to meet certain criteria of validity. Furthermore, to the best of our knowledge, no previous study has compared all days of the week against each other to test for differences in physical activity besides from weekend/weekday differences. If such differences exist, it becomes increasingly important that valid physical activity data are recorded throughout the whole measurement period for each individual, which is highly unlikely. In addition, only very few studies have examined seasonal variation in physical activity in children based on an objective method, thus detailed information on seasonal variation in physical activity are not available at the moment. Consequently, there is a need for more research in temporal sources of variation in physical activity.

Earlier studies of tracking of physical fitness and body fatness are, in all probability, not as limited by methodological issues as the earlier studies of physical activity. A variety of methods are available for estimating physical fitness and body fatness in large scaled epidemiological studies with an acceptable accuracy and validity. This is especially true for physical fitness. The hallmark of aerobic fitness is maximal O_2 uptake – a clearly defined physiological condition in which the limits of the O_2 delivery/uptake systems have been met [68]. The gold standard method of assessing aerobic fitness is considered to be directly assessed VO_2 max through the analysis of respiratory gases and several studies of tracking of physical fitness have used direct determination of aerobic fitness [14,18,40,43]. Alternatively, indirect methods of estimating aerobic fitness have been applied - predominantly with relatively high validity. For instance, McMurray et al. predicted VO_2 max from a cycle ergometer test validated up against treadmill measured VO_2 max with a correlation coefficient of r= 0.807 [39,69]. Studies of tracking of body fatness have mainly used BMI as an indicator of fatness [28-34], however skinfold measures have also been frequently used [23,28,29,33]. There are several limitations to the use of BMI and skinfold measures to indicate body fatness in children and youth. For instance, the use of skinfold measures is complicated by the fact that children are not chemically mature and the chemical composition of the fat-free body changes as the child passes through puberty. Thus, estimates of body fatness by skinfold measures may reflect the changing fat free body composition rather than change in actual fat content [70]. However, when controlling for maturational status, ethnicity, and gender Slaughter reported that approximately 80 percent of the variation in body fatness, estimated using hydrostatic weighing, can be explained by skinfold measures [70]. The specific limitations to the use of BMI are reviewed in the method section of the present thesis, however despite limitations, several validation studies have concluded that BMI is a reasonable measure with which to assess fatness in children and adolescents [71-76]. Thus, there is reason to expect that earlier studies of tracking of physical fitness and body fatness, in general, have produced results of higher validity than earlier studies of tracking of physical activity.

The level of detail of the present knowledge of tracking of physical fitness and body fatness (and physical activity for that matter) is, however, rather limited. To the best of our knowledge, tracking has only been studied in representative samples of total populations and, apart from gender differences, no previous study has tested whether tracking varies significantly between subgroups of the population. An obvious candidate in this respect would be SES. It is well documented that the highest prevalence of several CVD risk factors including obesity, smoking and low physical activity is observed in adults of low SES [6,77,78] - in fact, CVD show a relatively clear socio-economic gradient in adults [79,80]. As pointed out by several authors, these socio-economic gradients are expected to develop at some point in childhood or adolescence [81,82]. Depending on how the gradients develop, SES could significantly influence tracking of CVD risk factors during childhood/adolescence and thereby affect the possibilities of early intervention. Thus, tracking of CVD risk factors across socio-economic strata should be considered an interesting research topic for future studies, especially considering the significance of SES as a risk factor of CVD [83].

1.4 Aim

The specific aims of the present thesis are:

1) to investigate the influence of gender, maturity state, seasonality, type of measurement day, and socio-economic status on objectively measured physical activity in children and adolescents. (Study I)
2) to study tracking of objectively measured physical activity from childhood to adolescence taking into account potential sources of variation and error in the estimates of physical activity, in order to obtain a more correct analysis of tracking of physical activity than has been performed earlier in the literature. (Study II)
3) to study tracking of physical fitness and BMI from childhood to adolescence, and to examine whether tracking of these risk factors are related to socio-economic status. (Study III)

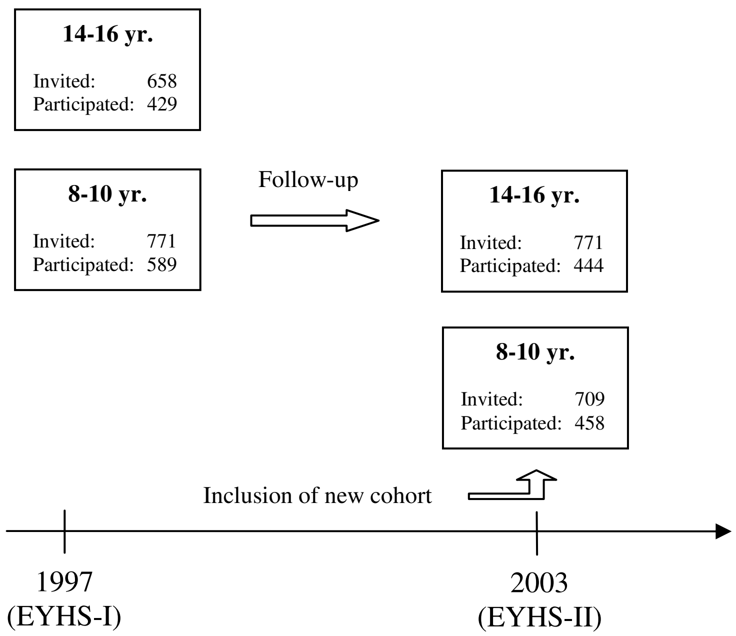
2 Materials and methods

As mentioned, the present thesis is based on data from EYHS and two measurement series have been conducted so far within the framework of EYHS in Denmark, as can be seen from figure 2, which provides a schematic representation of the design of the study. In 1997 a sample of 8-10-year-old children and a sample of 14-16-year-old adolescents participated in the EYHS-I. Six years later in EYHS-II the younger of the two EYHS-I-cohorts were re-examined and in addition, a new cohort of 8-10-year-old children was included, thus extending the design into a mixed longitudinal design.

The EYHS is a school based study and in EYHS-I all observations and tests were performed at the schools attended by the children. In EYHS-II all children and adolescents were transported by taxi (school by school) to the University of Southern Denmark and the Back Research Centre in Ringe, respectively, to be tested. Four to twelve children/adolescents were examined per day, and a measurement period of a whole school year was planned. However, the schools involved did not allow the 14-16-year-old adolescents to be disturbed during the examination period (i.e. May and June) and in EYHS-II, the data collection was not initiated until three months into the school year due to logistic issues. Thus, not all months of the school year were represented in the data.

Figure 2

Schematic diagram showing the design of the Danish part of the EYHS in the year 2003.



2.1 Sampling

The sampling frame was a complete list of public schools in the municipality of Odense. Schools were stratified according to the location and the socio-economic character 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 in the appropriate age band (8-10 yr. and 14-16 yr.) were allocated code numbers and randomly selected using random number tables. A more detailed description of the sampling procedure has been given elsewhere [19].

2.2 Subjects

In EYHS-I, 28 out of 35 schools were sampled to participate in the study and 25 schools participated. A sample of 771 8-10-year-old children and 658 14-16-year-old adolescents were drawn from the schools and 589 children (♀=310, ♂=279, 76 %) and 429 adolescents (♀=223, ♂=206, 65 %) participated in the study.

In EYHS-II, all of the 771 8-10-year-old children invited to participate in EYHS-I were re-invited as 14-16-year-old adolescents and a total of 444 (♀=251, ♂=193, 60 %) participated. In order to include the new cohort of 8-10-year-olds 25 out of 37 schools were sampled. Replacement schools were sampled to ensure a final sample size of 25 schools. 1 school did not wish to participate and was replaced. A total of 709 8-10-year-olds were invited to participate and 458 (♀=259, ♂=199, 65 %) participated. Table 1 provides an overview of the use of data from the Danish part of EYHS in the main analyses of the three individual studies included in the present thesis.

Table 1
EYHS data used in the main analyses of the three studies conducted.

	Subjects included	
	Part of EYHS	Age group
Study I	I	8-10 + 14-16
	II	8-10 + 14-16
Study II	I	8-10
	II	14-16
Study III	I	8-10
	II	14-16

2.3 Ethics

All procedures and methods in the EYHS conformed to the ethical guidelines laid down in the World Medical Association's Declaration of Helsinki and its subsequent revisions. The study was approved by the ethic committee of Vejle and Funen.

2.4 Representativeness

The permission from the ethical committee did not include a permission to contact non-responding subjects and subjects who chose not to participate in the study; however, in the following text a description is given of the indirect attempts carried out to examine the representativeness of the study samples.

In each case that a child chose not to participate in the EYHS-I, the form master was asked whether the child differed from the rest of the class in any way. Of all the children and adolescents not participating, one adolescent was described as being obese and physically inactive and one child was autistic. In EYHS-II we were extraordinarily offered access to anthropometric data on all 8-10-year-old children attending the schools invited to participate in the study, after the data collecting period had ended. We were given this opportunity since the school nurses at the schools routinely measured the children. When analysing results from these measurements, we did not find any differences in weight, height, or BMI between participants and non-participants.

For the follow-up sample of 14-16-year-old adolescents in EYHS-II, possible dropout effects were examined indirectly by comparing baseline values between the group of children who participated in both studies and the group who only participated in EYHS-I. For the three main variables of interest in this thesis (i.e. physical activity, physical fitness and body fatness) a significant dropout effect was observed only with respect to physical fitness, examined by the Wilcoxon rank-sum test. The children who only participated in EYHS-I possessed a lower mean fitness level than the children who participated in both studies.

2.5 Measurements

2.5.1 Physical activity

Habitual physical activity was measured objectively by the use of the uniaxial MTI Actigraph (Manufacturing Technology, Fort Walton Beach, FL) formerly know as the Computer Science & Applications (CSA) accelerometer. The Actigraph detects acceleration in the vertical plane with a dynamic range of 0.05-2.13 g. The Actigraph is band limited with a frequency response from 0.25 to 2.5 Hertz in order to discriminate human movement from non-physiological vibrations. The filtered acceleration signal is digitized and the magnitude is summed over a user-specified interval (epoch). At the end of each epoch, the summed value is stored in the memory and the numerical integrator is reset [84,85].

The MTI Actigraph has been validated in both children and adolescents using heart rate telemetry [86], indirect calorimetry [87-90] observational techniques [91] and doubly labelled water [92]. Ekelund et al. found that physical activity determined by activity counts was significantly related to total energy expenditure (TEE) ($r = 0.39$; $P < 0.05$), activity energy expenditure (AEE=TEE - basal metabolic rate (BMR)) ($r = 0.54$; $P < 0.01$), and daily physical activity level (PAL=TEE/BMR) ($r = 0.58$; $P < 0.01$) (n= 25, age= 9) [92]. Puyua et al. studied a group of 6-16 year old children (n = 26) by indirect calorimetry and found a significant correlation between the activity counts and activity energy expenditure (kcal/kg/min) (r=0.66) [87]. Eston et al. validated the MTI Actigraph against scaled oxygen uptake for walking and running on a treadmill and for unregulated play activities (i.e. hopping, catching, and crayoning) in children (n=30, age=8-11), and found a correlation coefficient of r=0.69 and r=0.85 for treadmill activity and unregulated play activities, respectively [90]. Furthermore, in a mechanical setting, the MTI Actigraph demonstrates good intra-instrument reliability (coefficient of variation < 4.4%) [93,94] whereas more inconsistent results have been observed as to the inter-instrument reliability. Metcalf et al. reported that inter-instrument variability never exceeded 5% in a mechanical setup [94], however Brage et al. observed a considerable higher inter-instrument variability and concluded that the level of inter-instrument reliability calls for calibration of each unit [93].

In the studies validating the MTI Actigraph, it has been generally concluded that the MTI Actigraph is a suitable instrument for epidemiological studies in children, as reflected indirectly by the frequent use of the Actigraph in recent epidemiological literature. However, there are several well-known limitations to the use of activity monitors, which it is important to be aware of. Some of the most frequently described limitations are summarized in the following:

- The output of the MTI Actigraph levels off during running at a speed of 8-9 km/h (≈8000 counts per minute (cpm)) in children, and as a consequence the instrument does not discriminate between higher running speeds than 8 km/h. The levelling off phenomenon is mainly due to the biomechanical characteristics of running, i.e., that average vertical acceleration is relatively constant across high running speeds [95].
- The MTI Actigraph is not waterproof and must be removed during water-based activities (e.g. bathing and swimming). Furthermore, the monitor underestimates the oxygen uptake during cycling by 30 to 90 percent, depending on the work rate [96], since cycling involves very little vertical movement of the hip.
- The MTI Actigraph lacks the ability to record upper body movement and cannot detect load carriage that would cause an increase in the energy expenditure [97].
- Wearing the Actigraph might modify the child's normal activity behaviour [98].
- The choice of epoch length influences the estimates of time spent in different intensity categories [99].

2.5.1.1 Protocol and data reduction

In the present study, participants were asked to wear the Actigraphs for at least five consecutive days, which included weekend days. The Actigraph was attached directly to the skin at the child's hip using an elastic belt. Subjects were instructed not to wear the Actigraph during sleep at night and during water-based activities.

The physical activity data were uploaded to a data reduction program developed within the framework of the Danish part of the EYHS. The program was set up to analyse the data at a daily basis and calculate the average daily physical activity intensity expressed as total cpm.

In order to distinguish between true intervals of inactivity and "inactivity" recorded when the monitor had been taken off, all MTI files were screened for periods of zero activity. Zero activity periods of 10 min or longer were interpreted as “MTI not worn,” and these periods were removed from the summation of activity by the data reduction program.

Not all subjects removed the Actigraph during sleep at night, and to avoid bias the data reduction program was set up to ignore activity between 12 am and 6 am with respect to the 8-10-year-olds. This procedure was not appropriate for the 14-16-year-olds since youth of this age are more likely to stay up at night, especially at weekends. As an alternative procedure, all activity files in the cohort of 14-16-year-old adolescents were manually screened, and where an activity file conformed to the criteria listed below, activity between 12 am and 6 am was ignored:

1. “normal” activity pattern throughout the day
2. abrupt change in the activity pattern at some point in the late evening/night towards a sporadic pattern of very low intensity activity throughout the night
3. an immediate change towards a “normal” activity pattern in the morning which was maintained throughout the following day

In EYHS-II in 2003 an additional initiative was undertaken to ensure the quality of data – each Actigraph was tested on a calibration machine before being sent off into the field in order to ensure that all MTI Actigraphs were in good working order.

Given the above-mentioned criteria, subjects who did not manage to record ≥600 min/d of activity for ≥3 d were excluded from further analyses.

2.5.1.2 Adjusting for temporal sources of variation in physical activity

In Study II, several corrections to the physical activity data were made in order to obtain a more correct analysis of tracking of physical activity than has been performed earlier in the literature. The corrections were based on results found in Study I, where potential sources of variation in habitual physical activity were examined. The results of Study I will not be presented in detail until the result section. However, in the following paragraphs a brief description will be given of selected findings from Study I followed by a description of the methodological initiatives these results have given rise to in Study II.

2.5.1.2.1 Within-week variation

In the Danish part of EYHS, individual estimates of physical activity are, in general, not based on measurements performed on the exact same days of the week, partly due to the fact that the measurement period was not standardized according to days of the week subjects were given the opportunity to wear the Actigraphs. Therefore, as a rule of thumb, the combination of days on which a given subject has been examined varies between the baseline and follow-up study. As a consequence, the results of Study II examining tracking of physical activity could be biased to the extent to which physical activity is influenced by the type of measurement day.

In Study I, the association between physical activity and the type of measurement day was investigated and the results showed that the habitual physical activity level of children and adolescents are significantly lower in weekends than on weekdays. However, it should be noted that in the 8-10-year-olds, Friday appeared like a weekend day in this context, as the lowest activity estimates in general were to be found on Fridays, Saturdays and Sundays for the 8-10-year-old children.

In order to obtain a measure of mean physical activity, which represented a whole week, a variable was created which combined weekend and weekday activity, such that the average of weekend activity was weighted by 2/7 and the average of weekday activity by 5/7 for the 14-16-year-old adolescents. For the 8-10-year-olds, the average weekend activity (including Fridays) was weighted by 3/7 and the average of weekday activity by 4/7. Individuals were excluded from analyses if they did not have data for both weekend and weekdays.

2.5.1.2.2 Between-months variation

In both EYHS-I and EYHS-II the measurement period lasted for almost an entire school year, which means that for a given subject the activity data at baseline and at follow-up could have been collected during entirely different seasons of the year. Thus, significant seasonal variation in the physical activity pattern of children and adolescents could introduce bias in the analyses of tracking of physical activity in Study II.

In Study I, seasonal variation in the physical activity level was studied and the results revealed a considerable and significant effect of seasonality for the 8-10-year-old children. To avoid bias in the tracking analyses as a result of seasonal variation, all individual activity estimates were rescaled to the same reference month by subtraction of the relevant effects of months reported in Study I.

2.5.1.2.3 Natural day-to-day variation and within instrumental measurement error

The method of using accelerometry to estimate physical activity is based on the underlying assumption that each human being has a relatively fixed average level of physical activity during a specific period of life and furthermore, that this average level can be estimated from relatively few days of recording. However, repeated measurements of daily physical activity on a given subject will vary around the subject's average level of activity even after controlling for general day-type differences. Physical activity level varies due to both natural day-to-day variations and instrumental measurement error. As a consequence, physical activity estimates derived by accelerometry will be more or less randomly error prone, depending on the magnitude of both day-to-day variation and instrument variability. However, it is in fact possible, at the same time to take both day-to-day variations in physical activity and within instrumental measurement errors into account in the tracking analyses, but it is more statistically demanding and for this reason, a detailed description of the method is given in the statistics paragraph.

2.5.2 Physical fitness

Physical fitness was determined by a progressive maximal cycle ergometer test, and defined as the maximal power output per kilogram raised to the 2/3 power (W_{max}*$kg^{-2/3}$). The cycle-ergometer used was an electronically braked Monark 839 Ergomedic, and two test protocols were used: a) for 8-10-year-olds, initial and incremental workloads were 20 W for children weighing less than 30 kg and 25 W for those weighing 30 kg or more; and b) for 14-16-year-olds, initial and incremental workloads were 40 W for girls and 50 W for boys. Workload was programmed to increase after every 3 min. Heart rate was recorded continuously (Polar Vantage, Kempele, Finland) throughout the test, and the test continued until the subject was no longer able to continue. Criteria for a maximal effort were

1. A heart rate above or equal to 185 beats per minute
2. Failure to keep a pedalling frequency of at least 30 revolutions per minute
3. Subjective valuation by the test personnel

The cycle ergometer was electronically calibrated once every test day and mechanically calibrated after being moved. Maximal power output (Wmax) was calculated as the watts in the last fully completed stage (W_1), plus the increment in watts (W_i) of the last incomplete stage multiplied by the number of seconds completed of the last incomplete stage (t_{ls}) divided by 180.

$Wmax= W_1 + (W_i \cdot t_{ls}/180)$

The test has been validated in both children and adolescents with a correlation coefficient of r=0.89 and r=0.90, respectively, to directly measured VO_2-max (unpublished data).

2.5.3 Anthropometry

Body height was measured to the nearest half centimetre using a stadiometer and body mass was determined by a beam-scale weight to the nearest 100 g. Body fatness was assessed by BMI, i.e. weight divided by height squared ($kg \cdot m^{-2}$).

The use of BMI as an indicator of fatness in children has been questioned and several limitations to the use of BMI as a measure of body fatness have been described in the literature. 1) BMI (like weight) is influenced by both the lean and the fat compartments of the human body and is a measure of lean body mass as well as a measure of fatness or obesity [100]. 2) BMI has been shown to be positively related to stature in children [100,101]. 3) BMI is significantly dependent of age and gender [100,101]. However, the International Obesity Task Force recently convened a workshop on childhood obesity to determine the most appropriate measurement to assess obesity in populations of children and adolescents and in spite of the limitations mentioned, they concluded that BMI is a reasonable measure with which to assess fatness in children and adolescents [76]. Furthermore, BMI has been validated against dual energy x-ray absorptiometry in children and adolescents with a relatively high correlation (r=0.67-0.79) to percent body fat [72-74]. Moreover, in the present thesis the dependency of BMI on age is probably not an important source of bias, since BMI values were only compared between subjects of almost exactly the same age.

An advantage of using BMI as a measure of body fatness in children is that absolute cutoff values can be used to define overweight and obesity. Cole et al. has established a standard definition for child overweight and obesity worldwide on the basis of BMI cut-points [102].

2.5.4 Pubertal stage

Pubertal stage was assessed according to Tanner stages [103] by a researcher of the same gender as the child, using brief observation. In both age and gender groups, pubertal stage were identified using a 5-point scale of pictures — girls according to breast development and boys according to pubic hair growth. When adjusting for maturity state the 5-point scale was used except from in Study I, where the 5-point scale was reduced to a 2-point scale primarily in order to ensure adequate power in the analyses conducted. With respect to the 8-10-year-old children in EYHS, data on pubertal stage were almost exclusively distributed within 2 stages (i.e. Tanner stage 1 and 2), therefore in Study I a dichotomous variable coded “1” if the pubertal stage was equal to 1 and “2” if the pubertal stage was equal to or greater than 2 was created. For the 14-16-year-old adolescents, nearly all data were distributed within 3 stages (i.e. stage 3-5), however due to a difference of opinion between the test personnel conducting the EYHS-I and EYHS-II, respectively, about how to distinguish between the Tanner stages 4 and 5, a dichotomous variable coded "1" if the pubertal stage was equal to or less than 3 and "2" if the pubertal stage was equal to or greater than 4 was used. A detailed overview of the distribution of Tanner data by gender, age group and year of study is provided in the results section (Table 2).

2.5.5 Socio-economic status

In both Study I & III socio-economic status was a main variable of interest, however two different definitions of SES were applied.

In Study I, SES served an analytical purpose besides being a main variable of interest. As described in the method section, EYHS is a school-based study and the individual schools were tested over a short and continuous period of time, usually 1-2 weeks. This gave rise to an analytical problem in Study I when investigating the influence of the month of measurement on physical activity, since a potential effect of months could be confounded by a potential effect of schools or school neighbourhoods on physical activity. It has been argued that household and neighbourhood factors could be important modifiers of physical activity [104], and although only a limited number of studies have investigated the association between neighbourhood characteristics and physical activity in children, the results indicate that both children and adults in low SES areas are less active than in high SES areas [105-108]. A key factor in the definition of SES of area of residence in earlier studies has been economical resources (e.g. per capita income). Thus, it was decided to base the classification of SES in Study I on the average gross income per inhabitant within the catchment areas of the schools attended by the subjects. This approach had two advantages. First, the findings of Study I could add significantly to the knowledge of whether neighbourhood context affect childhood behaviour. Secondly, a potential confounding effect of the school-based design of EYHS on the between-months variation in physical activity would be minimized when SES was included in the multivariate analyses in Study I. A list of the average gross income per inhabitant within the different school catchment areas in the Municipality of Odense was obtained from the local authorities. This information was acquired for the years 1997 and 2003. Combined data from both years was used to divide the catchments areas into three gross income groups – low, middle and high-income areas. The three subgroups contained approximately the same number of schools. The boundary between low and middle income areas was set at an average gross income of 187.000 Danish kroner per year (≈32000 US dollars) and the boundary between middle and high income areas was set at 198.500 Danish kroner per year (≈34000 US dollars). The stated values are average values of the 1997 and the 2003 gross income data.

In Study III SES was defined according to the occupational level of the adult female in the household at the first measurement point. Recent evidence suggest that the risk of being overweight in childhood/early adolescence is related to the SES of the mother rather than that of the father, thus the fathers were not considered in the definition of SES [109,110]. The mothers were categorised into two different socio-economic groups. The two groups represented blue-collar and white-collar occupation respectively and they were defined by *The International Standard Classification of Occupation* scheme [111], which holds nine major categories. The categories 1-4 correspond to "blue-collar" occupation while the categories 5-9 correspond to "white-collar" occupation. Mothers working as housewives (n=5) were classified as housekeepers (i.e. classified as belonging to the blue-collar group). Occupational data was assessed from questionnaires completed by the parents.

2.5.6 Computer-based questionnaire

A computer-based child's questionnaire (peach) was used to gather information about smoking habits, alcohol intake, diet preferences, and various types of physical activity including bicycling habits. Questions addressing psychosocial and environmental factors that influence children's physical activity were also asked.

2.6 Statistics

In **Study I**, the influence of gender, seasonality, type of measurement day, maturational status, and SES on objectively measured physical activity were studied using the following statistical model:

$$Y_{id} = a + b_1*X_1 + b_2*X_2 +...+ b_k*X_k + variation_i + variation_{iw} + error_{id}$$

where Y_{id} is the average physical activity level for subject i on day d, X_1 to X_k are the explanatory variables (i.e. months, days, maturational status, gender and SES). The first variation component (i.e. $variation_i$) was included in the model to allow each individual personal deviance from the model, capturing within correlation in the dataset. The variation component "$variation_{iw}$" allowed each subject his or her own individual weekend-weekday activity "identity". This component was included based on the earlier studies, which have examined weekend-weekday differences in physical activity and in general shown that differences are to be expected [63-66]. By adding the $variation_{iw}$ term to the model, individual differences in how the activity pattern is affected by the transition from weekday to weekend is taken into account. Finally, the $error_{id}$ component contains technical measurement error or "noise" on the individual day.

The model was fitted by a mixed model. In order to test for significant effects, Wald tests were applied. Specifically, we tested whether physical activity differed by level of the explaining variables in question.

Interaction terms between gender and the independent variables, respectively, were added in all models and in case of interaction, the parameters were estimated for the two genders separately.

Prior to running the statistical model a series of cross tabulations examining two-by-two combinations of the variables: gender, SES, maturational status, month of measurement, and day of measurement were produced in order to ensure, that the physical activity data were sufficient in quantity and distributed appropriately over gender, SES, maturational status, months, and days, such that valid statistical analyses of relationships could be performed. The cross tabulations were generated for each of the four age group and study year specific research samples, respectively.

In **Study II & III** the main subject of interest was the phenomenon “tracking”. In the epidemiological literature, tracking describes the relative stability of the longitudinal development of a certain outcome variable Y. There is no single widely accepted definition of tracking, but the following concepts are involved: (1) the relationship (correlation) between early measurements and measurements later in life, or the maintenance of a relative position within a distribution of values in the observed population over time – often referred to as the actually stability, and (2) the predictability of future values by early measurements [13].

In order to assess the crude stability of each of the CVD risk factors in concern (i.e. physical activity, physical fitness, and BMI) the following statistical model was used:

$$Y_{i2} = \beta_0 + \beta_1 Y_{i1} + \sum_{j=1}^{J} \beta_{2j} X_{ij} + \varepsilon_i$$

where Y_{i1} is the mean level of the specific risk factor in question at baseline for subject i, Y_{i2} is the mean level of the risk factor at follow-up for subject i, β_1 is the regression coefficient used as the stability coefficient, J is the number of covariates, X_{ij} is the covariate j of individual i and ε_i is the error term for subject i. The model can be fitted using traditional regression techniques with robust standard errors and is derived from a more general formula for assessing tracking outlined by Jos W. R. Twisk [13]. In order to account for scale differences all continuous variables were transformed into subgroup specific z-scores before entering the model. The use of standardized variables in the longitudinal analyses makes the coefficients vary between -1 and +1 and makes the stability coefficient interpretable as a longitudinal (partial) correlation coefficient [112]

The stability coefficient does not directly express the predictive value of being overweight, inactive or low fit early in life in relation to the risk of these conditions later in life. Therefore, to obtain an expression of predictability, logistic regression analyses were used to calculate the odds ratio which compares the odds of maintaining an unhealthy profile with the odds of developing an unhealthy profile during the measurement period.

In the absence of objective cut points for low physical activity and low physical fitness, subjects who belonged to the lower sex and age group specific quartile of physical activity and physical fitness, respectively, were defined as being inactive and unfit, respectively. The classification of overweight was based on BMI cut-points proposed by the International Obesity Task Force [102]. The cut points for overweight used included obese cut point values.

In **Study II** the models of assessing stability and predictability were fitted using both raw and adjusted estimates of physical activity. In paragraph 2.5.1.2 a description was given of how data in Study II was adjusted for a number of sources of variation in physical activity. A detailed description of how the analysis of tracking was adjusted for natural day-to-day variation and within instrumental measurement error is included here.

Natural day-to-day variation and within instrumental measurement error gives rise to random error in the physical activity estimates derived by accelerometry. Random error in a regression model, such as the model used to assess stability of physical activity, has a different effect on the slope coefficient depending on whether it is the dependent or independent variable that is considered. Random error in the independent variable biases the slope coefficient towards zero whereas random error in the dependent variable does not bias the slope coefficient but widens the confidence intervals on the slope coefficient [113].

In the analysis of stability of physical activity, the baseline sample of 8-10-year-olds from 1997 was used as the independent variable. Thus, attention was focused on this particular sample in the corrections of natural day-to-day variation and within instrumental measurement error.

The combined magnitude of random error due to day-to-day variation and within instrumental measurement error can be determined through a study of reproducibility. To be precise, the within subject standard deviation of repeated measurements of physical activity, which we shall denote *wsd*, provides an estimate of the combined magnitude of error in question [114]. In order to calculate *wsd* an analysis of reproducibility of a single day of physical activity registration was conducted on the baseline sample of 8-10-year-old children from 1997. The reproducibility of a 4 day period of measurements was also considered, since the mean number of valid activity registration days per subject was approximately 4. The within subject standard deviation was assessed by a mixed model with a fixed effect for subjects and days.

When *wsd* had been estimated, the coefficient of reproducibility (R) could be calculated as $R=1-(wsd^2/\text{total variance})$ and any subsequent linear regression analysis involving physical activity could be adjusted using the program eivreg in the statistical package STATA 9 [115].

Unfortunately, it is more demanding to correct for day-to-day variation and within instrumental measurement error in logistic regressions than in linear regressions, therefore only stability coefficients and not predictability odds ratios were adjusted in this respect.

In **Study III** tracking of physical fitness and BMI, respectively, were analysed within different groups of SES. Differences between stability coefficients were tested by the following test statistics:

$$Z = (\beta_1^* - \beta_2^*)\sqrt{(SE[\beta_1^*])^2 + (SE[\beta_2^*])^2}$$

where β_1^* and β_2^* are subgroup specific standardized regression coefficients. Differences in predictability between groups of SES were tested by interaction terms in the logistic regression model. Logistic regression models were also used to assess the risk of both developing and maintaining overweight/low physical fitness within the measurement period in different groups of SES.

In order to compensate for the dropout effect observed for physical fitness (described in section 2.4), individual measurements of fitness were weighted in all tracking and logistic regression models by the inverse probability of a subject turning up at the follow-up examination given the gender, SES and the fitness level at baseline [116].

All tracking analyses and logistic regression models in Study III were controlled for biological maturation state, and interaction terms between gender and the independent variables were added in all models to investigate whether different models should be fitted for the two sexes separately.

The statistical package STATA 9 were used for all analyses in this thesis.

3 Results

The following result section summarizes the results from the underlying three papers and is divided into four parts 1) basic descriptive statistics, 2) results evaluating sources of variation in objectively measured physical activity, 3) results evaluating tracking of objectively measured physical activity, 4) results evaluating tracking of physical fitness and BMI.

3.1 Descriptive statistics

Table 2 provides basic descriptive statistics for all participants in both EYHS-I and EYHS-II. For descriptive statistics calculated for the actual subjects included in the specific studies, the reader is referred to the result sections of the individual papers.

Table 2

Basic subject characteristics across study year, age group, and gender. Shown are means (SD), except for the categorical variables TS, SES (1) and SES (2) where frequencies are reported. Furthermore, non-normally distributed variables (i.e. Reg Days) are expressed as median (10^{th}-90^{th} percentile).

	EYHS-I						EYHS-II					
	8-10 yr.			14-16 yr.			8-10 yr.			14-16 yr.		
	N	Boys	Girls	N	Boys	Girls	N	Boys	Girls	N	Boys	Girls
Age years	589	9.7 (0.4)	9.6 (0.4)	429	15.5 (0.4)	15.5 (0.5)	458	9.9 (0.4)	9.8 (0.4)	444	15.8 (0.3)	15.7 (0.4)
Weight kg	589	33.9 (6.4)	33.2 (6.3)	429	63.5 (10.0)	57.0 (8.8)	458	34.5 (6.8)	34.2 (7.0)	444	65.2 (10.8)	58.0 (9.4)
Height cm	589	139.5 (6.3)	138.5 (6.3)	429	174.3 (7.0)	165.2 (6.4)	458	140.5 (6.9)	139.9 (6.5)	444	176.0 (7.4)	164.8 (6.9)
PA cpm	383	720 (240)	599 (210)	233	452 (167)	411 (143)	419	717 (205)	598 (173)	346	503 (186)	409 (145)
Reg Days	383	4 (3 – 5)	4 (3 – 5)	223	4 (3 – 5)	4 (3 – 5)	419	4 (3 – 5)	4 (3 – 5)	346	4 (3 – 5)	4 (3 – 5)
PF watt/kg	539	3.2 (0.6)	2.8 (0.5)	398	3.8 (0.6)	3.0 (0.5)	428	3.3 (0.5)	2.8 (0.4)	408	3.8 (0.5)	3.0 (0.4)
Max HR bpm	539	198.5 (7.2)	199.4 (7.0)	398	198.5 (6.7)	197.0 (7.3)	428	200.4 (7.6)	201.6 (8.1)	408	197.5 (7.2)	197.4 (6.6)
BMI kg/m^2	589	17.3 (2.4)	17.2 (2.5)	429	20.9 (2.6)	20.9 (2.8)	458	17.3 (2.4)	17.4 (2.7)	444	21.0 (2.8)	21.3 (3.1)
TS 1/2/3/4/5	578	274/0/ 0/0/0	295/9/ 0/0/0	424	4/4/6/ 13/178	0/1/11/ 60/147	455	199/0/ 0/0/0	199/5 2/ 4/1/0	444	0/1/12/ 180/0	0/0/12/ 189/50
SES (1) Blue/White	547	124/131	124/168	–	–	–	–	–	–	–	–	–
SES (2) low/mid/high	589	44/117/ 117	50/125/ 136	429	51/79/ 76	54/78/ 91	458	59/53/ 87	96/73/ 90	386	22/74/ 75	40/72/ 103

TS denotes tanner stage

PA denotes physical activity

PF denotes physical fitness

Reg Days denotes mean number of valid physical activity registration days

SES (1) was defined according to occupational level. This variable was only defined for the cohort of 8-10-year-olds in EYHS-I.

SES (2) was defined according to the average gross income per inhabitant within the catchment areas of the schools attended by the subjects. In the follow-up cohort, 58 subjects attended continuation schools in 2003 (EYHS-II), thus SES (2) was not determined for these individuals.

3.2 Sources of variation in physical activity

In Study I the influence of gender, maturity state, seasonality, type of measurement day and SES on habitual physical activity were examined in 8-10-year-old children and 14-16-year-old adolescents. All data from the Danish part of EYHS were included in the analyses (i.e. a total sample size of 1920 subjects). Of the 1920 participants, 1330 subjects had complete data for all parameters considered. The mean number of registration days per subject was approximately 4 leading to a total number of 5713 physical activity registration days.

Cross tabulations examining two-by-two combinations of the explaining variables did not reveal any marked asymmetry, however it was noted that not all of the categories of the maturity state variable were well represented, especially not in the samples of 14-16-year-olds. Furthermore, activity data were not collected throughout all months of the year and some months were poorly represented and had to be excluded from the analyses. The tables include an overview of the months that have been excluded from the analyses and the months that were not represented at all. The total number of subjects/physical registration days included in the multivariate analyses decreased from 1330/5713 to 1318/5624 after the exclusion of months that were inadequately represented.

The Tables 3-6 present results from the multivariate analyses. A separate table has been produced for each of the four age group and study year specific research samples.

3.2.1 Within-week variation

In the cohort of 8-10-year-old children, significant differences in physical activity were observed between measurement day types, as can be seen in Tables 3 and 4. Furthermore, the results revealed two clusters of days. The children appeared to be less active on weekends and Fridays than on the remaining days of the week, independent of gender and year of study.

A more detailed look at the results revealed some differences between the two genders and the two samples of 8-10-year-olds. In the sample of 1997 the wald-test showed a significant effect of the type of measurement day for boys while there was no significant effect for girls, indicating that the observed pattern in the effect of day types in girls in 1997 could simply be due to chance. In the sample of 2003 the effect of day types was significant for both boys and girls, however, as it was the case in the 1997 sample, differences between day types were in general largest for boys.

The within-week variations in habitual physical activity for the 14-16-year-old adolescents are shown in Tables 5 and 6. The general pattern in the results across gender and year of study was that weekend activity levels were lower than weekday activity levels. However, in contrast to the case of the 8-10-year-olds, Friday was no longer to be considered as an inactive day. In the adolescents examined in 1997, analyses revealed no interaction between the two sexes and the estimated effect of day types was highly significant. In resemblance, the effect of day types was also significant in the sample of 2003 for both boys and girls. However, in this sample analyses showed a significant interaction between gender and days, with the largest effect for boys, thus gender specific analyses were carried out.

3.2.2 Between-months variation

In the 8-10-year-old children a highly significant association was observed between physical activity and the month of measurement, and the analyses revealed no interaction between gender and months (Tables 3 & 4). In the sample of 2003 the highest activity levels were observed in the months of spring (i.e. March, April and May) while the remaining months, which were winter and autumn months, were more or less at the same level. This general pattern was also observed in the 8-10-year-olds examined in 1997. In addition, this sample contained activity registrations performed in the summer season (i.e. the months of June and August). However, it should be noted that the estimates of the months of summer were based on a limited number of observation days and even fewer subjects, thus less attention should be attached to these results in the final interpretations.

For the 14-16-year-old adolescents the effect of months was not significant in either the 1997 or the 2003 sample. However in the 2003 sample a borderline significant effect of months ($p=0.06$) was observed in boys, and in line with the results found in the 8-10-year-olds it was the months of spring, April in particular, that differed from the winter and autumn months by higher activity estimates (Tables 5 & 6).

3.2.3 Gender

The results revealed gender differences in the physical activity level independent of cohort and year of study, with a consistent pattern of higher activity estimates for boys compared to girls (Tables 3-6). The effect of gender was significant in all research samples with the exception of the cohort of 14-16-year-old adolescents in 1997, where the Wald-test showed a borderline significant effect ($p=0.0771$).

3.2.4 Maturational status

No significant effect of maturational status on physical activity was observed in this study and the estimated effects were small (Tables 3-6).

3.2.5 *Socio-economic status*

In the 8-10-year-old children, a significant effect of SES on habitual physical activity was only observed in the sample examined in 2003 (Tables 3 & 4). In this sample, the children from the middle-income areas were estimated to be the most active, while the remaining socio-economic groupings were estimated to be at the same level. In the 14-16-year-old adolescents, a general pattern of lower activity levels in the middle and high-income areas compared to the low-income areas was observed (Tables 5 & 6). However, the effect of SES was only significant in the 14-16-year-olds examined in 2003.

Table 3

Association between physical activity and explaining variables in 8-10-year-old children from 1997. Shown are differences in cpm (ß) between the reference category with a fixed value of 1 and each of the remaining categories of the explaining variable.

	N	ß (95 % CI) Girls		ß (95 % CI) both genders		ß (95 % CI) Boys		P
Day								0.39/<0.0001
Mon	164/154	1				1		
Tue	148/123	-48.3	(-96.1 -0.4)			-79.6	(-131.1 -28.1)	
Wed	75/65	-4.6	(-65.6 56.4)			-29.5	(-94.7 35.7)	
Thu	61/53	-2.8	(-71.8 66.2)			-95.6	(-168.4 -23.1)	
Fri	60/74	-42.4	(-111.9 27.2)			-141.2	(-204.8 -77.7)	
Sat	150/144	-26.1	(-78.5 26.2)			-181.6	(-235.0 -128.2)	
Sun	137/135	-42.6	(-96.1 11.0)			-160.8	(-215.1 -106.6)	
Month								<0.0001
Jan	205			1				
Feb	20			-				
Mar	201			148.5	(62.8 234.2)			
Apr	201			83.3	(-5.8 172.4)			
May	172			303.8	(218.1 389.5)			
Jun	44			63.4	(-58.1 185.0)			
Jul	3			-				
Aug	35			-14.5	(-167.9 139.0)			
Sep	273			162.6	(77.9 247.3)			
Oct	89			7.1	(-100.1 114.3)			
Nov	216			47.4	(-34.1 128.9)			
Dec	117			59.0	(-34.2 152.3)			
Gender								<0.0001
Girls	795			1				
Boys	748			195.5	(130.2 260.7)			
Maturity								0.86
TS = 1	1316	1				1		
TS > 1	227	-6.1	(-72.8 60.6)			-		
SES								0.97
Low	187			1				
Mid	643			5.1	(-79.3 89.6)			
High	713			8.5	(-69.8 86.7)			

TS denotes Tanner Stage

N denotes the number of observation days in each category.

The p-value refers to a test of the null hypothesis that no difference in physical activity exists between the categories of the explaining variable in question.

If no effect is reported for a given month and N differs from zero, data has been excluded due to an inadequate representation of the month in question.

Interaction terms between gender and each of the explaining variables were added in the model and in case of interaction, gender specific effects were reported.

Table 4

Association between physical activity and selected explaining variables in 8-10-year-old children from 2003. Shown are differences in cpm (ß) between the reference category with a fixed value of 1 and each of the remaining categories of the explaining variable.

	N	ß (95 % CI)		ß (95 % CI)		ß (95 % CI)		P
		Girls				Boys		
Day								0.0021/<0.0001
Mon	172/131	1				1		
Tue	108/81	11.6	(-41.3 64.6)			11.6	(-49.1 72.3)	
Wed	90/79	-3.4	(-61.4 54.7)			-1.9	(-65.3 61.5)	
Thu	110/82	0.5	(-54.2 55.1)			27.2	(-36.0 90.3)	
Fri	169/131	-58.6	(-106.0 -11.2)			-50.8	(-104.9 3.4)	
Sat	209/160	-70.4	(-115.4 -25.4)			-172.9	(-224.5 -121.4)	
Sun	178/137	-11.0	(-57.9 35.9)			-63.5	(-117.1 -9.8)	
Month								<0.0001
Jan	266			1				
Feb	191			-16.2	(-77.1 44.8)			
Mar	435			49.0	(-9.8 107.7)			
Apr	201			181.1	(104.0 258.2)			
May	336			144.2	(62.1 226.4)			
Jun	15			-				
Jul	-			-				
Aug	-			-				
Sep	-			-				
Oct	-			-				
Nov	212			25.0	(-39.2 89.3)			
Dec	196			21.6	(-50.6 93.7)			
Gender								<0.0001
Girls	1036			1				
Boys	801			140.7	(81.9 199.6)			
Maturity								0.2816
TS = 1	1618	1				1		
TS > 1	219	-30.6	(-86.2 25.1)			-		
SES								0.0129
Low	572			1				
Mid	554			63.2	(5.4 120.9)			
High	711			0.1	(-65.6 65.8)			

TS denotes Tanner Stage

N denotes the number of observation days in each category.

The p-value refers to a test of the null hypothesis that no difference in physical activity exists between the categories of the explaining variable in question.

If no effect is reported for a given month and N differs from zero, data has been excluded due to an inadequate representation of the month in question.

Interaction terms between gender and each of the explaining variables were added in the model and in case of interaction, gender specific effects were reported.

Table 5

Association between physical activity and selected explaining variables in 14-16-year-old adolescents from 1997. Shown are differences in cpm (ß) between the reference category with a fixed value of 1 and each of the remaining categories of the explaining variable.

	N	ß (95 % CI)	P
Day			<0.0001
Mon	189	1	
Tue	163	8.8 (-26.5 44.1)	
Wed	84	14.9 (-30.1 60.0)	
Thu	55	12.7 (-43.4 68.8)	
Fri	92	96.6 (51.6 141.6)	
Sat	177	-6.5 (-44.4 31.4)	
Sun	168	-37.1 (-75.3 1.1)	
Month			0.6345
Jan	170	1	
Feb	16	-	
Mar	121	-9.6 (-85.9 66.7)	
Apr	156	-31.2 (-101.4 39.0)	
May	9	-	
Jun	-	-	
Jul	-	-	
Aug	11	-	
Sep	126	-34.6 (-111.2 42.1)	
Oct	98	-59.1 (-143.1 24.9)	
Nov	169	-55.1 (-120.0 9.8)	
Dec	88	-17.9 (-100.2 64.3)	
Gender			0.0771
Girls	519	1	
Boys	409	37.4 (-4.1 78.8)	
Maturity			0.5043
TS ≤ 3	68	1	
TS > 3	860	25.4 (-49.1 99.8)	
SES			0.1476
Low	226	1	
Mid	316	-64.2 (-128.8 0.4)	
High	386	-42.6 (-100.3 15.1)	

TS denotes Tanner Stage

N denotes the number of observation days in each category.

The p-value refers to a test of the null hypothesis that no difference in physical activity exists between the categories of the explaining variable in question.

If no effect is reported for a given month and N differs from zero, data has been excluded due to an inadequate representation of the month in question.

Interaction terms between gender and each of the explaining variables were added in the model and in case of interaction, gender specific effects were reported.

Table 6

Association between physical activity and selected explaining variables in 14-16-year-old adolescents from 2003. Shown are differences in cpm (ß) between the reference category with a fixed value of 1 and each of the remaining categories of the explaining variable.

	N	ß (95 % CI) Girls			ß (95 % CI) Boys			P
Day								0.0396/<0.0001
Mon	115/99	1			1			
Tue	87/64	56.9	(7.0	106.8)	22.5	(-34.2	79.3)	
Wed	81/60	29.5	(-23.4	82.4)	39.6	(-20.3	99.4)	
Thu	86/68	27.4	(-25.0	79.9)	-54.5	(-111.9	2.9)	
Fri	122/101	3.4	(-43.4	50.1)	-25.0	(-75.6	25.6)	
Sat	126/100	-13.6	(-59.6	32.5)	-110.5	(-161.3	-59.8)	
Sun	122/85	-19.8	(-66.3	26.8)	-122.6	(-175.4	-69.8)	
Month								0.5138/0.0611
Jan	150/127	1			1			
Feb	122/105	50.2	(-16.6	117.0)	-17.7	(-86.6	51.2)	
Mar	159/120	28.6	(-39.8	97.1)	38.2	(-36.9	113.3)	
Apr	145/69	8.6	(-63.1	80.4)	111.0	(17.9	204.2)	
May	10/14	-			-			
Jun	0/5	-			-			
Jul	-	-			-			
Aug	-	-			-			
Sep	-	-			-			
Oct	-	-			-			
Nov	92/70	-21.3	(-101.0	58.3)	-32.5	(-119.4	54.3)	
Dec	71/86	31.9	(-53.2	116.9)	-14.4	(-96.4	67.7)	
Gender								0.0029
Girls	739	1						
Boys	577	131.1	(44.7	217.4)				
Maturity								0.9311
TS ≤ 3	66	1						
TS > 3	1250	-3.6	(-84.1	77.0)				
SES								0.0112
Low	206	1						
Mid	487	-57.8	(-112.1	-3.4)				
High	623	-79.3	(-131.2	-27.4)				

TS denotes Tanner Stage

N denotes the number of observation days in each category.

The p-value refers to a test of the null hypothesis that no difference in physical activity exists between the categories of the explaining variable in question.

If no effect is reported for a given month and N differs from zero, data has been excluded due to an inadequate representation of the month in question.

Interaction terms between gender and each of the explaining variables were added in the model and in case of interaction, gender specific effects were reported.

3.2.6 Natural day-to-day variation and within instrumental measurement error

The combined magnitude of natural day-to-day variation in physical activity and within instrumental measurement error of the MTI Actigraph was evaluated in Study II. Table 7 presents the results, and as can be seen from the table, the reproducibility of a four-day period of measurements was just below 0.80 for both boys and girls in 1997 and considerably better than the reproducibility of a single day of measurement. When repeating the reproducibility study on the sample 8-10-year-olds examined in 2003, significantly lower reproducibility coefficients were observed.

Table 7

Reproducibility coefficients (R) of a single day of measurement and a 4-day period of measurements in 8-10-year-old children from 1997 and 2003.

	1 day		**4 days**	
	R	**95 % CI**	**R**	**95 % CI**
8-10 yr. 1997				
Boys	0.45	0.39 – 0.50	0.74	0.71 – 0.76
Girls	0.46	0.40 – 0.51	0.78	0.75 – 0.80
8-10 yr. 2003				
Boys	0.36	0.29 – 0.42	0.65	0.62 – 0.68
Girls	0.36	0.30 – 0.41	0.68	0.65 – 0.71

3.3 Stability and predictability of physical activity

Tracking of objectively measured physical activity was studied in Study II, taking into account the significant temporal sources of variation in physical activity described in the above (i.e. seasonal variation, day-type variation, and natural day-to-day variation). The tracking analyses were conducted on the follow-up data available in the Danish part of EYHS (i.e. the sample of 384 subjects aged 8-10 years at baseline in 1997). Of the 384 subjects participating in both EYHS-I and EYHS-II, a total of 208 had valid physical activity data at both time points.

In Table 8, stability coefficients for physical activity are presented in both a crude and an adjusted version. As can be seen from the table, the results revealed a markedly different stability pattern depending on whether the crude or the adjusted model was considered. As a rule of thumb it has been suggested that stability coefficients of less than 0.30 show low stability and values from

0.30 to 0.60 show moderate stability, while values between 0.60 and 0.90 indicate moderately high stability [117]. Consequently, in this study the stability of mean physical activity estimated from the crude model was low and only borderline significant. However, when the adjusted model was considered the results showed moderate stability of physical activity and the stability coefficients were highly significant.

In Table 9, predictability odds ratios are presented. It should be noted when interpreting the predictability odds ratios that the adjusted model did not include an adjustment for day-to-day variation and within instrumental measurement error, in contrast to the case of the stability coefficients. As can be seen from the table the corrections for error/variation had a positive effect on the results, even though differences between the crude and the adjusted model were not as pronounced as was the case for the stability coefficients. The most pronounced effect for correcting data was observed for boys, where the predictability odds ratio doubled and reached borderline significance.

Table 8

Stability coefficients (β) of mean physical activity

	Crude model				**Adjusted model**			
	N	**β**	**95 % CI**	**p**	**N**	**β**	**95 % CI**	**P**
Boys	96	0.18	-0.02 – 0.38	0.078	72	0.53	0.25 – 0.80	<0.001
Girls	112	0.19	-0.001 – 0.39	0.052	97	0.48	0.23 – 0.72	<0.001

The adjusted model was controlled for seasonal variation, within-week variation, activity registration during night time sleep, within instrumental measurement error, and day-to-day variation in physical activity.

Table 9

Predictability odds ratios (OR)

	Crude model				**Adjusted model**			
	N	**OR**	**95 % CI**	**P**	**N**	**OR**	**95 % CI**	**P**
Boys	96	1.50	0.52 – 4.36	0.456	72	2.83	0.85 – 9.39	0.090
Girls	112	4.07	1.56 – 10.59	0.004	97	4.36	1.51 – 12.62	0.007

The adjusted model was controlled for seasonal variation, within-week variation, and activity registration during night time sleep.

3.4 Stability and predictability of physical fitness and body mass index

In Study III tracking of physical fitness and BMI was studied across SES groups, using the follow-up data in the Danish part of EYHS. Furthermore, the prevalence and longitudinal development of overweight and low physical fitness was examined.

Table 10 presents SES specific baseline distributions of the CVD risk factors considered. Due to the significant dropout effect observed for physical fitness, data are presented for the total sample of 8-10-year-olds in EYHS-I (n=589). Of the 589 children, 42 families did not provide the necessary information for the classification of SES and 50 subjects failed to meet the criteria for exhaustion in the physical fitness test. In general, results revealed a more favourable risk factor profile in the group of high SES compared to the group of low SES. However, only differences in mean physical fitness and in the prevalence of low physical fitness (boys only) were significant.

Table 11 presents the stability coefficients for physical fitness and BMI, respectively. When comparing stability coefficients between groups of SES no statistical differences were seen. However, the results indicated that the stability of physical fitness and BMI is moderate and moderately high, respectively, independently of SES.

In Table 12 predictability odds ratios for low physical fitness and overweight are presented. No significant differences in predictability odds ratios were observed between groups of SES.

Table 13 presents odds ratios for being overweight and having low physical fitness, respectively, at follow-up in the group of low SES compared to the group of high SES. A significant socio-economic gradient was observed with approximately twice as high odds in the group of low SES compared to the group of high SES. Furthermore, Table 13 also presents odds ratios of maintaining and developing overweight/low physical fitness in the group of low vs. high SES. As can be seen in the table, both the odds of maintaining overweight and developing overweight were more than twice as high in the group of low SES compared to the group of high SES, whereas the corresponding odds ratios for low physical fitness were 1.36 and 1.94, respectively. It should be noted, however, that only odds ratios regarding the development of overweight and low physical fitness came out significant and borderline significant, respectively.

To give an impression of the absolute probabilities from which the relative odds ratios in Tables 12 and 13 are based, Table 14 shows the raw percentages of subjects who remain “at risk” throughout the measurement period and the percentage of subjects who develop into “risk”.

Interaction terms between gender and the independent variables were added in all tracking and logistic regression models and came out “not significant”. Therefore, analyses were performed for the two genders simultaneously, but controlled for maturity state.

Table 10

Mean and prevalence of CVD risk factors by SES in 8-10-year-old children

	Low SES			**High SES**		
	Total group size	**At-risk prevalence % (95 % CI)**	**Mean (SD)**	**Total group size**	**At-risk prevalence % (95 % CI)**	**Mean (SD)**
Physical fitness						
Girls	107	30.84† (22.27-40.50)	8.75‡ (1.41)	160	21.25† (15.19-28.41)	9.15‡ (1.39)
Boys	114	32.46‡ (23.99-41.86)	9.94‡ (1.74)	122	15.57‡ (9.64-23.25)	10.50‡ (1.48)
Body Mass Index						
Girls	124	16.13 (10.34-23.81)	17.43 (2.63)	168	13.69 (8.88-19.83)	17.08 (2.45)
Boys	124	15.32 (9.48-22.89)	17.48 (2.71)	131	12.98 (7.74-19.96)	17.28 (2.23)

Physical fitness was measured in watt/kg$^{2/3}$ and BMI in kg/m^2

‡ denotes a significant difference between groups of SES (P<0.05)

† denotes a borderline significant difference between groups of SES (0.05< P <0.09)

Table 11

Stability coefficients β adjusted for maturity state and gender, reported for the two groups of SES separately and for the total sample

	Low SES			High SES			Total sample			
	n	**β**	**95% CI**	**n**	**β**	**95% CI**	**n**	**β**	**95% CI**	**P[a]**
Physical fitness	133	0.45	0.25-0.64	195	0.54	0.41-0.67	328	0.50	0.38-0.61	0.54
Body Mass Index	161	0.75	0.61-0.90	219	0.70	0.60-0.80	380	0.73	0.64-0.81	0.60

a: P-value for the test of whether stability coefficients differ between groups of SES

Table 12

Predictability odds ratios adjusted for maturity state and gender, reported for the two groups of SES separately and for the total sample

	Low SES			High SES			Total sample			
	n	**OR**	**95 % CI**	**n**	**OR**	**95 % CI**	**n**	**OR**	**95 % CI**	**P[a]**
Physical fitness	133	4.29	1.85-9.93	195	7.31	3.20-16.71	328	5.44	3.03-9.75	0.47
Body Mass Index	161	15.73	5.26-47.03	219	17.08	5.41-53.92	380	16.26	7.40-35.80	0.89

a: P-value for the test of whether odds ratios differ between groups of SES

Table 13

Odds ratios for specified risk events in the group of low SES compared to the group of high SES. Odds ratios are adjusted for maturity state and gender.

	n	OR	95% CI	P[a]
Physical Fitness				
Being low fit at T_2	328	1.77	1.04-3.01	0.034
Maintaining low PF	81	1.36	0.55-3.36	0.511
Developing low PF	247	1.94	0.95-3.95	0.069
Body Mass Index				
Being overweight at T_2	380	1.95	1.07-3.57	0.030
Maintaining overweight	46	2.09	0.60-7.26	0.245
Developing overweight	334	2.35	1.02-5.40	0.045

a: P-value for the test of whether odds ratios differ from 1

PF denotes physical fitness

Table 14

Unadjusted frequencies of subjects maintaining or developing risk within the measurement period. The probabilities are weighted for dropout.

	Low SES		High SES		Total sample	
	Maintain (n)	**Develop (n)**	**Maintain (n)**	**Develop (n)**	**Maintain (n)**	**Develop (n)**
Low PF	55.48 % (38)	21.36 % (95)	47.76 % (43)	12.28 % (152)	51.82% (81)	15.89 % (247)
Overweight	69.59 % (22)	12.52 % (139)	51.72 % (24)	5.41 % (195)	60.31 % (46)	8.60 % (334)

n denotes the number of subjects on which the percentages are based

PF denotes physical fitness

4 Discussion

4.1 Main findings

The major findings of the present thesis were that:

I. Objectively measured physical activity was significantly influenced by the type of measurement day, with a general tendency towards higher physical activity levels in weekdays compared to weekend days.

II. Significant seasonal variation in physical activity was found in 8-10-year-old children, with a range of 318 counts per minute between the most active and the most inactive month. A general pattern of higher physical activity levels in the months of spring than in the months of winter and fall was observed.

III. The reproducibility of a single day of physical activity registration was estimated to approximately 0.45, after controlling for day-type differences in physical activity.

IV. Physical activity was inversely related to socio-economic status in 14-16-year-old adolescents whereas no clear relationship was observed for 8-10-year-olds.

V. Tracking of physical activity from childhood to adolescence showed stability coeffients of approximately 0.19, when calculated on the basis of raw estimates of physical activity derived by accelerometry. However, when taking into account the significant temporal variations in physical activity (i.e. natural day-to-day variation, seasonal variation and day-type variation), stability coefficients were estimated to approximately 0.50 for both boys and girls, respectively.

VI. Stability coefficients for physical fitness and BMI were estimated to be 0.50 and 0.73, respectively, from childhood to adolescence. When comparing tracking effects of physical fitness and BMI between groups of SES, no statistical differences were seen.

VII. At an age of 8-10 years social gradients were present in both the mean level of physical fitness and in the prevalence of low physical fitness, whereas no significant social gradient was observed for BMI and overweight. At the age of 14-16 years, the odds of being overweight and the odds of having low physical fitness were approximately twice as high in the group of low SES compared to the group of high SES. The social gradient in overweight observed at the age of 14-16 years developed as a result of more than twice as high odds ratios of both maintaining and developing overweight during the follow-up period in the group of low SES compared to the group of high SES.

4.2 The temporal aspect of physical activity

According to our knowledge, no previous study has examined how physical activity varies by the single days of the week in children and adolescents. However, several studies have investigated whether weekday activity levels differ from weekend activity levels [63-66]. All of these studies have reported significantly lower activity levels in weekends compared to weekdays, except for the study of Trost et al. [63] who reported the opposite result in young children – which could be due to cultural or age related differences between the populations being studied. Our results showed a general tendency towards lower activity levels in weekends than in weekdays, but the two clusters of days were not tested up against each other in the result section. At that point, the main objective was to estimate the effect of each of the single days, and merely test whether there was a significant effect of day types. However, a post hoc test showed that the observed tendency towards low activity levels in weekends was significant with respect to each of the included subgroups of children except for the 8-10-year-old girls in 1997 - in line with the results of the earlier applied Wald-test.

Seasonal variation in the activity pattern of children and adolescents has not been comprehensively addressed in the literature. A search through several databases including Medline and Sports Discus revealed very few studies addressing this subject [58-62], and only the study of Goran et al. [61] and Fisher et al. [62] were based on objective measurements of physical activity. However, the located studies generally confirmed the results found in adults [118-121], for whom the subject is more adequately addressed, namely that activity levels are higher during the months of spring and summer compared to the months of winter and autumn. A post hoc analysis was carried out to test whether the activity levels during spring and summer were significantly higher than during winter and autumn in the present study. In the 8-10-year-old children the results showed a highly significant difference, independent of gender and year of study, whereas a significant effect was observed only for boys in 2003 for the 14-16-year-old adolescents. However, the interpretation of the results should take into account that the summer period was not represented at all in the cohort of 14-16-year-old adolescents and the months of spring were less represented than in the case of the 8-10-year-olds, since the schools involved did not allow the ninth grade students to be disturbed during the examination period. Furthermore, it could be a source of bias that the 14-16 year olds were preparing their school leaving examination during the months of spring considered, since they might have been more sedentary during these months than they would have been under other conditions.

The magnitude of day-to-day variation and within instrumental measurement error found in this thesis was comparable to the results reported in earlier studies on data collected in the mid 1990s. Trost et al. examined the reproducibility of physical activity in several age groups of children and adolescents, and for the population of 1-3 grade children they reported reproducibility coefficients of 0.46 and 0.77 for a 1-day and a 4-day period, respectively [63]. Janz et al. studied a population of 7-15-year-old children and reported reproducibility coefficients of 0.42 and 0.75 for 1-day and a 4-day period, respectively [67]. In the present study, the calculations of reproducibility were performed on the baseline sample of 8-10-year-old children from 1997. As a post hoc analysis we repeated the reproducibility calculations on the sample of 8-10-year-olds examined in EYHS-II in

2003. Both the results from the main analysis of reproducibility and the post hoc analysis were presented in Table 7. The results indicated that a secular trend in the physical activity pattern of children had occurred during the period from 1997-2003, such that children aged 8-10 in 2003 exhibited significantly less day-to-day variation in physical activity than was the case in 1997. A possible explanation for this finding could be that childhood physical activity patterns have become more organized in recent years such that, compared to earlier years, participation in exercise is less spontaneous and to a greater extent carried out in structured settings at certain days of the week. The literature contains a number of references indicating that during the latter part of the 20th century, "free play" or unstructured games have given way to organized sports [122,123]. By concentrating exercise at certain days of the week rather than exercise is spread throughout the week, day-to-day variations in physical activity would in all likelihood increase.

The results of our post hoc analyses should, of course, be replicated in other studies before any significant importance can be attached to the findings, however if the findings hold true then the problem of day-to-day variation becomes even more relevant in future studies in this age group.

4.3 The association between physical activity and socio-economic status

The results showed a general pattern of lower activity levels in the middle and high-income areas compared to the low-income areas in the cohort of 14-16-year-old adolescents. In the 8-10-year-olds, however, this pattern was not observed - if anything SES and physical activity was unrelated for this age group. The finding of an inverse relation between SES and physical activity in the adolescents was surprising. Earlier studies in adults have shown that the highest prevalence of several CVD risk factors including obesity, smoking and low physical activity is observed in groups of low SES [6,77,78,106,108]. Studies conducted on younger people, on the other hand, have reported more inconsistent findings [124,125], however the controversy relates to whether SES and physical activity are positively associated [82,105,107,126,127] or not related at all [128-130]. Only very few studies have reported an inverse association [131].

Due to the unexpected finding in this study, a series of post hoc analyses were carried out to study the cycling habits of the various socio-economic groupings within the dataset. The reason for carrying out these tests was that the MTI Actigraph underestimates the oxygen uptake during cycling by 30 to 90 percent, depending on the work rate [96], and studies in both adults and adolescents indicate that cycling habits can vary by socio-economic conditions [132,133]. Moreover, the Danish population is one of the populations in the world that most frequently uses a bike for transportation; on average every adult Dane bicycle 400 km per year and children bicycle significantly more than adults [134-136]. As a consequence, cycling could bias the results concerning the association between physical activity and SES in this study. Detailed data on general cycling habits was only collected in 2003; thus, only data from 2003 was used in the post hoc analyses. In the 8-10-year-old children no significant difference was observed in the frequency of bicycling by SES (P=0.73), indicating that cycling probably did not have a biasing effect on the results of the 8-10-year-olds. However, in the 14-16-year-old adolescents a highly significant difference in the frequency of bicycling was observed between the lowest income areas and the two highest income areas – as shown in Table 15.

Table 15

Percentage distribution of how often 14-16 year-old-adolescents bicycle by SES in 2003.

	Max 1-2 times/week	**Almost every day**	**Every day**
Low SES	50.00	15.22	34.78
Mid SES	18.75	26.79	54.46
High SES	12.06	24.82	63.12

$P<0.001$ when testing the null hypothesis that the frequency distribution did not vary by SES (tested by Fishers exact test).

An additional post hoc test was carried out to examine the association between physical fitness and both SES and cycling habits in the 14-16-year-old adolescents, and the results showed a highly significant positive association in both cases. The results of these post hoc analyses clearly indicate that due to the Actigraphs inability to pick up bicycling activities, the estimates of physical activity in the cohort of 14-16-year-olds suffers from differential measurement error. Consequently, the association between physical activity and SES cannot be assessed correctly for the 14-16-year-old adolescents in the present thesis.

It should be emphasized, that the post hoc analyses, just described, only tested for significant differences in the frequency of bicycling between groups of SES, and did not take into account potential differences in the duration and intensity of bicycling by SES. Thus, caution is still needed when interpreting the results of the 8-10-year-olds, although the results revealed no difference in the frequency of bicycling by SES for this cohort. In fact, the finding that no general association was apparent between SES and physical activity for the 8-10-year-olds must be considered unexpected in light of the results of Study III, showing a positive association between physical fitness and SES for this age group. It is possible that the inconsistency can be explained by bias (i.e. socio-economic differences in bicycling habits). However, a second possibility is that the different definitions of SES applied when studying the influence of SES on physical activity and physical fitness, respectively, explains the inconsistency. Different definitions of SES might not to the same extent capture the factors responsible for the development of social gradients.

Having seen how the analyses involving SES was biased by the Actigraphs inability to register bicycling activities, it is natural to pose the question whether other of the important analyses involving physical activity in the present thesis could be biased by this limitation of the Actigraph. A Danish questionnaire survey concerning children's bicycling habits showed that children and adolescents bicycle more often on weekdays than on weekend days and more often when the weather is warm compared to cold weather [137]. Thus, the reported differences between types of days and seasons would only have been more pronounced if the Actigraph had been able to estimate cycling activities correctly. Likewise, the estimated tracking effect of physical activity would, in all probability, be negatively influenced by the problem of differential measurement error and the real tracking effect of physical activity would probably be higher than that reported. However, as a direct consequence of the problem of differential measurement error it was not possible to stratify the analysis of tracking of physical activity by SES, which would have been both natural and relevant in this thesis.

4.4 Tracking of physical activity

Tracking of objectively measured physical activity was studied both with and without adjustments for temporal sources of variation in physical activity. The results revealed a markedly different tracking pattern depending on whether the crude or the adjusted model was considered. In general, both stability coefficients and predictability odds ratios were low and not significant in the crude model, whereas highly significant moderate tracking effects was observed in the adjusted model.

When comparing the results obtained in the present thesis with those of earlier studies it is important to keep in mind, that the conditions for studying tracking depend on the length of the follow-up period used – the shorter the length, the better the chance of observing high tracking coefficients. This phenomenon has often been described in the literature on tracking, for instance Malina reported in a review study that: *over a span of 3 years during adolescence, inter-age correlations for activity are moderate at best, but over spans of 5 and 6 years, the inter-age correlations are lower* [38]. In order to obtain the best possible reference for comparing the results of the present study, we conducted a short review of the literature concerning tracking of physical activity within the years of childhood and/or adolescence, and we included only studies where the length of the follow-up period was comparable to the length in the present study. In the literature reviewed, stability coefficients ranged from -0.08 to 0.43, and all except one of the localized studies consistently reported stability coefficients under 0.31 [25,39,40,138,139]. Thus, the adjusted stability coefficients for physical activity observed in this study were larger than those generally reported in the literature. In fact, the adjusted stability coefficients were more comparable to the stability coefficients reported for physical fitness in the present thesis and in the recent literature [39-41].

The finding that physical activity and physical fitness "tracks" to the same degree seems plausible in light of the fact that the two parameters, physiologically speaking, are closely linked together. It has been argued earlier that physical fitness is a more stable parameter over time than physical activity because of the genetic component of aerobic power and the large behavioural component of physical activity [39]. However, at the same time, a number of authors have called attention to the fact that low tracking coefficients for physical activity might just reflect our difficulties in assessing physical activity in a valid and precise manner in large scaled epidemiological studies [14,39]. Physical activity is a very complex behaviour to quantify – much more complex than is the case for physical fitness. Several accurate methods of assessing physical fitness are available for epidemiological studies including direct assessments of VO_2 max through the analysis of respiratory gases [14,18,40,140,141] and physical fitness is less influenced by the type of measurement day and day-to-day variations compared to physical activity. The test-retest intraclass correlation coefficient for directly estimated VO_2 max has been estimated to be 0.96 in children [142]. Thus, a direct comparison of the naive tracking effect of physical activity and physical fitness could lead to a biased conclusion.

The results of the present study clearly demonstrated that both stability coefficients and predictability odds ratios are highly sensitive to potential sources of variation and measurement error. Four initiatives were carried out to reduce error in this study, however, future studies could also benefit from taking into consideration the significant inter instrument variability of the MTI Actigraph described earlier in the literature [143]. In the present study we did not have the opportunity to adjust for inter instrument variations but we are preparing a manuscript on the subject for later publication.

4.5 Tracking of physical fitness and body mass index

Tracking of physical fitness and BMI was studied both independently and in relation to SES. In general, results showed no significant differences in tracking of physical fitness and BMI, respectively, between the two groups of SES – both the stability coefficients and the predictability odds ratios did not differ significantly between groups of SES. However, when studying tracking independently of SES, the results showed highly significant moderate and moderately high tracking effects for physical fitness and BMI, respectively.

To the best of our knowledge, no earlier study has analysed and compared tracking of CVD risk factors between children from families of different SES. Thus, it is not possible to compare the current findings to the work of others. Even when looking at the results independently of SES, few studies are directly comparable to our study, but despite differences in methods and ages of the populations being studied, a review of relevant literature revealed that our conclusion regarding tracking of BMI and physical fitness, independent of SES, is in line with previous studies [23,26,33,40,144].

The fact that no significant differences were observed in predictability and stability coefficients between groups of SES did not indicate that SES was unrelated to physical fitness and BMI. In fact, when studying separate aspects of the term tracking (i.e. maintenance and development of risk) as well as total prevalence of risk, social gradients were observed, as shown in Table 13. It should be noted that the odds ratios for maintaining overweight and low physical fitness did not came out significant; however, the interpretation of the results should take into account that the analyses of maintenance of risk had less statistical power than the analyses of development of risk and prevalence of risk. A post hoc power calculation revealed low statistical power to detect odds ratios of 2 or less in the analyses of maintenance of risk. Thus, the reported odds ratios in these particular analyses could represent true differences between groups of SES, although estimated to be non-significant.

The finding that the predictability odds ratios were unrelated to SES, although social gradients were observed in the risk of maintaining and developing risk, can easily be explained logically. The higher estimated odds for maintaining risk in low versus high SES individuals tended to move predictability odds ratios in direction of higher values in low SES individuals; however, the equally higher odds of developing risk in low SES individuals tended to move in the opposite direction, leaving predictability odds ratios independent of SES. The net result was, however, that children of low SES were more likely to be at risk at follow-up compared to children of high SES; therefore, children of low SES should be recognized as a main target for preventive strategies.

5 Perspectives and Conclusions

The major findings of the present thesis were as follows 1) objectively measured physical activity was significantly influenced by temporal factors i.e. seasonality, type of measurement day, and day-to-day variation. 2) Tracking of physical activity from childhood to adolescence was low when calculated from raw unadjusted estimates of physical activity; however, when adjusting for temporal sources of variation in physical activity highly significant moderate stability coefficients were observed (ß≈0.50). 3) Physical fitness and physical activity showed the same overall stability from childhood to adolescence (ß=0.50), whereas BMI demonstrated higher stability over time (ß=0.73). 4) SES was not significantly related to tracking of physical fitness and BMI. However, when studying separate aspects of the term tracking (i.e. maintenance and development of risk) as well as total prevalence of risk, social gradients were observed. Finally, the study revealed a methodological problem in studying the influence of SES on physical activity assessed by accelerometry. Due to the fact that the MTI Actigraph underestimates the oxygen uptake during cycling and cycling habits varied by SES, the estimates of physical activity suffered from differential measurement error.

These findings have several important health scientific implications. From a methodological point of view, the considerable temporal sources of variation in physical activity observed in this thesis can seem quite problematic for epidemiological studies. Individual measurements of habitual physical activity are carried out within a limited time interval; in most studies within a period of no longer than a week, due to logistical considerations and a limited storage capacity of the MTI Actigraph when using an appropriate epoch setting. The actual number of physical activity registration days per subject, however, will be even less after data have been cleaned according to the criteria of validity and considerable differences can arise in the combinations of day types between subjects - even in cases where the measurement period has been standardized to a whole week. Thus, the significant differences in habitual physical activity between types of measurement days will be a relevant issue in many research projects. The exact adjustment procedure used in this thesis has, to the best of our knowledge, not been applied earlier in the literature, but could be used in a number of other research contexts in order to help standardizing the individual physical activity estimates. The adjustment for day-to-day variation and within instrumental measurement error could also have widespread application in future studies. The optimal way of handling the problem of day-to-day variation in future studies is, of course, to base individual physical activity estimates on as large a number of recording days as possible. However, in studies where physical activity estimates are based on few days of recording, the erroneous impact of day-to-day variation and within instrumental measurement error can often be limited by using the method described in this study, depending on the specific research question.

In large-scale epidemiological studies the length of the overall measurement period is often several months due to the large number of subjects being tested. Thus, seasonal differences in habitual physical activity can also be an important source of variation in these studies - especially in developmental studies of physical activity. When studying developmental research questions, such as tracking of physical activity, it makes sense to standardize the point in time where physical activity is assessed for all subjects. However, in studies of association it will often be more important that physical activity and associated variables are estimated at the same point in time for each individual subject, rather than physical activity measurements being carried out at the same point in time for all subjects - for instance when the association between physical activity and various blood parameters are examined.

As a general recommendation, future research involving objectively measured physical activity should consider whether temporal sources of variation could give rise to bias in the analyses conducted. Although not equally relevant in all research questions, completely ignoring temporal sources of variation in physical activity could form the basis for substantial bias in individual comparisons of physical activity, in particular, and in analyses of association in which physical activity is included.

An additional methodological issue is the Actigraphs inability to register bicycling activities correctly. On the basis of the present thesis it should be recommended, that future studies examining the influence of SES on objectively measured physical activity in populations where bicycling is common, also performs a test to see if cycling habits vary significantly by the SES groups defined. If so, the method of accelerometry could very well be inappropriate for answering the specific research questions, unless actions are taken to combine accelerometer data with valid information on bicycling activities.

From a health care point of view the most important aspect of the findings presented in this thesis is the indirect evidence of a linkage between childhood health and the development of life style related diseases. The moderate and moderately high tracking effects of physical activity, physical fitness, and BMI, respectively, indicates that inactivity, low physical fitness, and overweight tend to be established in childhood and follow the children into later stages of life, where these conditions are known to be independent risk factors for lifestyle related diseases as for instance CVD. As an implication, childhood inactivity, overweight and low physical fitness should be recognized as possible targets for intervention strategies - not only to improve health in childhood but also in order to lower the prevalence of these risk factors in later stages of life. In this context, the results further suggest that weekend leisure time and the seasons of autumn and winter might be appropriate targets for physical activity intervention programs. Furthermore, the results provided evidence that children of low SES should be considered as a particularly important target for preventive strategies. The finding that significant socio-economic gradients in the prevalence of overweight and low physical fitness developed no later than during the age range from 9-15 years suggest, that preventive initiatives aiming at reducing the occurrence of new cases of these conditions in the group of low SES during childhood/early adolescence, should be considered as a potentially important preventive strategy for reducing socioeconomic inequalities in health.

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