Ph.d. Thesis

Atherosclerotic Cardiovascular Risk Factors in Danish Children and Adolescents. A Community based approach with a special reference to Physical Fitness and Obesity.

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Abbreviations:

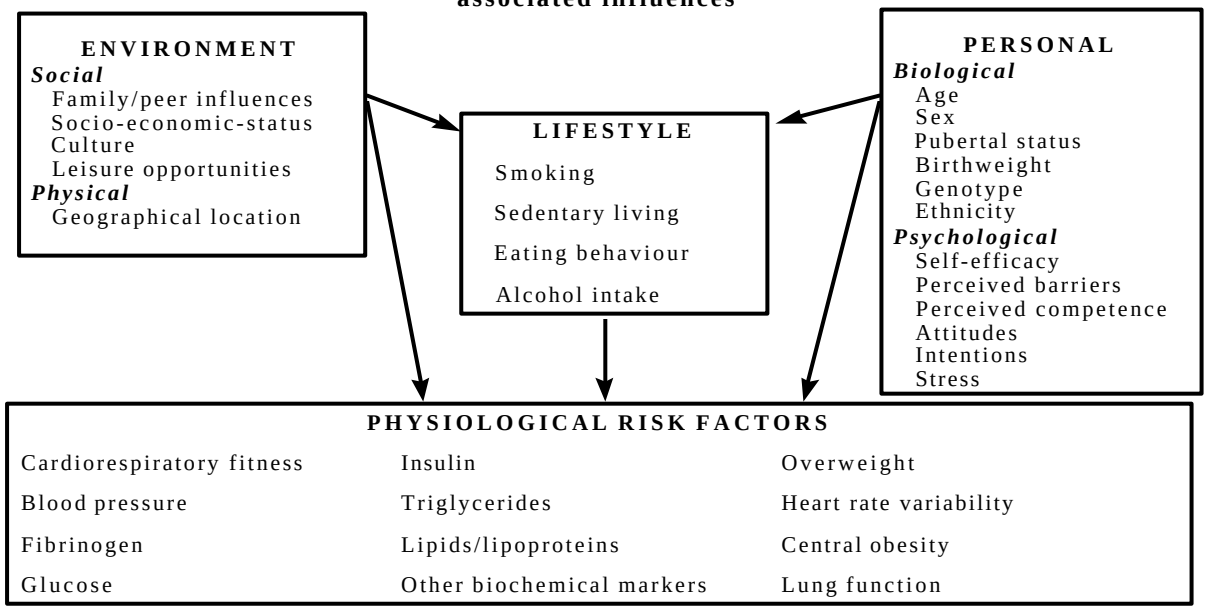
ACVD:	Atherosclerotic cardiovascular disease.
BMI:	Body mass index.
BP:	Blood pressure.
CI:	Confidence interval.
CVD:	Cardiovascular disease.
EYHS:	European Youth Heart Study.
HDL	High density lipoprotein.
HR	Heart rate.
LDL:	Low density lipoprotein.
NIDDM:	Non insulin dependent diabetes mellitus.
PF:	Physical fitness.
PI:	Ponderal index.
S:	Serum.
SD:	Standard deviation

1. Foreword:

During the nineties a group of exercise scientists from all over Europe met and decided to investigate multiple cardiovascular disease (CVD) risk factors including cultural and genetic influences in children. This ph.d.-thesis is based on results from the Danish part of this study, the European Youth Heart Study (EYHS).

The EYHS is an international multicenter study of cardiovascular disease risk factors in children. The aim of the study was: To study the nature, strength and interactions between personal, environmental and lifestyle influences on CVD risk factors in children of differing age, sex, culture and ethnicity. The risk factors and its associated influences are schematically illustrated in fig. 1.

Fig. 1. **Schematic representation of candidate CVD risk factors and associated influences**



The aims of the study are:

Within each study location:

1. To assess the age and sex specific magnitude and prevalence of a range of CVD risk factors
2. To assess the age and sex specific relationships between personal, environmental, lifestyle and physiological risk factors, and their associated influences

Between study locations:

1. To assess the age and sex specific differences in the magnitude and prevalence of personal, environmental, lifestyle and physiological risk factors
2. To assess how relationships between personal, environmental, lifestyle and physiological risk factors, and their associated influences, vary between study populations

From the combined database:

1. To assess whether patterns of children's CVD risk factors reflect the adult patterns of the disease
2. To determine the age and sex specific dose-response relationships between exposure to parameters which influence risk and the observed physiological levels of risk.

The study is designed as a mixed longitudinal study, with planned follow up and inclusion of a new childhood cohort every 6th year.

2. Introduction and background :

Atherosclerotic cardiovascular disease (ACVD) is increasingly recognised as a paediatric problem [1]. Even though the clinical manifestations of ACVD typically appear in adulthood rather than childhood, pathological studies have reported that advanced atherosclerotic lesions are identifiable in young children [2]. Anatomical changes in the aorta, coronary arteries and kidneys of youth and young adults are related to risk factor levels in childhood [2], and evidence exists that children maintain their levels of blood pressure (BP), obesity, serum lipids and lipoproteins in relation to their peer from childhood to young adulthood [3-5]. This persistency of an individual's risk factor level in relation to his or hers peers is defined as "tracking".

Risk factors for ACVD are manifest in childhood, with studies in the USA [6] England [7] and Northern Ireland [8] indicating that approximately 70% of twelve-year-old children have at least one modifiable ACVD risk factor. Behavioural risk factors for adults are associated with childhood experiences; in particular the early establishment of physical activity patterns, taste preferences, and cigarette smoking [9].

Primary prevention strategies for ACVD have therefore become a national priority in many countries and, logically, this should begin in childhood [10].

2.1 Clustering of atherosclerotic cardiovascular disease risk factors:

Clustering of ACVD risk factors is defined as the coexistence of several ACVD risk factors in the same person. Clustering of risk factors was originally described in adults by Reaven [11] and has been variously termed either the 'Metabolic Syndrome', 'Insulin Resistance Syndrome', or 'Syndrome X'. Typically, the syndrome is characterised by the co-existence of most of the following conditions: Abdominal obesity, hypertriglyceridaemia, dyslipidaemia, hypertension, and hyperinsulinaemia. Such an adverse profile is recognised as predisposing non-insulin dependent diabetes mellitus (NIDDM), atherosclerosis, and increased ACVD morbidity and mortality. The mortality due to ACVD is nearly the double of the normal population in the patients suffering from the metabolic syndrome, and these patients have a 3-4 times increased risk of getting ACVD [12]. This makes subjects with the metabolic syndrome a main target group for treatment and prevention of ACVD. It has been suggested, that sedentary living and low cardiovascular fitness may further exacerbate the condition [13].

It is well known that ACVD risk factors persist from childhood into adulthood [14], and it is therefore prudent to consider whether clustering of ACVD risk factors can be observed in children. Although the emergence of abnormal risk factor levels, as judged by adult criteria, begins in young adulthood, it is not normally seen in children [14]. However it is possible that adult 'patterns' of risk factor profiles - e.g. clustering tendencies - may be observable in children. Chu et al. [15] have suggested that ACVD risk factors do appear during adolescence in the obese. If so, childhood may be considered as a vital period for primary ACVD prevention programmes.

There is some supporting evidence for this hypothesis. For example, childhood risk factor profiles have been shown to reflect the adult patterns of the disease [16]. A measure of clustering has been shown to be observable in adolescents [17], and some authors have suggested, that obesity may be one of the key causative factors [14;18]. In particular, obesity correlates with multiple risk factors in the Insulin Resistance syndrome and ACVD [19]. Childhood obesity has been observed to increase the risk of adult metabolic syndrome [20], and it has also been reported that 50% of obese children will become obese adults with especially a high risk of metabolic syndrome and ACVD [20].

The close relationship between hyperinsulinaemia, hypertension and development of ACVD is now almost universally accepted [21-23], and it has been suggested that high insulin levels precedes the development of an atherogenic risk factor profile and predict future BP among children and adolescents independently of age and body mass [24]. Thus individuals with higher serum (S) insulin values have been reported to possess higher levels of other ACVD risk factors [22], and high S-insulin during childhood predicts high S-triglyceride levels 6 years later [24]. Hypertriglyceridemia is strongly correlated to obesity and also widely accepted as an important independent ACVD risk factor [25;26]. Elevated total S-cholesterol and low high density lipoprotein (HDL) levels are both widely recognised as independent ACVD risk factors and the ratio between them as one too [27]. In addition the ratio HDL:S-cholesterol has the advantage of summarising complex associations of the serum lipid pattern into a single numerical approximation.

Epidemiological studies have shown that elevated cholesterol levels, particularly elevated low density lipoprotein (LDL) cholesterol, are a major established risk factor for the development of ACVD [28;29]. Evidence also suggests that the atherogenic potential of a high level of LDL is increased by insulin resistance, the metabolic syndrome and manifest NIDDM [12;30]. There is a large amount of clinical data available to indicate that lowering total or LDL-cholesterol levels reduces the risk of cardiovascular events and mortality [29;31]. Studies have also demonstrated a decrease in both atherosclerotic associated morbidity and mortality in the NIDDM patient after lowering of LDL [30].

Clustering of ACVD risk factors has been suggested to persist from childhood to adulthood [32]. This phenomenon has been described by several authors [33-36]. Adult multifactorial ACVD risk status can be predicted from childhood risk factor levels [35]. Berenson et al. [34;37] have also noted that the risk factor profile during childhood is predictive of adult ACVD, and a recent Danish study (follow-up of the Odense Schoolchild study) has confirmed the tracking tendencies of ACVD risk factors and the clustering of the risk factors [38]. It is therefore prudent, to consider whether clustering of ACVD risk factors can be observed in children. Some studies have been performed to determine if clustering of ACVD risk factors is apparent in childhood [15;32;39;40].

2.2 Physical fitness:

Cardiorespiratory fitness is an important independent predictor of atherosclerosis in middle-aged men [41]. In addition, high levels of physical activity and physical fitness (PF) have been associated with a lower prevalence of ACVD risk factors and a lower ACVD morbidity in epidemiological studies [42;43]. Fitness shows, as most other risk factors, tracking over time [33], and the evidence suggests that an inverse relationship exist between PF and cardiovascular risk in adults. The evidence in children is more indeterminate, though some studies have shown a bivariate relationship to risk factors in children [44;45], and some studies have shown a multivariate relationship to BP [46;47].

2.3 Secular Trends:

2.3.1 Trends in fitness:

In children, secular trends of fitness has only been described by Dollman et al [48]. They found a lower fitness in 10-11 year old children in the middle of the nineties than in 1985.
No up to date study regarding secular trends in fitness of adolescents was found, by a search strategy with the terms fitness and adolescents together with secular, trends, tendency, tendencies and evolution performed in the databases Medline and Sports Discus.
It is very important to study and describe trends of PF in children, because tracking from childhood to adolescence and from adolescence to young adulthood is evident [33;49]. PF tracks moderately, and unfit children and adolescents are prone to be unfit adults [4;50]. Therefore

the results from secular trend studies would provide the first warning signals of low PF in groups of the subsequent adult generation.

2.3.2 Trends in obesity:
Obesity has been shown to increase in many countries [51;52], and obesity is clearly related to ACVD risk and ACVD risk factors [20;53;54].In a Danish study obesity in childhood has been found to be associated with low insulin-sensitivity index values in young adulthood [55]. It is an increasing problem in children and adolescents as well as in adults [56], and it has a high degree of tracking [20;57].
A lot of these studies have used the body mass index (BMI) (kg/m^2) to measure obesity. Though this is problematic, because body mass changes with height raised to the third power as described by Asmussen et al [58]. In addition it has been found by some authors, that skinfold measurements and the ponderal index (PI) (kg/m^3) are better suited for estimating body fat than the BMI [3;59]. This applies especially to children and adolescents, where BMI should be used with caution when assessing the body composition of children aged 8-16 years [60]. The most precise approximation of body composition assessed in field studies has been shown to be by skinfold thickness, which represent a comparatively simple and reasonably accurate assessment [61]. This should especially be taken into account in a longitudinal study. However the PI of children and adolescents has also been shown to be a good predictor of future overweight in adulthood, and better than the BMI [3]. As in PF the results from secular trend studies of obesity would provide the first warning signals of an increasing number of obese subjects and increasing degree of obesity in groups of the subsequent adult generation.

2.4 Parental educational level:
Educational level has been shown to be inversely associated with ACVD and ACVD risk factors in adults [62;63]. The same unfavourable pattern of ACVD risk factors has been found in children and adolescents in relation to parental educational level [17;64;65].
Parental educational level could also be used to assess if the sample is generally representative. This can easily be done, by comparing the educational levels of the parents of the sampled children with that of the Danish population, which is available from the home page of the Danish statistical bureau on the internet [66].

2.5 Smoking:
Smoking is recognised as one of the most important life style risk factors of ACVD [67]. Cigarette smoking has been shown to be strongly correlated to atherosclerotic lesions in aorta and the coronary arteries in young men [68]. In addition smoking has also been shown to be associated with unfavourable levels of S-cholesterol, LDL, HDL, and S-triglyceride in adults and adolescents [17;69].
The social difference in smoking has been regarded as one of the major causes of the social difference in ACVD [70]. A tendency towards women smoking more and starting earlier has been reported, thereby increasing the risk of ACVD in women as a group considerably [71].

2.6 Inherited risk:
Genetic studies and studies of familial aggregation of risk factors, provide evidence that children born to families with a high prevalence of hypertension are themselves at risk for development of hypertension [72]. Children born to families with at least one member who has either hyperlipidemia; low HDL; essential hypertension; or a family history of premature CVD are known to have high levels of CVD risk factors [10;72;73].

2.7 Aims of this study:
In Danish children and adolescents to
1. Investigate whether the phenomenon of clustering of risk factors of ACVD is observable.
2. Investigate the influence of physical fitness on ACVD-risk factors and clustering of risk factors.

3. Describe and discuss the implications of the secular trends of PF and obesity.

3 Subjects:

The study was school based. All observations and tests were performed at the schools that children attended. Eight to 12 children were examined per day, and the study was performed during one whole school year, from august 10th 1997 to June 12th 1998.

3.1 Sampling:

3.1.1 Sampling considerations:

Our primary need was to establish a study population which was locally representative and could be clearly defined. In addition it should be a population for which sampling frames existed, and it should be socially diverse. As the study was to be conducted within schools, the final choice of populations was governed in part by the nature and distribution of schools and access to a suitable sampling frame.

3.1.2 Sampling procedures:

Schools was stratified according to school type (age range, selection procedures), location (urban, suburban, rural) 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 (clusters) were the schools. The sampling frame for schools was a complete list of public schools in Odense, from which schools were selected using probability proportional to school size. Each school on the list was allocated a weighting equivalent to the number of children enrolled who were eligible for selection into the study. Three replacement schools were sampled from the list, to allow for schools refusing to participate and to act as reserves, in the event of low response rates.

The secondary units were the children within the schools, and equal numbers of children were selected from each school. Children within the appropriate age bands (8-10 yr. and 14-16 yr.) were allocated code numbers and randomly selected using random number tables. Previous experience of studying children of similar age and using similar methodology (including blood sampling), suggests that a 90% response rate would be likely from schools and a 75-80% response rate from children and/or parents [8;74].

3.2 Ethics:

The study was approved by the local ethics committee and performed by the rules stipulated by the Helsinki declaration. All children gave verbal consent and their parents gave written consent.

3.3 Subjects:

Twenty-eight out of 35 schools were randomly sampled, and 25 agreed to participate. Of the three non participating schools, one was rural, one was urban from middleclass area and one was urban from a low income area. All three schools gave interference with the educational process as reason for not participating.

A total of 1356 children and adolescents were invited to participate in the study, and 1325 (97.7%) responded. Of these 1020 (75.2%) and their parents participated in the study. Five hundred eighty nine children (37.0% of the third grade population), 279 boys and 310 girls, and 225 female and 206 male adolescents (36.1% of the ninth grade population) participated. Mean age of the children were 9.6 years and mean age of the adolescents were 15.5 years.

In each case of a child and parents not participating, the form master was asked, whether the child differed from the rest of the class in any way. Thirty-five children and adolescents, who had given consent to participate, were ill and 4 chose on the day of the examination not to participate. Of all the children and adolescents not participating, 1 adolescent was described as being obese and physically inactive and 1 child was autistic. The remaining children and adolescents were all described as being normal with a normal level of physical activity in comparison with their class peers.

4. Methods:

4.1 Physiological measurements:

Body height to the nearest mm and body mass to the nearest 100g were determined by standard anthropometric methods [75], using a stadiometer and a beam-scale type weight. Body fatness was estimated by three methods. The PI [3], which is bodymass divided by height in meters raised to the third power (kg/m^3), was used in the comparison between the three studies to assess secular trends, because this was the only common measurement between the three studies. The waist-hip ratio was used in the assessment of the metabolic syndrome in children as described by The World Health Organisation [76]. Waist and hip circumferences were measured at the largest circumference of the abdomen below the ribs and over the throcanter major to the nearest mm. The sum of four skinfolds was used in the Danish part of EYHS (biceps, triceps, subscapular and suprailiac skinfold) [77]. Skinfolds were measured with Harpenden callipers over the m.triceps brachii, m.biceps brachii, subscapularly, and superior to the spina iliaca anterior superior. The jaws of the callipers were placed around the skinfolds 1cm below where it was held by the thumb and first finger. The observer waited for 2-3 seconds before taking the reading and kept hold of the skinfold whilst making the measurement. Care was taken not to get muscle inside the skinfold. Measurements were performed on the left side of the body with the child standing. Two measurements were taken on each position. If there was a difference of more than 2 mm, a third measurement was taken, and the mean of the 2 closest measurements was then used. Measurements were made in rotation (1 measurement on each site, then repeated). The measurements on the arm were done with the arm hanging and the subject in the anatomical position. Measurement was performed over the centre of the muscle on a line drawn between olecranon and acromion. On the anterior side of the humerus the measurement was taken over m.biceps brachii. The skinfold was measured at the midpoint between olecranon and acromion. Subscapularly, the skinfold was measured under the angulus inf. on a line with a 45 degrees downward tilt compared to the vertical line. The skinfold above Spina Iliaca anterior superior was measured 3-5 cm above the spina with 45 degrees inferior medial tilt on the anterior axial line.

Puberty stage was assessed according to Tanner [78].

4.2 Blood sampling and analysis:

After an over night fasting, intravenous blood samples were taken in the morning 8-8.30 A.M. from the antecubital vein, one hour after application of an anaesthetic cream (lidocaine/prilocaine - Emla cream, Astra). Blood were aliquoted and separated within ½ hour. Samples were stored at -80?C until the transport to the central laboratory for analysis. A single certified laboratory (Dept. of Chemical Pathology, Bristol Royal Infirmary, Bristol, England) was used for all analyses.

4.3 Blood pressure:

BP was measured in the sitting position using the subject's left arm, with the Dinamap adult/paediatric and neonatal vital signs monitor, model XL (Critikron, Inc., Tampa, FL). The use of an automated instrument avoids the potential observer variation associated with non-automated instruments. The Dinamap monitor has been validated in children [79] against direct radial artery readings (mean error 0.24 systolic, 1.28 diastolic, 0.10 mean pressure). These errors were smaller than the errors observed using an ausculatory method. The children were introduced to the monitor, and were sitting quietly resting by them selves at least 5 min before the measurements were made. Five measurements were taken 2 minutes apart and the mean of the last three was used in the analysis.

The BPs were taken using a standard pressure cuff. The cuff sizes were either adult or child. The width of the cuff was at least 40% of the circumference of the upper arm, and the cuff was long enough to encircle the upper arm completely with or without overlap [80;81].

4.4 Parents educational level:
Through a questionnaire delivered to the schools and brought home by the children, information was obtained from both parents or guardians on their level of education and income. Parents educational level was defined by the guidelines from the Danish Statistical bureau [66].

4.5 Smoking:
Smoking was assessed through a computerised questionnaire. The children and adolescents were asked if they had ever smoked, if they smoked now and how often they smoked at the moment. Answer possibilities were "yes" or "no" for the first two questions. If the subjects answered "no" to the first question, they were not asked the two last questions. The possible answers of the last question were every day, at least once a week, and less than once a week.

4.6 Possible inheritance of ACVD risk:
Parental atherosclerotic related diseases were assessed by questionnaire. Parents were asked if they had had either myocardial infarction, stroke, angina pectoris, atherosclerosis or hypertension diagnosed.

4.7 Physical fitness:
PF was determined by a watt max test. The watt max test is a progressive maximal cycle-ergometer test that has been validated in both children and adults with a high correlation coefficient (r=0.9) to directly measured VO_2max [82]. The workload is increased every third mi??nute until exhaustion. The children started at either 20 watts, if their body mass was less than 30 kg, with a rise in workload of 20 watts for each 3 minutes; or 25 watts with a rise in workload of 25 watts for each 3 minutes. Adolescent females started at 40 watts, and the workload was raised with 40 watts for each 3 minutes, whereas adolescent males started at 50 watts, and the workload was raised with 50 watts for each 3 minutes.
The cycle-ergometer was a computerised Monark 839 Ergomedic, and it was pre-programmed to increase the workload every third minute. The workload was increased until exhaustion, and the time and heart rate (HR) were registered. Polar Vantage NV was used to register time and HR. Criteria for exhaustion were HR above 185 beats per minute (bpm), that the child could not keep a pedalling frequency of at least 30 revolutions per minute (rpm) or more, as well as a subjective judgement by the observer that the child could no longer keep up, even after vocal encouragement. All children and adolescents were tested by the same person.
The number of children and adolescents meeting the criteria of exhaustion were 298 childhood girls, 276 childhood boys, 216 adolescent girls and 205 adolescent boys. The number of children and adolescents excluded, because of not meeting the criteria for exhaustion, were 17 childhood girls, 19 childhood boys, 13 adolescent girls and 8 adolescent boys..
The maximal power output (Wattmax) was calculated as the watts in the last fully completed workload (W_l), plus the increment in watts (W_i) of the last step divided by 180 sec multiplied by the number of sec completed of the last step (t_{ls}).
$Wattmax = W_1 + (W_i \times t_{ls} / 180)$
PF was assessed as the maximal power output per kg body mass (watt/kg).

4.8 Secular trends:
The investigation of secular trends was performed between 3 representative studies performed 12 and 15 years apart:

1. The Danish Youth and Sport Study performed by L. B. Andersen et al. in 1983-84 [83].
2. The Odense School Child Study performed by H.S. Hansen et al. 1985-86 [46].
3. Danish Part of The European Youth Heart Study 1997-98.

The tests used for assessing PF were maximal work tests (the watt max test) in all three studies.

4.8.1 The Odense Schoolchild Study:
Eighty-one point five percent of all third grade school children in Odense, 1369 children participated in Odense Schoolchild Study in 1985-86. The anthropometric measurements, height and body mass were measured wearing light indoor clothes and shoes, the PF test used was exactly the same as in the present study [46].

4.8.2 The Danish Sport and Youth Study by L.B. Andersen:
Seven hundred female and 550 male adolescents aged 15-18 years were participated. They were a random sample from schools throughout Denmark. Subjects attended 36 classes from 18 high schools (gymnasium), 9 classes from different vocational schools and 9 classes from trade schools. The number of classes sampled in a region was proportional to the number of pupils attending the different types of schools. All pupils in the classes participated. The sample was compared to a random sample of the whole population at the same age. Variables included height, weight, physical performances as measures of strength and aerobic capacity, and no significant differences were found.
The anthropometric measurements, body mass and height were measured to the nearest 500 grams and nearest cm. The PF test used, was the watt-max test, with a constant pedalling frequency, and an increase of 35 watts every two minutes until exhaustion [84].

4.9 Risk factor indices:
4.9.1 Clustering of ACVD risk factors:
Five apparently independent risk factors (LDL, obesity as sum of four skin folds, systolic BP, inherited risk as parental ACVD related disease, and smoking) were selected to assess the degree of clustering of unfavourable ACVD risk factors. The rational for selecting the five risk factors was, that the risk factors are all recognised as some of the most important independent risk factors and that in children the relationship between the risk factors is not clear.

4.9.2 Clustering of risk factors connected to the metabolic syndrome:
A risk factor index of risk factors connected to the metabolic syndrome (S-insulin, S-triglyceride, HDL, Obesity and systolic BP) was also used for clustering analysis. The rational for selecting the five risk factors was, that the risk factors all are biological risk factors which in adults are related. So the risk factors were selected to find out whether the interrelationship also was detectable in Danish children and adolescents If an interrelationship existed it would be analysed to find out whether any "common causes" were observable (e.g. physical fitness, obesity).

4.10 The metabolic syndrome in children and adolescents:
In addition the five risk factors were used to assess if any of the children or adolescents could be categorised as having the metabolic syndrome. The values used were the values advised by the report "Type 2 diabetes and metabolic syndrome, diagnosis and treatment" [85] and The World Health Organisation [76]: Fasting S-insulin > 55pmol/ l=8 ? IU in pre-puberty children and 14 ? IU in children and adolescents who had started puberty (55 pmol/l=8 ? IU, as advised by the report, but 14? IU (95 percentile as described by Dunger et al [86]) was used to adjust for the effect of puberty, because S-insulin raises with puberty and returns to childhood levels in adulthood [86;87].) together with at least two of the following; fasting S-glucose>6.1 mmol/l, BP>140/90, fasting S-triglyceride>1.7 mmol/l, HDL<1mmol/l, waist hip ratio > 0.9 in males and 0.85 in females. All criteria except the obesity measure (the waist hip ratio) are according to the report "Type 2 diabetes and metabolic syndrome, diagnosis and treatment"[85]. The obesity measure is according to WHO [76] criteria, because the obesity measure from the report, a waist circumference longer than 90 cm in males and 80 cm in females, makes no sense in children and adolescent. Children and adolescents are still maturing and growing and have not reached their final "size", therefore the waist-hip ratio was used as a more "correct" obesity measure.

5. Quality Control:

5.1 Data Entry:

Data entry was manual except for the computerised questionnaire and the results of the analysis of the blood samples. All data entries were checked by a second person, and corrected, if they differed from the original results, written down during the examination of the children. An additional control of all outliers was performed. The outliers in the database were checked once more against the original results written down during the examination of the children. The outliers were corrected if they differed from the original results.

Data entry of the questionnaire for the children and adolescents was automatic. They answered the questions on a computer, where the data automatically were downloaded to the harddisk and a floppy disk. At the end of the study the questionnaire database was merged with the main database. The results of the analysis of the blood samples were received by electronic mail from the laboratory, and merged electronically with the main database. This eradicated the possibilities of error by manual data entry.

5.2 Anthropometric measurements:

The stadiometer was calibrated once a month with a metal anthropometric tape. The beam balance weight was calibrated every time it was moved. The skinfold calliper was calibrated either at each school or each time it was transported. The anthropometric measurements were made by the same male investigator on the boys and by two female investigators on the girls. The inter- and intra-observer reliability of the anthropometric measurements were checked continuously during the study, and inter- and intra-tester Pearson correlation coefficient of 0.988 and 0.998 were found between skinfold measurements. Body height and body mass were not tested this way, because inter- and intra-observer values were found to be identical in a pilot study performed for training purpose.

5.3 Maximal work test on the computerised cycle ergometric Monark 839 Ergomedic:

5.3.1 Calibration of the bike:

Each morning the ergometer was switched on and electronically calibrated according to the manufacture instructions. The ergometer was also mechanically calibrated every time it was moved.

5.3.2 Adjustment of the bicycle ergometer:

The seat height on the bicycle ergometer was adjusted so that the heel was flat on the pedal, when the leg of the child was extended. The child could choose the pedal frequency which was most comfortable for them (usually between 70-90rpm).

5.3.3 Heart rate:

A Polar HR monitor was fitted to the child and set to record HR beat by beat. It was ensured that the chest belt was tight and for the 9 year olds it was placed around or above the nipples to receive an adequate signal. Two sets of receivers were used at each test in case one should fail. One was chosen as the main receiver and the other as backup. The measurements from the main receiver were used unless it failed, then the backup was to be used. However no receiver failed during the study.

5.3.4 Validation of the maximal work test, the watt-max test:

A validation study was performed on a subsample of the participating children and adolescents. To validate the accuracy and validity of the watt-max test, and to create the algorithms for calculating VO_2max from the maximal watt performed by the children and adolescents. The subjects pedalled the same cycle-ergometer as used in the main study, the computerised Monark 839 Ergomedic.

They performed the test twice two days apart and the subjects were randomised to direct measurement of VO_2max at either first or second test round. The cycle ergometer was as in the main study pre-programmed to increase the workload every third minute. The workload was increased until exhaustion, and the time and HR were registered. Polar Vantage NV was

used to register time and HR. Criteria for exhaustion were HR above 185 beats per minute (bpm), that the child could not keep a pedalling frequency of at least 30 revolutions per minute (rpm) or more, and a subjective judgement by the observer that the child could no longer keep up, even after vocal encouragement. When getting VO2max measured directly the respiratory exchange ratio was also measured. All subjects who reached the criteria for exhaustion, above 185 bpm, 30 rpm and the subjective judgement also had a respiratory exchange ratio exceeding 1.00. All children and adolescents were tested by the same person (NW).
Hansen et al [82] have determined the appropriate increase in the work load during the test in children. At the same time both the accuracy and validity of the test and a method for calculation of VO_2max from Watt-max was performed [82]. The same procedure had a few years earlier been performed in adolescents by Andersen et al [84]. A correlation coefficient between calculated VO_2max and directly measured VO_2max of 0.98 was found by Hansen et al in children [82] and 0.90 by Andersen et al in adolescents [84]. In a validation study on a subsample from the present study, correlation coefficients of 0.92 in children and 0.96 in adolescents between calculated VO_2max and directly measured VO_2max were found.

5.4 Blood sampling and analysis of blood samples:
The child was lying down during blood sampling to prevent a vaso-vagal reaction. Blood samples were taken from the right arm, because BP was to be measured on the left arm. The child was put at ease and asked about the last time he or she had had something to eat or drink. If any of the children had eaten, they were asked to come back the following day to have the blood sample taken.
Even though a certified laboratory were used, a quality control was performed by sending 8 blood samples as doubles with each batch, checking that the analysis had reached the same results on the identical samples. No significant differences were found.

6. Statistics:

6.1. Sample size:
6.1.1 Bivariate analyses:
The target difference was the minimum difference between groups, it was the aim to be able to demonstrate as a significant difference, was termed. Based on epidemiological studies meaningful target differences and variability as standard deviations (SD) have been established for BP, S-cholesterol, and HDL [8].[47;74] Calculations were based in each case on the comparison of two independent groups of equal size, using a 2-tailed test. Sample sizes were estimated using 1-?=0.80 and ?=0.05.
For blood pressure 33 subjects were needed in each gender and age group with a target difference of 5 mmHg and a SD of 7mmHg. Thirty eight subjects were needed in each gender and age group for comparing S-cholesterol with a target difference of 0.5 mmol/l and a SD of 0.75mmol/l. The same number of subject were also needed in each gender and age group for comparing HDL with a target difference of 0.2 mmol/l and a SD of 0.3 mmol/l. One-hundred and forty-two subjects were needed in each age and gender group for comparing PF with a target difference of 0.2 watt per kg and a SD of 0.6 watt per kg.

6.1.2 Multivariate analyses:
The multivariate analysis performed were multiple linear regression and multiple logistic regression.
Multiple regression analyses require a minimum of 10 subjects per independent variable entered into any particular model [88]. A maximum of 20 independent variables per model were anticipated, indicating a sample size of 200 per age/gender group.

6.1.3 Summary:
From the above data it was concluded that 200 subjects per age and gender group would give an high level of power for the projected bivari-

ate analyses and an acceptable level of power for the multivariate analyses.
Possible 'cluster effects' necessitated a 'design effect' to be incorporated which was estimated at 1.25, thus increasing the sample size to 250 per age/sex group (n=1000).
A maximum non-response rate of 25% was anticipated, and therefore an oversampling of at least by this amount was performed. Consequently the total number of sampled needed was at least 1333 subjects.

6.2 Descriptives:
Mean values and SD of physical characteristics, of values for blood samples and BP were used to describe the population. S-triglycerid and S-insulin were described with 95% confidence intervals (CI), because of normalised values from skewed distributions. ANOVA was used to test for difference and interaction between gender and age groups.

6.3 Clustering of risk factors:
The degree of clustering of risk factors was assessed using an expected versus observed approach, where the expected number was the number expected if the risk factors were independent of each other. A subject was defined as having clustering of risk factors, if he or she had three or more risk factors. The expected frequency was calculated using the binomial probability formula $((n! / r!(n-r)!)p^r(1-p)^{n-r})$, and the null hypothesis was that no interrelationship existed. Chi-square analysis was used to test if the observed frequencies were higher than expected.
The interrelationship between risk factors was analysed using multivariate statistics.

6.3.1 Clustering of ACVD risk factors:
6.3.1.1 Children:
If a child belonged to either the top quartile of LDL, sum of four skinfolds or systolic BP, or if a parent had a ACVD related disease then it was defined as having a risk factor. Smoking was not used as a risk factor in children, because only very few (one or two) children could be expected to smoke. The subjects were assigned to 5 risk factor categories group (zero through four) depending on how many risk factors they possessed. Subjects with 3 or more risk factors were defined as clustering subjects. The other subjects were defined as non-clustering subjects. An expected frequency of 5.1% was calculated.

6.3.1.2 Adolescents:
If an adolescent belonged to either the top quartile of LDL, sum of four skinfolds or systolic BP, or if a parent had a ACVD related disease or if the subject smoked then it was defined as having a risk factor. The subjects were assigned to one of six risk factor categories (group zero through five) depending on how many risk factors they possessed. Subjects with three or more risk factors were defined as clustering subjects. The other subjects were defined as non-clustering subjects. An expected frequency of 10.4% for three or more risk factors was calculated.

6.3.2 Clustering of risk factors of the metabolic syndrome:
Two risk factor indices of the risk factors related to the metabolic syndrome were created, one for each age group.
If a subject belonged to either the top quartile of S-insulin, S-triglyceride, sum of four skinfolds and systolic BP, or the bottom quartile of HDL then it was defined as having a risk factor. It was possible to have between 0 and 5 risk factors. Subjects with 3 or more risk factors were defined as clustering subjects. The other subjects were defined as non-clustering subjects. A frequency of 10.4% for having three or more risk factors would be expected in each age group if the risk factors were independent and no interrelation existed.

6.3.3 Multivariate analyses of interrelationship between risk factors

6.3.3.1 ACVD risk factors:
The interrelationship between the risk factors was assessed using multiple regression with each of the continuous risk factors as dependent

variable and logistic regression with the two dichotomous variables as dependent variables. All risk factors in the index were used as single independent variables, adjusting for gender, age and puberty. The coefficients of the logistic regression was obtained using logit, hereby obtaining coefficients instead of odds ratios.

6.3.3.2 The metabolic syndrome:
The interrelationship between the risk factors of the metabolic syndrome was assessed using multiple regression with each of the risk factors as dependent variable and all other risk factors as single independent variables, adjusting for gender and puberty.

6.4 Metabolic syndrome in children and adolescents:
The number of children and adolescents with the metabolic syndrome diagnose was assessed using the criteria from the report "Type 2 diabetes and metabolic syndrome, diagnosis and treatment" [85] and WHO guidelines [76], as described earlier.

6.5 Parents educational level:
6.5.1 Correlation of the parental educational levels to that of Danish population:
Parents educational levels were compared to the educational levels of the total Danish population by differential plot with 95% CI, and a non-parametric test for trend of the differences was performed across the levels. The correlation between the parental educational levels and that of the total Danish population was assessed using non-parametric correlation (Spearman's rho).
Parents belonged to one of seven educational groups as described by the Danish statistical bureau [66]: primary school (10^{th} grade), secondary school (12^{th} grade), secondary vocational school, vocational education, short further education, college education (equivalent to bachelor), university education (master). The educational level of all parents were used when comparing with the total Danish population.

6.5.2 Multivariate analysis of the relationship between parental educational level and ACVD risk factors in children and adolescents:
For multivariate analysis the seven groups were divided into three groups: 1. Low: Basic schooling of 9-12 years, no further education (group 1-3 in the original classification). 2. Medium: Vocational and short further education (group 4-5 in the original classification). 3. High: Bachelor's degree or higher (group 6-7 in the original classification). If a child's two parents had different educational levels, the highest of the two was selected for multivariate analysis. If a child only had one parent or only one of them had provided the information, the educational level of that parent was selected.
Associations between parental educational level and risk factors were obtained using multiple regression, with the risk factors and fitness as dependent variables in the analysis, independent variables were four skin folds as continuous , and gender, smoking, puberty, age group, inherited disposition and educational levels as categorical. Non-smoking, lowest puberty stage, no inherited disposition and high educational level were reference categories.
The odds of having three or more ACVD risk factors were calculated using logistic regression with gender, puberty, age group and educational levels as independent categorical variables and with high educational level as reference category.
The odds of having three or more risk factors of the metabolic syndrome were calculated using logistic regression, with gender, puberty, age group and educational levels as independent categorical variables and with high educational level as reference category.

6.6 Smoking
Associations between smoking and risk factors were obtained using multiple regression with the risk factors and fitness as dependent variables in the analysis. Sum of four skin folds was continuos independent variable and gender, smoking, puberty were independent categorical variables. Non-smoking was reference category.

The odds ratios for having three or more risk factors of the metabolic syndrome were calculated using logistic regression with smoking, gender, and puberty used as independent categorical variables. Non-smoking was used as reference category.

6.7 Possible inherited disposition:
6.7.1 Parental diseases.
Parental diseases assessed were analysed one by one and an index (disease or no disease) taking all diseases into account was created. The index and the single diseases were used as parental disease variables to assess and adjust for possible inherited risk tendencies.

6.7.2 Association between parental disease and risk factors.
Associations between known parental diseases and risk factors were obtained using multiple regression with the risk factors as dependent variables in the analysis and four skin folds as continuos independent variable. Gender, smoking, puberty, inherited disposition (parental disease) and educational levels were independent categorical variables. Non-smoking, no inherited disposition and high educational level were reference categories.
The odds ratios for having three or more risk factors of the metabolic syndrome were calculated using logistic regression with gender, puberty, age group, and inherited risk as independent categorical variables. No inherited disposition was the reference category.

6.8 Fitness and risk factors:
6.8.1 The multivariate association between fitness and risk factors:
Multiple regression analysis was used for assessing the relationship between fitness and risk factors. Two methods were applied. Stepwise procedure was used to find the significant independent variables, and a calculation with all variables entered was performed to adjust for all variables.

Two models were tested.

1. Dependent variables were all risk factors except obesity. Independent variables introduced into the models for all dependent variables were PF, gender (female=0, male=1), the sum of four skin folds, start of puberty or not (not started=0, started=1) and exact age at time of examination.

2. Obesity expressed as sum of four skin folds was entered as the dependent variable. PF, gender (female=0, male=1), start of puberty or not (not started=0, started=1) and exact age at examination time were entered into the stepwise selection as independent variables.

6.8.2 Fitness and clustering of risk factors:
The expected and observed number of risk factors in the different quartiles of PF were calculated using the approach used for the clustering analysis of the total sample. Bivariate differences between observed and expected number of risk factors were tested using chi-square analysis. The null hypothesis was that the risk factors were not interrelated and if so a frequency of 5.5% would be found in the childhood quartiles and 10.4% in the adolescent quartiles.
Multivariate odds ratios for having three or more risk factors in relation to PF were calculated using logistic regression. The odds ratios were calculated for the separate quartiles of PF. The reference quartile was the quartile with highest PF. The calculation was adjusted for gender and puberty stage. This was illustrated by an odds ratio plot with 95% CI.
To increase the robustness of the multivariate approach odds ratios were also calculated for having two or more risk factors from the ACVD index.
A non-parametric test [89] was used to test for trend across the quartiles of PF.
Fitness by number of risk factors is shown graphically with 95% CI to illustrate the difference between the risk factor groups. A non-parametric test for trend [89] across the risk categories was performed.

6.8.3 Illustration of differences in serum values by fitness:
Differences in serum values between the highest and lowest quartiles of fitness were assessed and illustrated by the use of difference plot with 95% CI. A non-parametric test [89] was used to test for trend across the quartiles of fitness.

6.9 Secular trends:
6.9.1 Fitness:
Comparisons of PF were made between the calculated VO_2max using the algorithms obtained in the individual validation studies. The results were plotted by the deciles and compared in the gender and age specific groups. Deciles were used to assess if only part of the population had changed fitness level. A systematic error would show up; for example a higher fitness of equal magnitude in all deciles would indicate a systematic error.
PF is inversely proportional with age in adolescent girls [90] and obesity is highly associated to fitness. A skewed distribution of age was evident in the Danish youth and Sport Study, with the mean age being 1 year older than in the Danish part of EYHS.
Therefore to assure the validity of the above described graphical comparison, a multiple linear regression was used to adjust fitness for any difference in age distribution and obesity between two of the studies (EYHS and The Danish Youth and Sport Study).

6.9.2 Obesity:
Comparisons of obesity were made using the calculated ponderal indices. The results were plotted by the deciles and compared in the gender and age specific groups. Ponderal index were used because height and body mass were assessed in all three studies, whereas skinfolds were not taken at the same positions and body mass index was not ideal for use in children and adolescents [60;61;91].

6.10 Statistical software and general considerations concerning multivariate analyses:
All calculations were performed on a personal computer with STATA 6.0 for windows. For graphical illustrations the results were put into an EXCEL-97 spreadsheet and the graphs then produced with EXCEL-97.
STATA provides the opportunity of using "robust standard errors", which has "the ability to relax the assumption of independence of the observations. This means it can produce "correct" standard errors (in the measurement sense) even if the observations are correlated'[92]. The "robust" option was applied in all multiple regression and logistic regression calculations.
The level of significans was set at p=0.05 or by 95% CI.

7. Results:
7.1 Descriptives and differences between groups:
Nine-hundred and thirty nine subjects had blood samples drawn, 278 female and 247 male children, 214 female and 200 male adolescents. Mean values and SD of physical characteristics and values for blood samples and BPs are shown in table 1. S-triglycerid and S-insulin are shown with 95% CI, because of normalised values from skewed distributions.
Significant differences were found between genders in all variables with the exception of HDL and diastolic BP and between age-cohorts in all variables with the exception of the ratio between HDL and S-cholesterol. Significant interaction was found between gender- and age-cohort in all variables with the exception of S-insulin, S-triglycerid, S-cholesterol and LDL (table 1).
Among the children, 92 (30%) of the girls had started puberty, whereas all of the boys were staged as pre-puberty. Among the adolescents, only four boys and one girl were rated as not having started puberty.

Table 1: Physical characteristics, physical fitness, serum values and blood pressure.

	Children			Adolescents			ANOVA F-values		
Gender	No.	Girls (SD)	Boys (SD)	No.	Girls (SD)	Boys (SD)	Gender	Agegroup	Interaction
Body height (cm)	589	138 (6)	139 (6)	431	165 (7)	174 (7)	153^{+}	5567^{+}	98^{+}
Body mass (kg)	589	33 (6)	34 (6)	431	56 (9)	63 (10)	56^{+}	2905^{+}	35^{+}
Four skin folds (mm)	572	39 (19)	34 (17)	431	52 (19)	37 (18)	73^{+}	51^{+}	17^{+}
Fitness (watt/kg)	539	2.8 (0.5)	3.2 (0.6)	400	3.0 (0.5)	3.8 (0.6)	261^{+}	110^{+}	37^{+}
Max heart rate	539	199 (7)	199 (7)	400	197 (7)	199 (7)	0.5	0.8	0.2
S-Insulin ?IU/ml	525	7.9 (7.4-8.4)	7.0 (6.5-7.5)	414	12.7 (12.0-13.4)	12.4 (11.5-13.3)	4*****	244^{+}	1
S-triglycerid (mmol/l)	525	0.8 (0.7-0.9)	0.7 (0.6-0.8)	414	1.0 (0.9-1.1)	0.9 (0.8-0.9)	24^{+}	64^{+}	0
S-cholesterol (mmol/l)	525	4.6 (0.7)	4.5 (0.7)	414	4.3 (0.8)	4.1 (0.6)	17^{+}	52^{+}	3
HDL (mmol/l)	525	1.5 (0.3)	1.5 (0.3)	414	1.4 (0.3)	1.3 (0.3)	0	64^{+}	14^{+}
LDL (mmol/l)	525	2.7 (0.6)	2.6 (0.6)	414	2.5 (0.7)	2.3 (0.6)	14^{+}	47^{+}	0
HDL/cholesterol	525	0.32 (0.06)	0.34 (0.07)	414	0.32 (0.07)	0.33 (0.07)	10^{+}	3	5*****
Diastolic BP (mm Hg)	588	63 (6)	64 (6)	431	65 (6)	64 (6)	1	11^{+}	4*****
Systolic BP (mm Hg)	588	104 (7)	106 (8)	431	109 (8)	119 (11)	99^{+}	279^{+}	50^{+}

Means, SD and F-values from ANOVA of selected variables, means and 95% CI of S-triglycerid and S-insulin after back conversion from normalisation. *$p<0.05$, $^{+}p<0.01$

7.2 Clustering of risk factors:
The cut points of S-variables and obesity used for identifying the most unfavourable quartiles in the risk factors selected for risk indices are shown in table 2.

Table 2: Cut points of risk factors:

Cut points	Children		Adolescents	
Gender	Girls	Boys	Girls	Boys
Insulin micro (IU/ml)	10.5	9.2	15.5	15.9
S-triglycerid (mmol/l)	1.07	0.9	1.3	1.2
HDL (mmol/l)	1.2	1.2	1.1	1.1
LDL (mmol/l)	3.1	3.0	2.8	2.7
Four skin folds (mm)	46.5	39.0	60.6	42.5
Systolic BP (mm Hg)	108	110	114	126

Cut of points of the unfavourable quartiles (risk factor groups) of S-insulin S-triglycerid, HDL, LDL, sum of 4 skin folds and systolic BP

The ACVD and metabolic syndrome risk factor indices are shown in table 3. No more children and adolescents than expected had 3 or more ACVD or metabolic syndrome risk factors. In addition the observed number of subjects with four and five ACVD risk factors did not differ significantly from the expected. The multivariate analyses of the ACVD risk factor interrelation showed few significant relations (table 4). Only obesity was significantly related to LDL and systolic BP. All other variables were not significantly interrelated. All regression coefficients in multivariate analysis of risk factors of the metabolic syndrome, except between systolic BP and HDL, and between Systolic BP and S-triglyceride were significant (table 5).

Table 3.

	ACVD		Metabolic syndrome	
Gender	Children	Adolescents	Children	Adolescents
0 risk factors	189	129	168	118
1 risk factors	196	152	169	161
2 risk factors	99	83	107	77
3 risk factors	24	34	45	37
4 risk factors	3	7	16	14
5 risk factors		0	6	7
Total	511	405	511	414

Number of children and adolescents in ACVD and metabolic syndrome risk categories.

Table 4. Regression coefficients between risk factors of ACVD.

	Dependent variables				
Independent variables	LDL	Sum of 4 skinfold	Systolic BP	Smoking	Inherited risk
LDL		3.3 p=0.004	0.03 p=0.950	-0.17 p=0.425	-0.01 p=0.945
Sum of 4 skin-folds	0.004 p= 0.0039		0.06 p=0.0001	-0.001 p=0.946	0.01 p=0.131
Systolic BP	0.0002 p=0.950	0.29 p=0.0002		-0.02 p=0.170	0.02 p=0.007
Smoking	-0.07 p=0.399	0.99 p=0.743	-2.2 p=0.1111		0.01 0.986
Inherited risk	-0.003 p=0.946	2.2 p=0.150	1.8 p=0.008	-0.03 p=0.946	

Regression coefficients with significance level between risk factors from the ACVD index adjusted for gender, puberty and age.

Table 5. Regression coefficients between risk factors of the metabolic syndrome:

	Dependent variables				
Independent variables	S-insulin	S-triglycerid	HDL	Systolic BP	Sum of four skinfolds
S-insulin		0.18 p<0.0001	-0.03 p=0.045	1.2 p=0.004	5.1 p<0.0001
S-triglycerid	0.79 p<0.0001		-0.19 p<0.0001	1.5 p=0.056	7.3 p<0.0001
HDL	-0.21 p=0.039	-0.36 p<0.0001		1.5 p=0.1234	-9.8 p<0.0001
Systolic BP	0.010 p=0.004	0.003 p=0.052	0.002 p=0.1238		0.3 p<0.0001
Sum of four skinfolds	0.010 p<0.0001	0.003 p<0.0001	-0.002 p<0.0001	0.07 p<0.0001	

Regression coefficients with significant levels in children between risk factors of the metabolic syndrome, adjusted for gender, puberty and age.

7.3 Metabolic syndrome in children and adolescents:

The metabolic syndrome by adult values could be diagnosed in four childhood girls, three childhood boys, three adolescent girls and six adolescent boys.

7.4 Association between physical fitness and risk factors:

7.4.1 Associations assessed by multivariate analysis:

Multivariate analyses were performed as multiple linear regression to assess the association between PF and the risk factors (Table 6). Stepwise procedure was used to find the significant independent variables. Table 6 shows the significant regression coefficients and R^2 of the models obtained through multivariate linear

regression with stepwise selection, and with risk factors in children and adolescents as dependent variables.
PF was the variable most consistently associated with the risk factors in the children, and it was significant for all risk factors except BP and HDL. In the adolescents the sum of four skin folds was the variable most consistently associated with the risk factors and included in all models, except for diastolic BP and S-cholesterol.

The regression model, with all independent variables entered in one step, produced no change in the models of the childhood cohort. In adolescents some of the models changed. S-cholesterol: Only age was a significantly associated, sum of four skin folds was no longer significant when adjusting for gender, PF and start of puberty. Ratio between HDL and S-cholesterol: Sum of four skin folds was the only significantly associated factor, gender was no longer significant when adjusting for age, PF and start of puberty.

Table 6: Significant regression coefficients and R^2 in multiple linear regression with stepwise selection, for risk factors as dependent variables.

	Children			Adolescents		
Dependent variable	Independent variables	Coefficients	R^2 of the model	Independent variables for	Coefficients	R^2 of the model
Sqrt. S-insulin	Watt per kg Puberty start	-0.48 0.22	0.16	Gender Four skin folds Watt per kg	0.26 0.007 -0.27	0.08
Ln S-triglycerid	Age Watt per kg	-0.08. -0.15	0.07	Four skin folds	0.004	0.06
S-cholesterol	Age Watt per kg	-0.20 -0.11	0.03	Gender Age	-0.19 -0.19	0.04
HDL-cholesterol	Gender Four skin folds	1.57 -0.003	0.06	Gender Four skin folds Watt per kg Age	-0.06 -0.004 -0.08 -0.08	0.09
LDL-cholesterol	Watt per kg	-0.16	0.02	Four skin folds	0.005	0.03
Ratio HDL and S-cholesterol	Gender Watt per kg	0.02 0.02	0.07	Four skin folds	-0.001	0.08
Diastolic BP	No variables significant			No variables significant		
Systolic BP	four skin folds Gender start of puberty	0.07 2.4 2.1	0.06	Four skin folds Gender	0.09 10.43	0.21
Sum of four skin folds	Watt per kg start of puberty gender	-18.5 13.8 5.5	0.41	Watt per kg	-20.22	0.48

7.4.2 *Clustering and Odds of having risk factors by physical fitness:*

7.4.2.1 Bivariate analysis of clustering of risk factors by physical fitness:

Six children were expected to have 3 or more risk factors in each fitness quartile. No significant clustering was found in the three highest fitness quartiles, whereas in the lowest a significant clustering was found, with 16 (95% CI: 9 ; 15) children having 3 or more risk factors. In adolescents clustering was also only evident in the lowest fitness quartile. Nineteen (95% CI: 12 ; 28) adolescents were found with 3 or more risk factors, eight were expected.

7.4.2.2 Multivariate odds ratios of ACVD risk factors:

The cut points of the continuous variables used for evaluating the amount of clustering are shown in Table 2. Fig. 2a-b show the odds ratios for having three or more ACVD risk factors and fig 4a-b show the odds ratio for having two or more ACVD risk factors by the quartiles of PF with 95% CI. In children there were significantly higher odds ratios in the quartile with lowest fitness for having three or more risk factors of ACVD than in the two top quartiles (fig. 2a). In adolescents there were significantly higher odds ratios in the quartile with lowest fitness for having three or more risk factors of ACVD than in the top quartile (fig. 2b). In children the quartile with lowest fitness had significantly higher odds ratios than the three other quartiles for having two or more risk factors (fig. 3a). In adolescents there was significantly higher odds ratios in the quartile with lowest fitness for having three or more risk factors of ACVD than in the two top quartiles (fig. 3b).

There was a significant negative trend in number of risk factors across quartiles of fitness in all gender and age groups ($p<0.01$).

Fig. 4a-b show the PF by number of ACVD risk factors. There was a significant negative trend in PF with increasing number of risk factors in both age groups ($p<0.0001$).

Fig. 2a-b.

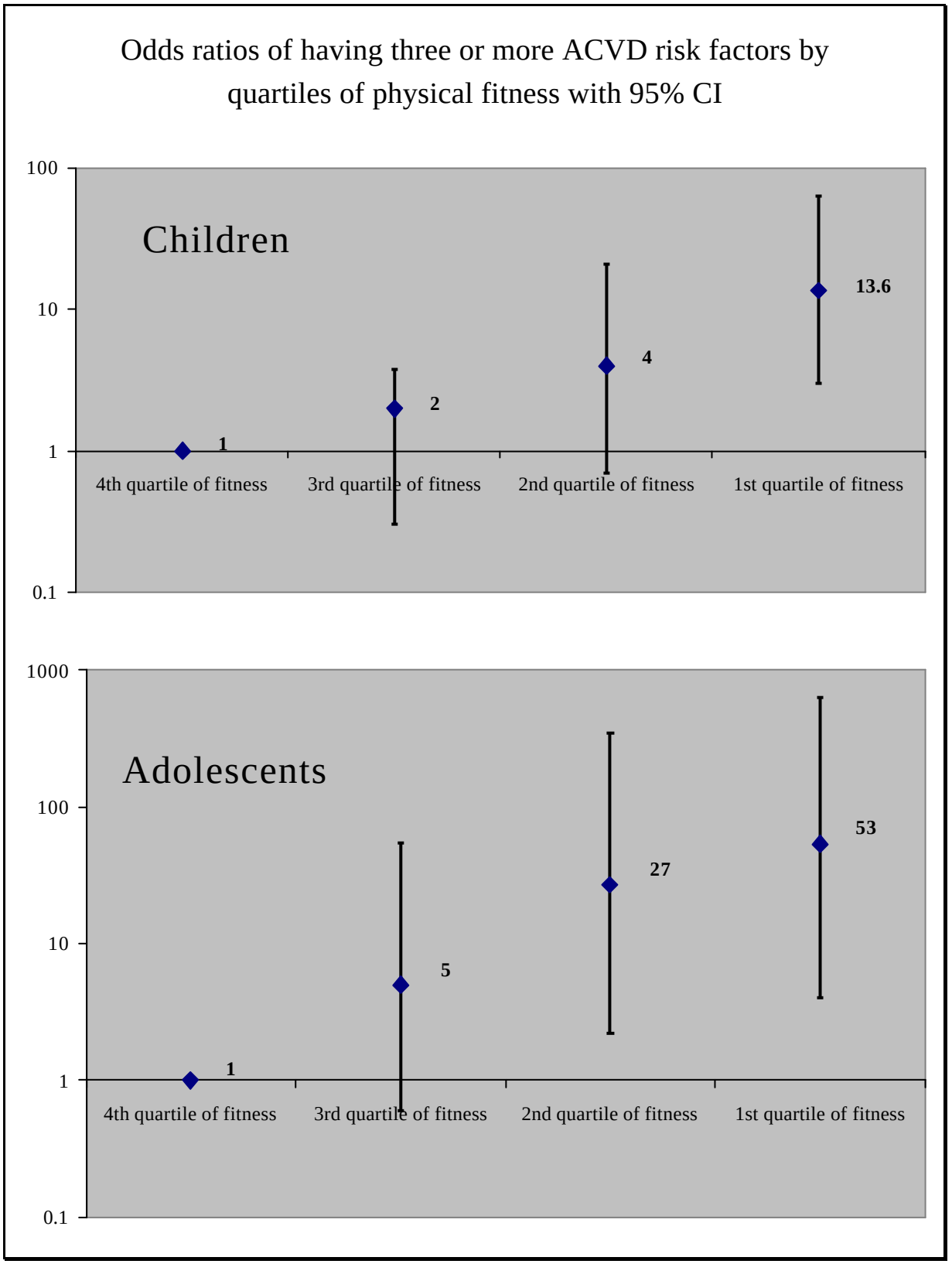

Odds ratios of having three or more ACVD risk factors by quartiles of physical fitness with 95% CI
Children
100
10
1
0.1
1
2
4
13.6
4th quartile of fitness
3rd quartile of fitness
2nd quartile of fitness
1st quartile of fitness
Adolescents
1000
100
10
1
0.1
1
5
27
53
4th quartile of fitness
3rd quartile of fitness
2nd quartile of fitness
1st quartile of fitness

Fig. 3a-b

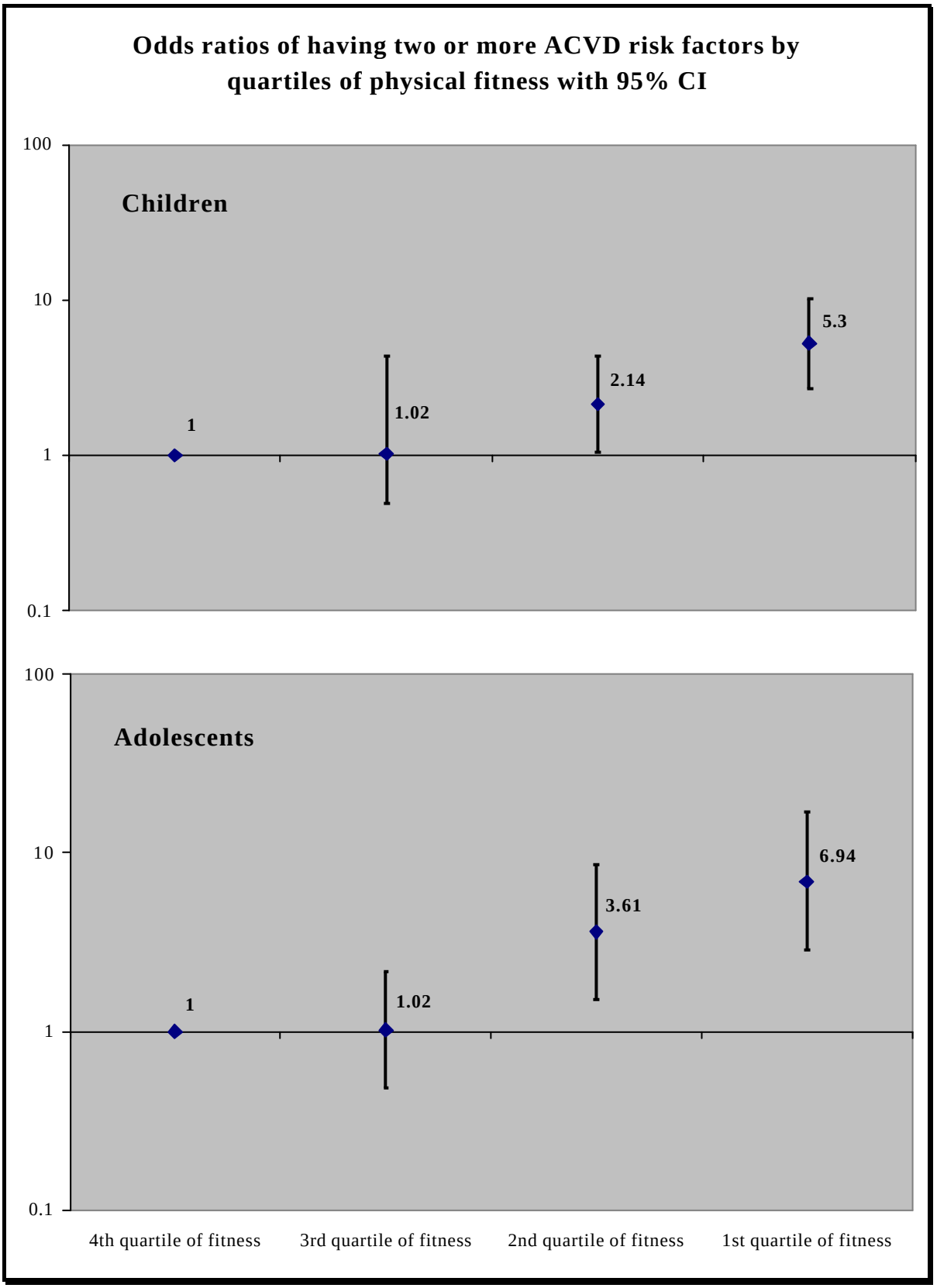
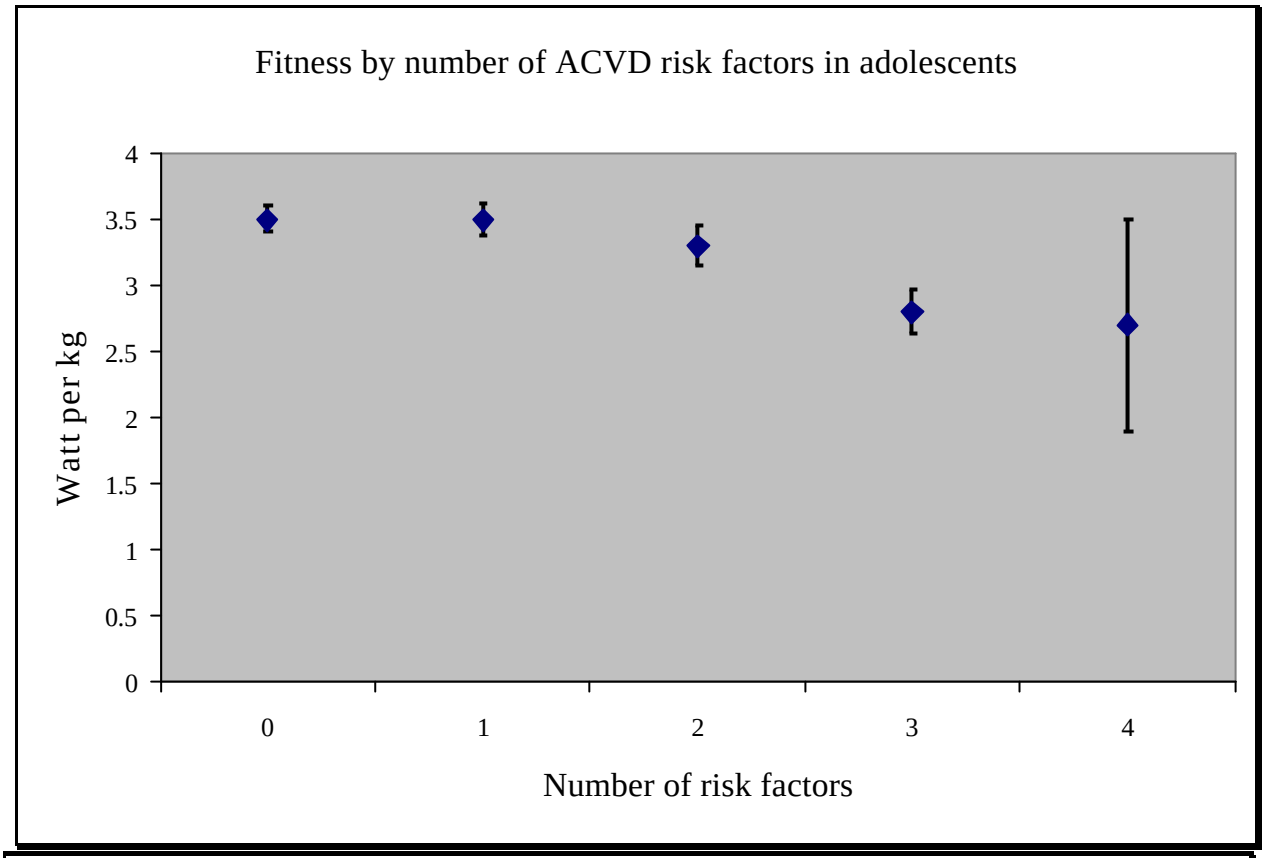
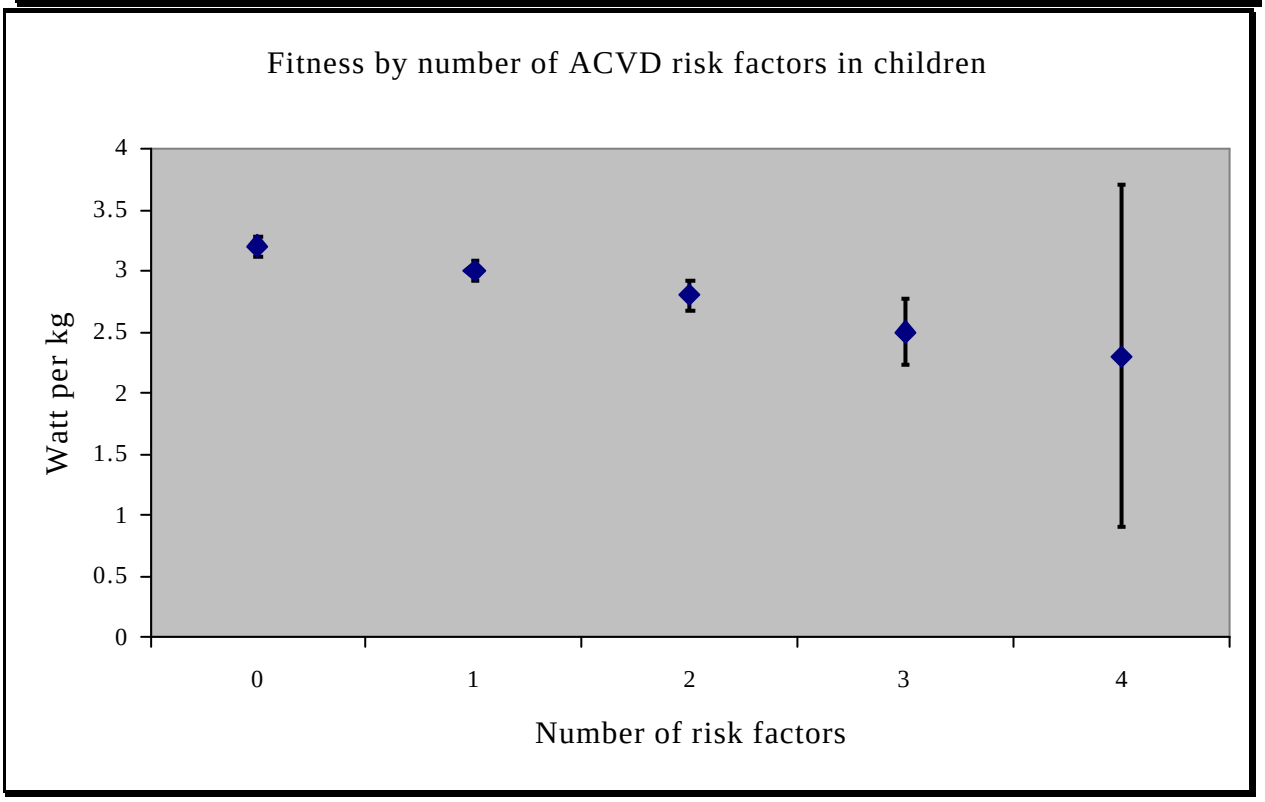

Odds ratios of having two or more ACVD risk factors by quartiles of physical fitness with 95% CI
Children
100
10
1
0.1
1
1.02
2.14
5.3
Adolescents
100
10
1
0.1
1
1.02
3.61
6.94
4th quartile of fitness
3rd quartile of fitness
2nd quartile of fitness
1st quartile of fitness

Fig 4a-b.


Fitness by number of ACVD risk factors in adolescents
Watt per kg
4
3.5
3
2.5
2
1.5
1
0.5
0
0
1
2
3
4
Number of risk factors


Fitness by number of ACVD risk factors in children
Watt per kg
4
3.5
3
2.5
2
1.5
1
0.5
0
0
1
2
3
4
Number of risk factors

7.4.2.3 Bivariate analysis of clustering of risk factors of the metabolic syndrome:

The cut points of the continuous variables used for evaluating the amount of clustering are shown in Table 2.

Significant clustering of risk factors of the metabolic syndrome was found in the lowest quartile of PF in children. A total of 40 (95% CI: 30 ; 51) children had clustering of risk factors where 12 were expected if the risk factors were not interrelated. No significant clustering was found in the three top quartiles of PF in children. The same pattern was found in the adolescents. Significant clustering was only found in the quartile with the lowest fitness, where 26 (95% CI: 18 ; 36) adolescents had clustering of risk factors, only 10 were expected.

7.4.2.4 Multivariate odds ratios of risk factors of the metabolic syndrome:

Risk factors of the metabolic syndrome clustered in children with the lowest PF

Fig 5a-b show the odds ratios for having three or more risk factors of the metabolic syndrome by the quartiles of PF with 95% CI. In children there were significantly higher odds ratios in the quartiles with lowest fitness than in the other three quartiles. In adolescents the quartile with lowest fitness had significantly higher odds ratios than the top two quartiles.

Fig. 6a-b show the PF by number of risk factors of the metabolic syndrome. There was a significant negative trend in PF with increasing number of risk factors of the metabolic syndrome in both age groups ($p<0.0001$).

Fig. 5a-b

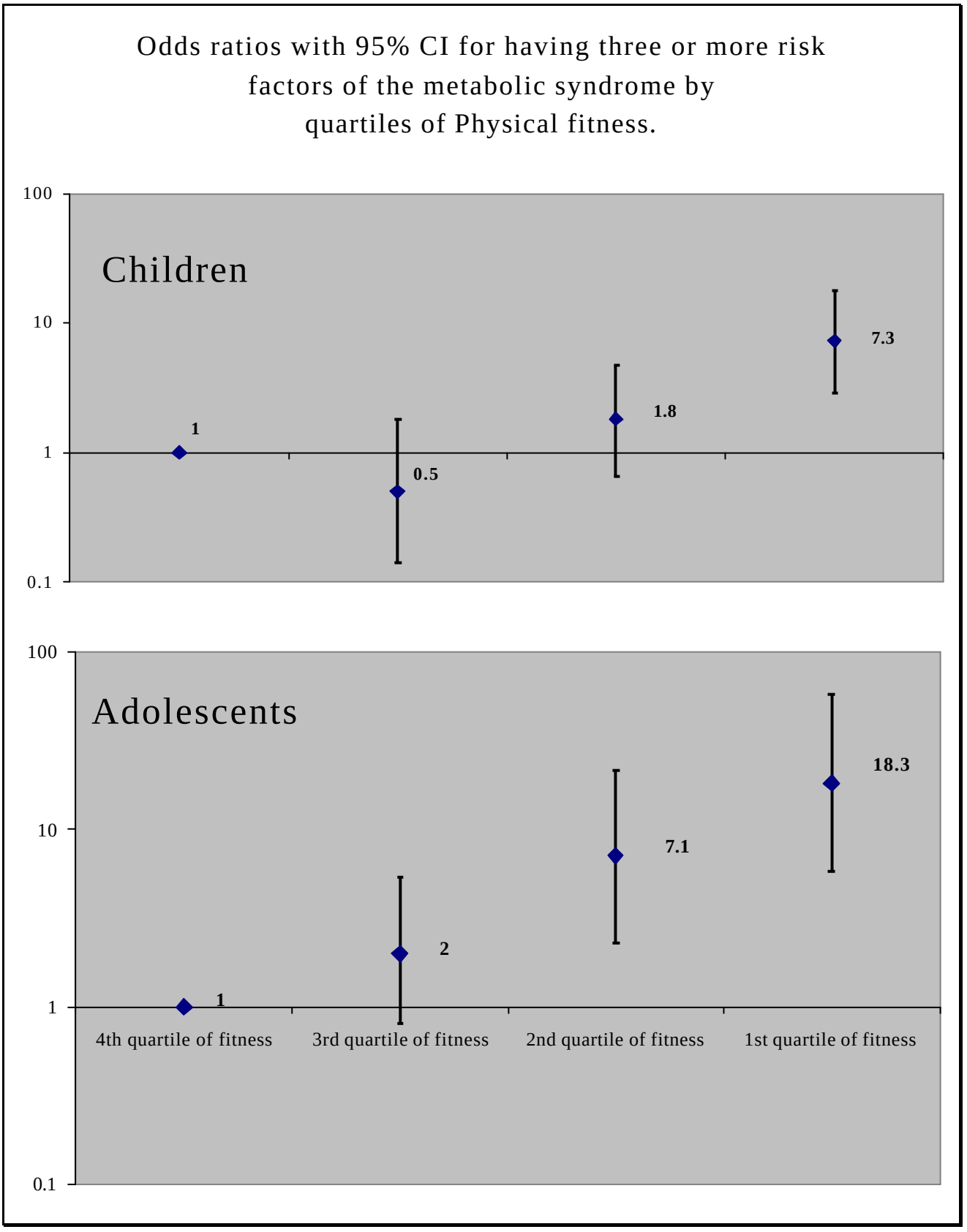
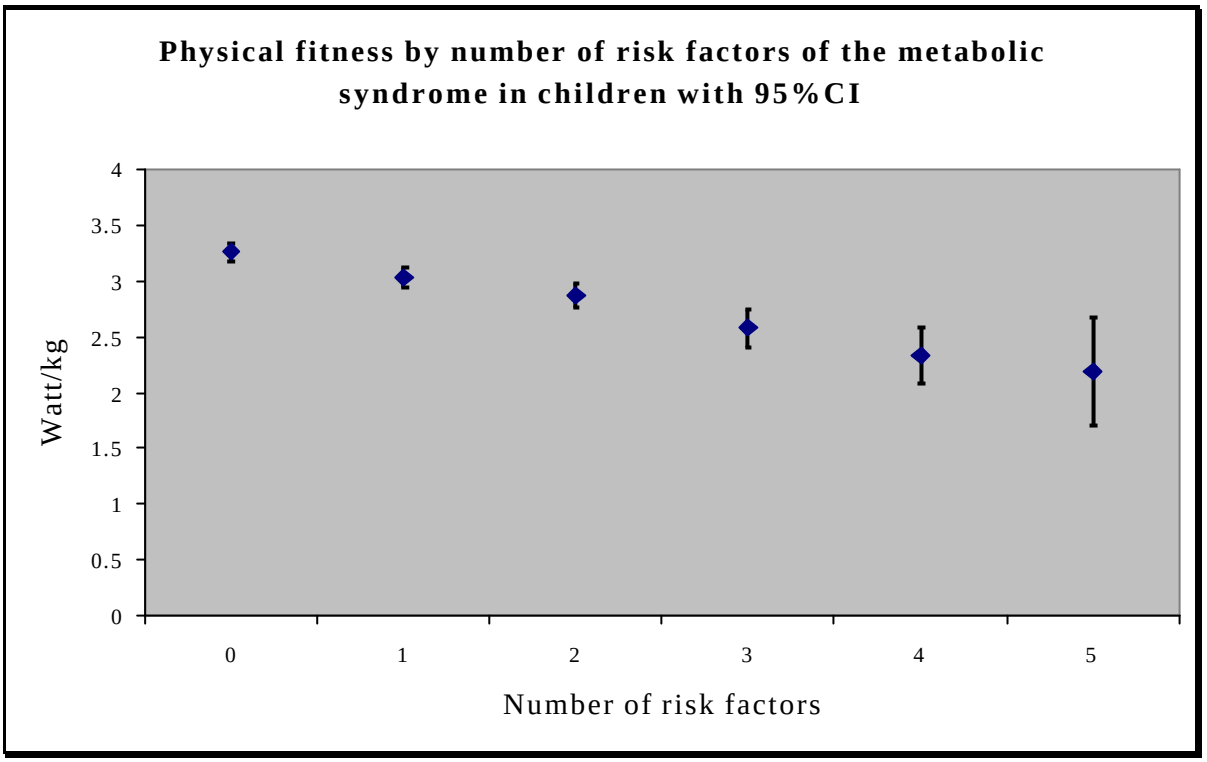
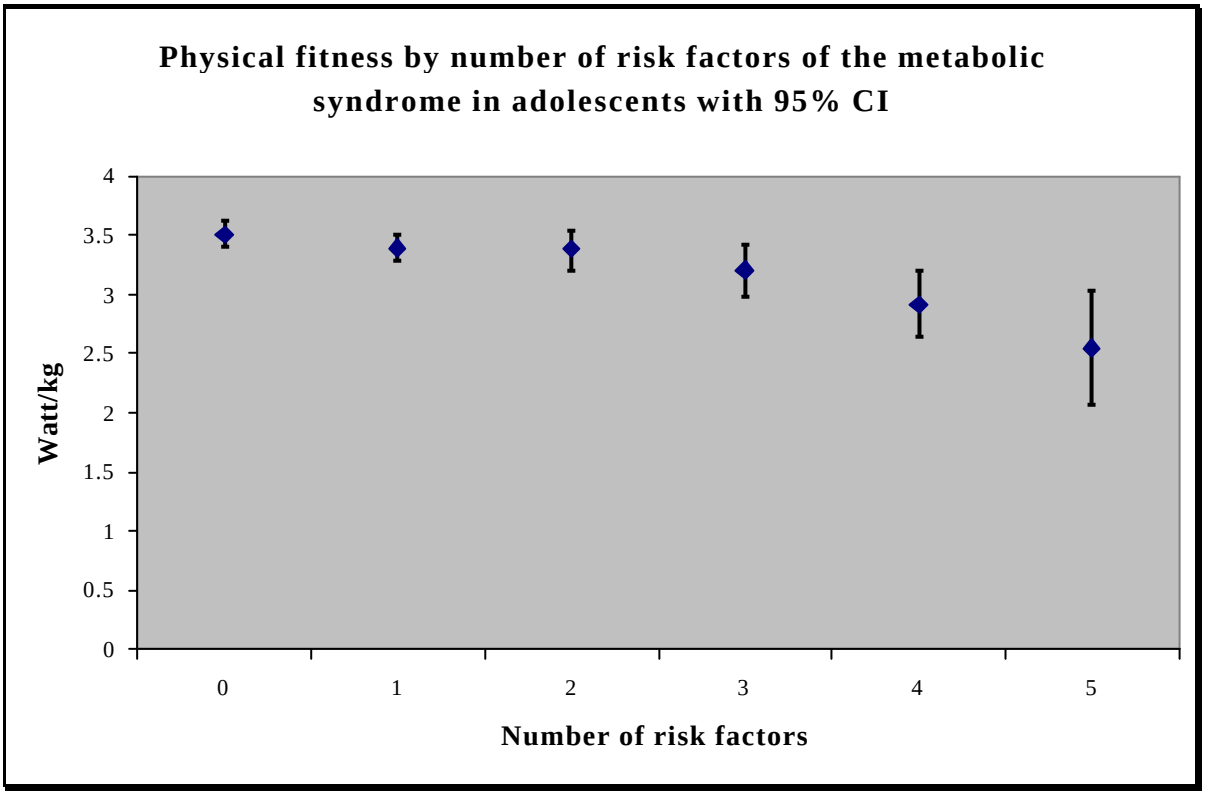

Odds ratios with 95% CI for having three or more risk factors of the metabolic syndrome by quartiles of Physical fitness.
Children
100
10
1
0.1
1
0.5
1.8
7.3
Adolescents
100
10
1
0.1
1
2
7.1
18.3
4th quartile of fitness
3rd quartile of fitness
2nd quartile of fitness
1st quartile of fitness

Fig. 6a-b



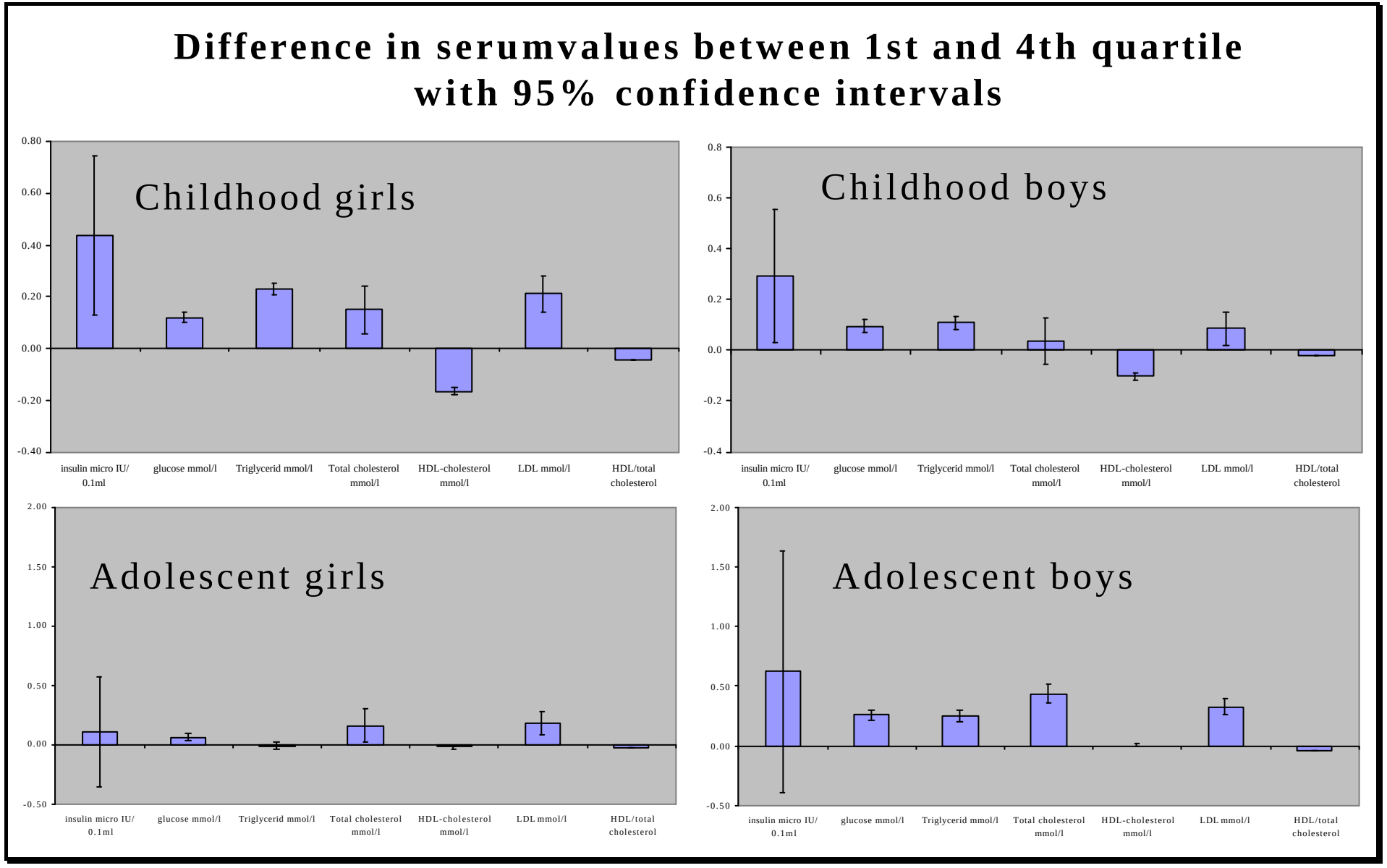


A significant negative trend in fitness by number of risk factors were found in both children and adolescents ($p<0.0001$).

7.4.3 Difference in risk factors by fitness: An age and gender specific significant positive trend by quartiles of fitness was found in the ratio between HDL and S-cholesterol. Significant negative trends were found for S-insulin, S-triglycerid, S-cholesterol, LDL and sum of four skin folds. A significant negative trend for systolic BP was found only in children. The trend in adolescents was also negative but not significant. No significant trend was found in diastolic BP.

The difference in serum values of the 1st quartile and the 4th quartile of PF is illustrated with a difference plot with 95% CI in Figs. 7a-7d. The differences in serum values between the highest and lowest PF quartiles of children were highly significant except in S-cholesterol (Fig. 7a and 7b). The differences for the adolescent boys were significant except for S-insulin and HDL. In the adolescent girls the differences were not significant for S-insulin, S-triglycerid and HDL (Fig. 7c and 7d).

Fig Fig. 7a-7d.


Difference in serumvalues between 1st and 4th quartile with 95% confidence intervals
Childhood girls
Childhood boys
Adolescent girls
Adolescent boys
insulin micro IU/ 0.1ml
glucose mmol/l
Triglycerid mmol/l
Total cholesterol mmol/l
HDL-cholesterol mmol/l
LDL mmol/l
HDL/total cholesterol

7.5 Secular trends:

7.5.1 Childhood girls:

7.5.1.1 Secular trends in fitness:

No significant difference was found in overall fitness of today compared to 12 years previously. But a different distribution was found. The top four deciles had a higher PF today and only the bottom four quartiles had a lower PF than 12 years previously (fig. 8a). The difference between the highest and lowest PF level had increased significantly.

7.5.1.2 Secular trends in obesity:

No overall difference was found in obesity, but the 2 highest deciles of 1997 had a significantly higher PI than the 2 highest deciles of 1985. The 7 lowest deciles of 1997 had a significant lower PI than the 7 lowest deciles of 1985 (fig. 9a). So the difference in PI between the highest and lowest PI level has increased significantly.

7.5.2 Childhood boys:

7.5.2.1 Secular trends in fitness:

The childhood boys had an overall significantly lower PF today than 12 years previously. However the lower PF was only evident, when comparing the lowest 7 deciles. The top 3 deciles had the same fitness as 12 years ago (fig. 8b). Thus the difference between the highest and lowest PF level has increased significantly.

7.5.2.2 Secular trends in obesity:

No overall difference in PI was found, but the 2 highest deciles of 1997 had a higher PI than the 2 highest deciles of 1985, and the 5 lowest 1997 deciles had a lower ponderal index than the 5 lowest 1985 deciles (fig 9b). Thus the difference in PI between the highest and lowest PI level has increased significantly.

7.5.3 Adolescent girls:

7.5.3.1 Secular trends in fitness:

The adolescent girls of 1997 had a significantly better overall fitness than adolescent girls of 1983. At the lowest deciles of fitness no significant difference was found, but from the second through the tenth decile the adolescent girls of 1997 had a better fitness level than the adolescent girls of 1983 (fig. 8c). Thus the difference between the highest and lowest PF level increased significantly.
Multiple regression showed a significantly higher fitness of adolescent girls in 1997-98 compared to 1983-84.

7.5.3.2 Secular trends in obesity:

An overall significant higher PI was found in the girls group from 1997. The group from 1997 had a higher PI in 4^{th} through 10^{th} decile. In the 1^{st} through 3^{rd} decile no significant difference was found (fig. 9c). Thus there has been an significant increase in the difference in PI between the highest and lowest PI level.

7.5.4 Adolescent boys:

7.5.4.1 Secular trends in fitness:

No difference was found in overall fitness in adolescent boys, but there were differences in the separate deciles. In the two lowest deciles the adolescent boys of 1997 had a significantly lower fitness than the adolescent boys of 1983. The third through the seventh deciles of the boys of 1997 were at a significantly higher fitness level, and at the eight through the tenth deciles the boys from 1983 had a significantly higher fitness level (fig. 8d).

7.5.4.2 Secular trends in obesity:

An overall significant higher PI was found in the adolescent boys from 1997. The group from 1997 had a higher PI in 2^{nd} through 10^{th} decile. In the first decile no difference was found (fig. 9d). Thus the difference in PI between the highest and lowest PI level has increased significantly.

Fig. 8a-8d.

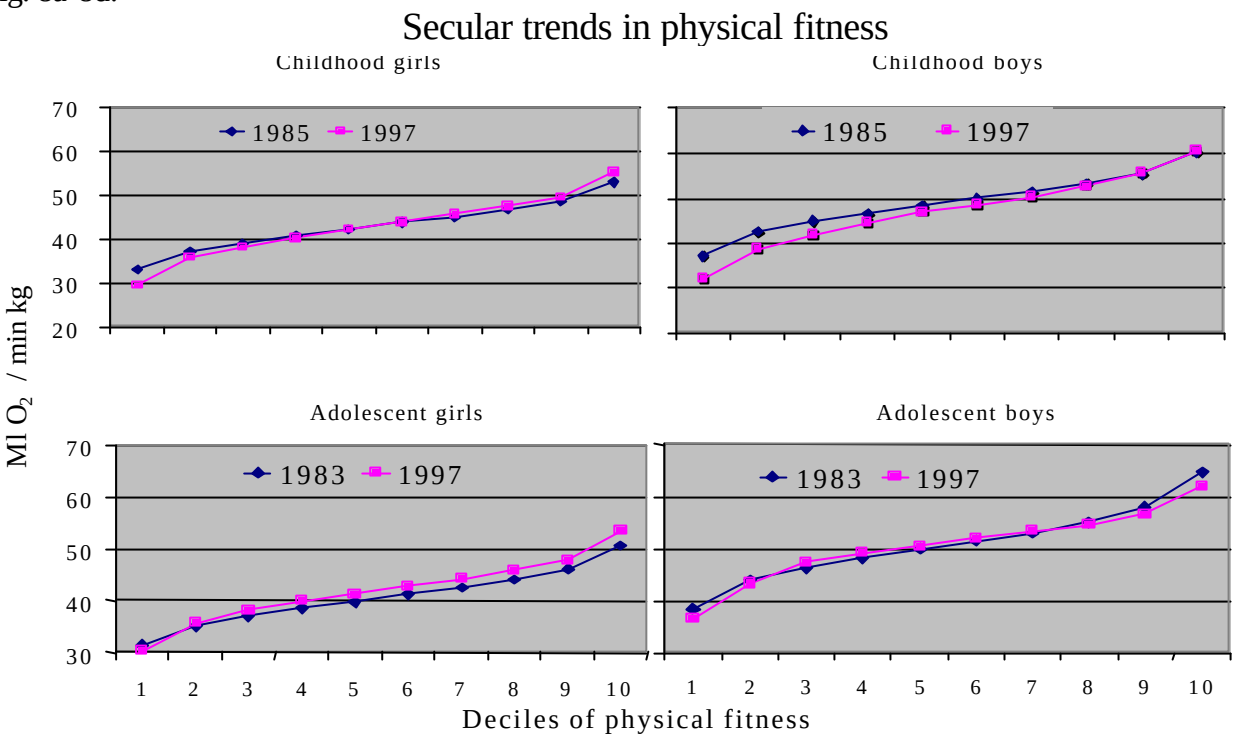
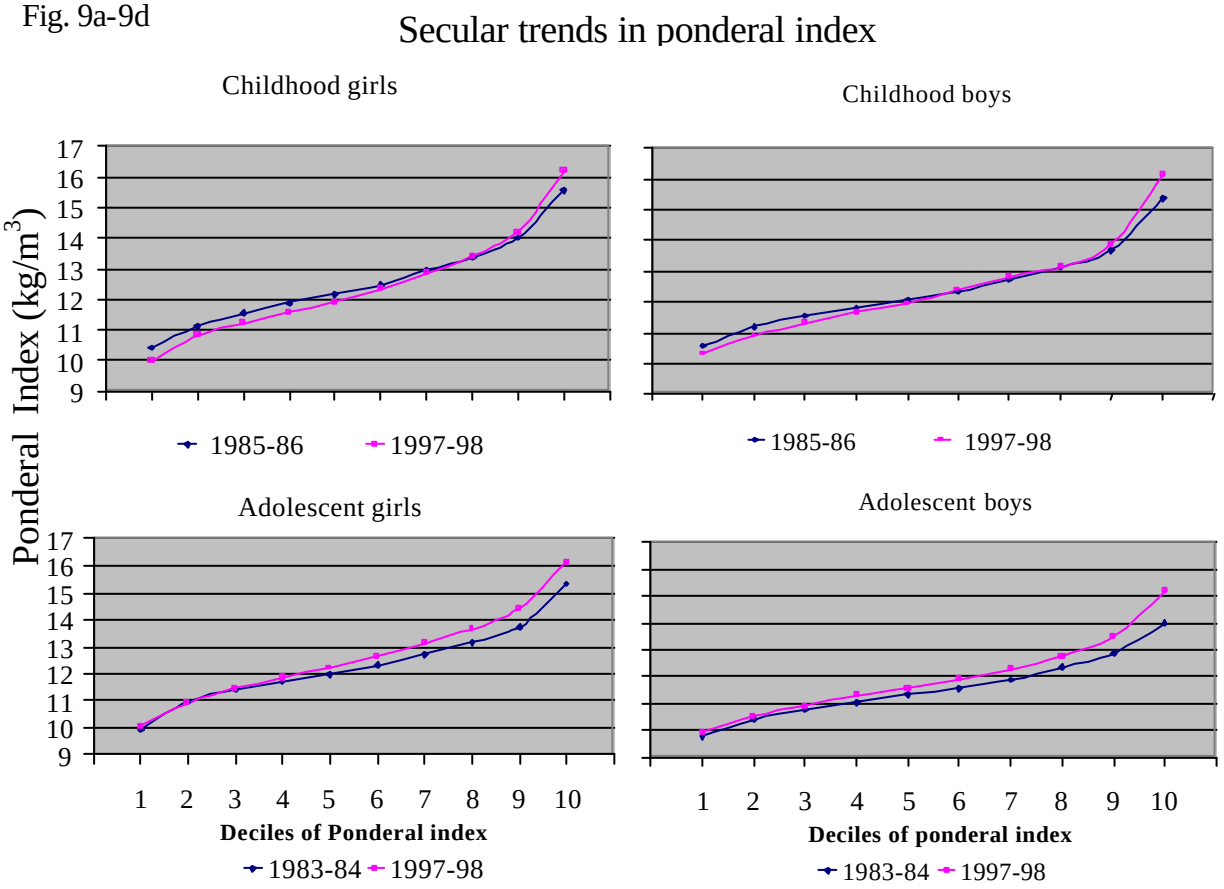


Fig. 9a-9d



8. Questionnaire obtained information:

8.1 Parents educational level:

A total of 1837 parents answered the questionnaire about educational level. The distribution of parental educational level differed from the total Danish population inside each group (fig. 10). A significant correlation coefficient of 0.96 ($p<0.0001$) between distribution of educational level in the study and the total country was found [66]. Although there is a tendency toward a higher educational level in the study, the trend toward a higher educational level is not significant ($p=0.174$).

The parental educational level was significantly negatively associated with S-insulin, S-triglycerid, and significantly positively associated with PF (table 8).

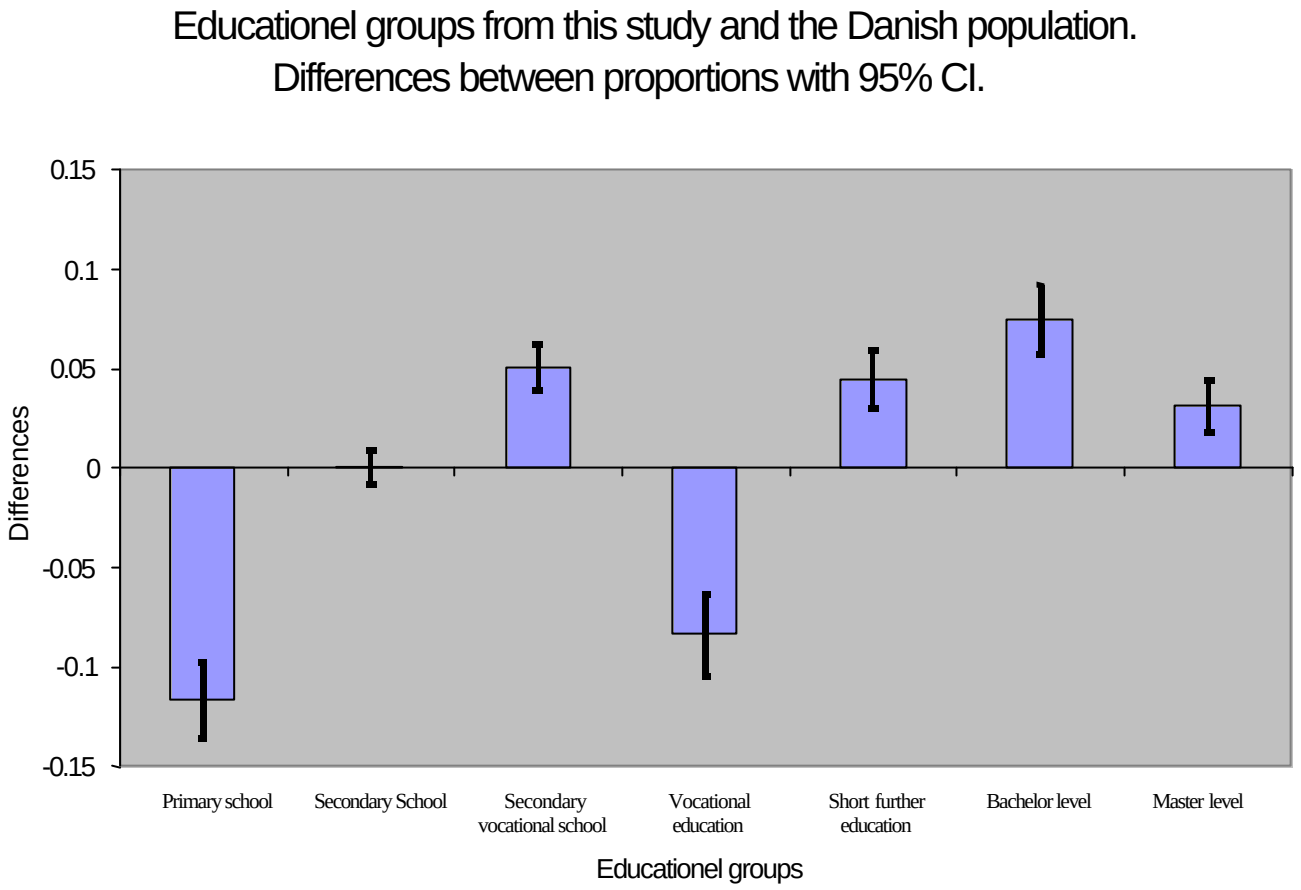
With the educational levels divided into three, and using the high level as reference level, a significant odds ratio for 3.0 (95% CI 1.5 ; 6.0) of having 3 or more ACVD risk factors was found for the group of parents who had the lowest educational level. The odds ratio for the middle level of education was 3.1 (95% CI 1.6 ; 5.9).

Table 8.
Statistical significant coefficients with 95% CI for educational levels. Risk factors not shown are not significantly associated to any educational level.

Dependent variable	Coefficient of middle educational level	95% CI	Coefficient of low educational level	95% CI
S-insulin	1.1*	0.04 - 2.2	1.2*	0.2 - 2.1
S-triglycerid	-0.01	-0.07 - 0.05	0.08*	0.01 - 0.14
S-cholesterol	0.13*	0.02 - 0.25	0.13*	0.01 - 0.26
Obesity	4.7**	1.2 - 8.1	4.3**	1.3 - 7.3

* $p<0.05$, **$p<0.01$.

Fig. 10.



8.2 Smoking:
All children and adolescents participating answered the questionnaire. No children were smokers. Of the adolescents, 39 (17.8%) girls and 25 boys (12.4%) were smokers.
The only significant regression coefficients for the association of smoking with any risk factors in adolescents was a coefficient of 0.14 (95%CI 0.03 ; 0.26) towards S-triglyceride. The odds ratio of having 3 or more risk factors of the metabolic syndrome was not significant (1.4 ; 95% CI 0.6 ; 3.0).

8.3 Inherited risk :
Distribution of parental disease is shown in table 7. A total of 1904 parents, representing 1004 children (98.4%), answered the questions on disease. Of these 216 had at least one atherosclerotic related diseases.

Table 7.
Atherosclerosis and atherosclerosis related disease in parents.

	Fathers	Mothers
Myocardial infarction	7	3
Stroke	4	4
Angina pectoris	10	5
Hypertension	90	104
Atherosclerosis	17	26

In children total parental disease was significantly associated to S-insulin (p=0.001), S-triglycerid (p=0.041), and diastolic BP (p=0.030) in multivariate analysis. Parental hypertension was associated to S-insulin (p=0.005), S-triglycerid (p=0.045) and diastolic BP (p=0.020) in multivariate analysis.
None of the other parental diseases were significantly associated to risk factors in children.
In adolescents parental disease was not significantly associated to any risk factors.
The odds ratio of having 3 or more risk factors of the metabolic syndrome was 1.6 (95% CI 0.8 ; 3.1) for total parental disease. No significant odds ratio was found for any single disease

9. Discussion:

9.1 External validity and quality of the selected measurements:

9.1.14 External validity of the study:

The external validity of the results is potentially biased by non-response. Non-responders often differ from responders in demographic characteristics and prevalence of risk factors [93]. Generally non-responders have higher levels of risk and are less likely to describe them selves as healthy [93;94]. The high response level of this study, together with a high response levels of the "Danish Youth and Sport Study" and "Odense Schoolchild Study", indicates that the results are valid and comparable. This is further supported by two things: First the majority were non-participants and not non-responders. the form masters had assumed the responsibility of getting the children and parents to answer. More than 97% of the children and parents answered one of the letters asking them to participate. Secondly; all the formmasters were asked if any of the non participating and non-responding children and adolescents differed in any way from the participating. Two subjects were reported to do so, one child was autistic and one adolescent was very obese and inactive.
We were not allowed to contact any of the non-participants. The permission from the ethical committee do not include a permission to contact the non-responding subjects in the study. It is possible that such a permission could have been acquired. But due to ethical reason and because of a reasonably large participation rate it was chosen not to apply for such a permission. With a lower participation rate a non-participant study would have been indisputable.

9.1.2 Quality of measurements:

9.1.2.1 Blood samples:

To minimise the variability in the serum values, the blood samples were drawn between 8 and 8:30 in the morning after an overnight fast. Through a very rigorous routine every morning, where the children were served breakfast after the sampling of blood, we made sure that no one had had any thing to eat at the school before the blood samples were drawn.
The analysis of the blood samples was performed at a certified laboratory, chosen by the EYHS scientific committee. At the time of decision only one certified laboratory was working in Denmark (the Clinical Chemistry dep. at Hvidover Hospital), and they could not provide all the planned analysis. Therefore the Department of Chemical Pathology, Bristol Royal Infirmary, Bristol was chosen as the central laboratory. They had experience in the planned analysis. They were certified and they had/have quality control routines that would ensure a very low between studies variance. The analysis were performed by established, validated and well controlled methods. The precision between batches of blood samples were high, the coefficients of variation were low for the analysis.
Much was done to ensure that the data would not only be valid on a group level, but if possible also one individual level. The blood samples were however made as single measurements, which makes it impossible to account for intra-individual variation. So all results have to be interpreted taking this into account,

especially when making conclusions on the individual level.

9.1.2.2 Anthropometric measurements:
All anthropometric measurements followed guidelines and was performed by trained personal. A high level of quality was achieved. Routine quality control during the study with control visits from the scientific committee were the tools for the achievement of high quality. The results with repeated measurements support this, e.g. a mean difference of less than one mm between the mean of 3 measurements and the single measurements were found in the measurements of hip and waist (99% CI -0.4 mm ; 0.5 mm). The same low level of error was found for the measurements of skinfolds. With the highest mean difference of 0.04 mm (99%CI 0.02 mm ; 0.06 mm). The very low intra variability of the measurements adds to the precision of the correlation's between the risk factors.

9.1.2.3 Blood pressure:
Blood pressure was measured using the Dinamap adult/paediatric and neonatal vital signs monitor, model XL (Critikron, Inc., Tampa, FL.), according to published guidelines for assessing blood pressure in children [80]. The use of an automated instrument avoided the potential observer variation associated with non-automated instruments. This was especially important considering that the international study is a multicenter study. The Dinamap machine also has been validated in children against direct radial artery readings and has a higher precision and smaller errors than the ausculatory method (mean error 0.24 systolic, 1.28 diastolic, 0.10 mean pressure) [79]. The measurements are therefore reproducible and valid for internal comparison. External comparisons are less valid, since most other studies have used the ausculatory method, and the machine has a tendency to underestimate especially the diastolic blood pressure compared to the ausculatory method [79].

9.2 Selection of risk factor indices and risk factors:
A risk factor index of five apparently non interrelated risk factors of ACVD in children was created to asses clustering of risk factors. The apparent non interrelation could be questioned, but in children no conclusive evidence was found concerning the interrelationship between the selected risk factors.
In addition a risk factor index of the metabolic syndrome was created too. Presence of the five risk factors obesity, dyslipidaemia, hypertriglyceridaemia, hyperinsulinaemia and hypertension are known as the metabolic syndrome [13;95;96]. The purpose was to asses the number of children and adolescents in risk of getting the metabolic syndrome. The mortality due to CVD is 40-50% in the general population, but 70-80% in the patients suffering from the metabolic syndrome or NIDDM, and these patients have a 3-4 times increased risk of getting CVD [30;97]. This makes the patients with the metabolic syndrome and NIDDM a main target group for treatment and prevention of ACVD, and make the decision to use these five risk factors in a clustering analysis logical from a clinical and preventive point of view, even though they are known to be interrelated.
The effect of clustering of risk factors is important, because the combined level of risk from more than one risk factor increases the risk by multiplication rather than just addition [71]. "A striking example is a male smoker with s-cholesterol above 6.2 mmol/l and a diastolic BP above 90 mmHg. He has over a six year period a 14 fold greater mortality due to coronary heart disease, compared to a non-smoking man with diastolic BP and S-cholesterol below these limits" [71].

Fasting serum insulin is an accepted indirect measure of insulin resistance [85;98;99], although currently there are no reference values for either adolescents or children.
Insulin facilitates glucose uptake, and the organ responsible for the majority (80%) of the glucose clearance is skeletal muscles [100;101]. The primary cause of fasting hyperglycaemia is

an elevated basal glucose production in the presence of hyperinsulinaemia. Whereas decreased insulin-mediated glucose uptake by muscle and impaired suppression of hepatic glucose production by insulin contribute almost equally to postprandial hyperglycaemia [102]. Hyperglycaemia provides a compensatory mechanism for the defect in nonoxidative glucose disposal in nondiabetic obese. However, this compensation is lost when overt NIDDM ensues [103].

The result of insulin resistance and hyperinsulinaemia is an increased synthesis and decreased clearance of VLDL and LDL. This leads to an atherogenic lipid profile with elevated VLDL and LDL, hypertriglyceridaemia and lowered HDL [104;105]. In addition subjects with insulin resistance often has a shift in the LDL sub-groups towards an increased level of small dense atherogenic LDL-particles [106]. An increase in lipid oxidation may contribute to the defects in glucose oxidation and nonoxidative glucose uptake in obese subjects with insulin resistance [103].

Insulin increases extremity blood flow in healthy subjects, whereas this is not the case in patients with NIDDM [107]. It has been shown that this sub-normal extremity blood flow in NIDDM patients can be improved by exercise and increased PF [100]. S-insulin also has a direct effect on blood volume by enhancing sodium retention in the kidneys [108;109]. Insulin in physiological doses activates the sympathetic nerve system through a central mechanism, persistent hyperinsulinaemia increases the activity of the sympathetic nerve system. This could be one of the mechanisms behind elevated BP.[109;110].

In epidemiological studies hyperinsulinaemia measured both as fasting s-insulin and 2 hours post prandial S-insulin has been found to be an independent risk factor or risk indicator of ACVD [111-113]. In a longitudinal study fasting S-insulin has been found to be an independent ACVD risk factor even when adjusted for other known risk factors [113].

Elevated fasting S-insulin could be considered a marker of insulin resistance and should therefore be considered either a key risk factor or a key risk indicator in the developing of atherosclerosis in both children and adolescents and of ACVD in adults [12;23;24;113;114].

LDL is an established risk factor of ACVD. dyslipidaemia with increased levels of LDL combined with low levels of HDL and high levels of triglyceride are regarded as one of the strongest indicators of increased risk of ACVD [115]. Several studies have reported differences in LDL particle size, density and composition between ACVD patients and healthy controls. Recent prospective studies have confirmed that the presence of small, dense LDL particles was associated with more than a three-fold increase in the risk of ACVD [111]. In addition studies including large epidemiological studies, have demonstrated that a predominance of small dense LDL is one of the constellation of interrelated risk factors that characterise the insulin resistance syndrome [116;117]. Cross-sectional and prospective studies have shown that small, dense LDL is a risk factor for NIDDM itself, and that this association may also be attributable to the metabolic syndrome [116]. This higher level of small dense LDL particles in metabolic syndrome and NIDDM patients may explain some of their increased risk of ACVD.

A large amount of clinical data indicate that lowering of LDL levels reduces the risk of cardiovascular events and mortality [29], and recent large studies have shown that reduction of LDL is beneficial, even in post-infarction patients with a relatively normal total cholesterol level [118].

Triglyceride has also emerged as a significant independent risk factor [26]. In addition levels of S-triglyceride has been found to track moderately, with levels in childhood and adolescence correlating significantly with levels in adulthood [119].

K. Kaas Ibsen et al [40] reported percentile values for a reference groups of school children in 1980. Unfortunately their blood samples were drawn without prior fasting, which makes direct comparisons with the present study difficult, in addition different chemical analysis

methods were applied making comparisons even more difficult. Even so all the fasting values from this study are at the level of the none fasting values of the former study from 1980. This could suggest that S-lipid levels have increased in children and adolescents.
The ratio of HDL and S-cholesterol is recognised as an important measure of ACVD risk. Low HDL and high S-cholesterol levels are independently important risk factors [26;120] and studies have shown the ratio to be one of the most important biological risk factors [26;121]. Furthermore, both S-cholesterol and HDL, and the ratio of the two track significantly from childhood through adolescence to adulthood [22;119]. For this reason the ratio has to be considered an important risk factor in childhood and adolescence.
Obesity is one of the ACVD risk factors most thoroughly described in the literature, relating strongly to ACVD in adults and nearly all known ACVD-risk factors in both children and adolescents [122-124]. Moreover obesity is a factor predisposing to the metabolic syndrome, insulin resistance, and NIDDM [23;125], and the trend of an increasing degree and prevalence of obesity observed in Denmark and most other countries in the western world is followed by a parallel increase in incidence of NIDDM [52;126]. As a recognised risk factor of several diseases strongly correlated to ACVD, it was therefore imperative to include obesity in any clustering analysis.
Hypertension has also been thoroughly described in the literature and it is one of the major risk factors for ACVD in adults [127], with the evidence suggesting that the association may be a causal one [128]. An elevated BP is also suggested to be part of the pathogenesis of nephropathia, retinopathia and neuropathia in subjects with insulin resistance and type 2 diabetes [129-133]. The tendency for BP measured during childhood to remain in the upper centiles and to an early indicator of essential hypertension in later life have been presented [6;38]. Evidence is accumulating, that the development of primary hypertension is the consequence of a process that begins early in life [36].

9.3 Metabolic syndrome:
The presence of the five risk factors obesity, dyslipidaemia, hypertriglyceridaemia, hyperinsulinaemia and hypertension are known as the metabolic syndrome [13;96] and the observation that individuals with metabolic syndrome have increased risk for ACVD [96;134], make the decision to use these five risk factors in a clustering analysis logical, even though the risk factors probably are interrelated[18;24;122]. But it is important from a clinical, epidemiological and preventive viewpoint to recognise the population with the metabolic syndrome and the childhood population with elevated risk of getting the metabolic syndrome.

9.3.1 The prevalence of metabolic syndrome in children and adolescents:
It is notable that by adult cut off points it is possible to diagnose the metabolic syndrome in 16 (1.6%) children and adolescents. The diagnose has to be viewed very carefully. It is not possible to diagnose the metabolic syndrome by just one blood sample. The intra-individual variation is too high. In addition all adolescents have started puberty, which induces alternation in insulin sensitivity and also changes fasting insulin levels [86]. Though this has been taken into account in the diagnosis, the cut point used is 14 ?IU, which is above the 95% CI of fasting S-insulin concentrations of 15 year old adolescents reported earlier [86], and just on the 99% CI of this study (mean 13.2 99% CI 12.4 ; 14.0). So even if it was an uncertain diagnosis, 16 children and adolescents had risk factor values, which in a clinical setting would have given them the diagnosis "Metabolic syndrome", if the values had been confirmed by one more measurement of the risk factors.
The results of the study on children of parents who died early of ischaemic heart disease by K. Kaas Ibsen [135], showed that first measurements of elevated S-lipid values could be repeated in 68-87% of the children, and support that most of the 16 subjects with clinical meta-

bolic syndrome actually have the syndrome. However longitudinal studies are necessary to evaluate this.

9.3.2 Consequences:
The consequences of the above described metabolic abnormalities are often serious. Insulin resistance and NIDDM are well known diseases associated with the Metabolic syndrome [97;136;137]. Subjects with NIDDM have a range of complications related to the micro and macro vascular changes from atherosclerosis, these include 3-4 times increased risk of ACVD [112], diabetic nephropathia [130;131;138], neuropathia [132;133], and retinopathia [132]. Moreover ACVD is the most common cause of death in patients with the Metabolic syndrome and NIDDM, with a 2 times higher rate of cardiovascular mortality than the normal population. [85;112].

9.4. Clustering tendencies of risk factors in children and adolescents:
Several ACVD risk factors in children and adolescents have been assessed. A number of studies have looked at either single risk factors or combinations of risk factors, and some have looked for clustering tendencies [123;139;140]. It is known that having multiple ACVD risk factors increases the risk of getting ACVD [95;96;141].
Neither the risk factors of the ACVD risk factor index nor the biological risk factors of the metabolic syndrome clustered more than expected in the total population. It is not so surprising that the ACVD risk factors do not cluster, but considering the amount of evidence confirming that an interrelationship exist between the risk factors of the metabolic syndrome it is very surprising that no clustering was evident in this study in the total population. It could be because of the chosen clustering definition, with clustering defined as three or more risk factors out of five. Some of the studies on clustering of risk factors in children and adolescents which have been able to show clustering, used only three risk factors to determine clustering [38;142].

9.5 Physical fitness:
9.5.1 Association between physical fitness and risk factors:
The present epidemiological study on children and adolescents confirms the findings from previous studies on adults [36;42;57;143;144]. PF is associated with individual cardiovascular risk factors, and in the subjects with low PF a significant clustering of risk factors from both indices was found. This together with the results of the multivariate analyses, with physical fitness as the variable with the best association to the biological risk factors in children and with highly significant increased odds ratios for having 3 or more risk factors in both children and adolescents with low PF, suggests that the interrelation and clustering of the biological risk factors can be explained by a low PF, associated with decreased insulin sensitivity and obesity. The question is what comes first and what is the cause. The fact that both S-insulin and obesity are the two factors most closely related to PF suggests that a "common cause" could be low physical activity. Clustering is a relatively uncommon feature, and the odds ratios are therefore probably equal to the relative risk of this low fit group of children and adolescents.
The cause of increased risk of clustering of non-biological risk factors in subjects with low PF could be social factors, smoking or unhealthy habits which are known to cluster in adolescents from families with low socio-economic status [17].

9.5.2 Difference in risk factor levels by physical fitness :
In both childhood boys and girls significant differences between the groups with the lowest and highest degree of fitness were found in all measured S-levels except S-cholesterol in boys. The children with the lowest fitness had unfavourable values compared to the children with the highest values. Similar results were found in adolescents, although especially a large difference in LDL was present in both genders. It is also important that significant trends toward

unfavourable values of all S-values were found in all gender and age groups.
It is known that serum insulin is highly dependent on age, puberty stage, and gender [21;145]. The differences between the fitness groups in the children, were not age, gender or puberty dependent. However in the adolescents the variation was large probably because of puberty and the results have to be interpreted with this in mind [86;146].
The difference between the high and low fit categories together with the significant trend toward unfavourable values in the least fit, could be a sign of a beginning insulin resistance in children and adolescents with low fitness. This could result in an increased synthesis and decreased clearance of VLDL and LDL, which would lead to an atherogenic lipid profile with elevated VLDL and LDL, hypertriglycidaemia and lowered HDL [104;105]. In addition a shift in the LDL sub-groups towards an increased level of small dense atherogenic LDL-particles has been reported and could be evident in the low fitness group [106].
The well fit children and adolescents probably have an increased capillarisation and metabolic capacity of their muscles resulting from a higher level and intensity of physical activity as described by Saltin et al in adults [100]. High physical activity with an increase in fitness has been shown to increase the HDL/LDL ratio, by increasing HDL and lowering LDL, probably due to an increased amount and activity of lipoprotein-lipase [100]. If the high fitness group maintains their level of fitness an increased capillarisation of the muscles induced by the exercise they perform, probably will decrease or maintain a low BP [147].
Some of the subjects with low fitness have a highly increased risk of getting NIDDM in young age.
The sub-normal extremity blood flow in NIDDM patients can be improved by exercise and increased PF [100], and at the same time the hyperinsulinaemia can be totally or partly normalised by exercise [148]. These facts makes the obese children with low fitness a main target for preventive strategies.

Systolic BP was not associated with PF when assessed by multiple regression in either children or adolescents. This could be explained by the fact that the association was too weak to show with the number of subjects in the study, or because the relationship is not linear but curvilinear, with a negative trend in subjects with low fitness and no trend in subjects with high fitness [149]. Though curvilinear relationship was not investigated, because the statistical premises to perform the analyses was not apparent.
The association between serum lipids and PF in children is supported by other studies in both adolescents and children [150;151], in prospective controlled trials it has not been possible to show the association [152]. The studies performed generally lack sufficient statistical power [151;153] and "empirical dose-response data relating the effects of physical activity to blood lipids in children and adolescents are non-existent" [151].
Obesity was the risk factor with the best association with PF in both children and adolescents, which is in accordance with other studies [44;45;150]. This together with the close interrelationship between obesity and S-insulin, PF and S-insulin, also supports the theory of low physical activity as the explanation of high risk factor levels.

9.5.3 Importance of type of fitness test with a reliable measure of fitness:

Several tests of PF exist, but the most precise measure in children and adolescents is a direct measurement of VO_2max by the Douglas bag technique [154]. But this is not applicable in epidemiological studies, where the attempt is to evaluate the maximal aerobic power on a large number of subjects. A good field test with a high correlation to VO2max is required to reduce the degree of error. Most of the field tests consist of maximal running tests covering several distances or differing in duration. One of the best known is the Cooper test [155], but the correlation of the Cooper-test with VO_2max found in the literature is ranging from r=0.20 to r=0.90 [156]. One reason for these differences

is that test performance is very dependent upon subject motivation ad running experience [154].
An indirect maximal fitness test with a good correlation with directly measured VO_2max is the best alternative for epidemiological research of youth. The associations between risk factors and fitness estimated by sub-maximal fitness tests are questionable, because the R^2 of the estimation from sub-max tests and the true VO_2max is approximately 0.42 [157]. The R^2 for the indirect max tests range from approximately 0.81 to 0.90 [84];[82].
The strength of this study is the large number of randomly sampled children, who probably are representative of the general Danish population of these age groups. Very few studies have included so many children performing maximum PF-tests [46;158]. Furthermore the associations observed previously between risk factors and fitness may be weak, because fitness was estimated using sub-maximal tests. In addition the reproducibility of the sub-maximal tests are low [159]. Correlation as low as 0.38 between test and retest in sub-maximal tests have been reported, even if standard procedures were used [159]. This low reproducibility and validity of the sub-maximal tests in estimating VO_2max may be the reason of much of the unexplained variance in risk factors, when a poor VO2max estimation is used. The R^2 of the estimation of the true VO_2max from the indirect maximal PF test was 0.86 for children and 0.92 for adolescents in the present study and the correlation between test and retest was 0.95, which is comparable to what has been found previously by Hansen et al[82] and Andersen et al. [82;159].

9.6 Secular trends:
9.6.1 Secular trends in fitness:
Secular trends of PF have only been described by Dollman et al. [48]. They reported a significant lower PF today compared with the eighties. They used a different test, the 1 mile run/walk test to estimate VO_2max, and as they also stated themselves, the reproducibility of the mile run/walk test is poor. An improvement of 37-48 sec. has been reported over 3 trials with in of two weeks, presumably due to improved tactical awareness rather than training effect [160]. In a validation study Rowland et al. found that the "1- mile run performance in children may not serve as a strong indicator of cardiovascular fitness" [161]. With this in mind, Dollman et al still found a lower fitness in 10-11 year old children of today of the same magnitude as was found in the 8-10 year old children in this study and also a trend toward a greater difference between the low fitness and high fitness groups, creating a polarisation of the children [48].
The negative trend of PF of the children with low PF in this study is put into perspective by the positive trend in PF of the adolescent girls. The positive trend could be questioned because of the difference in age distribution between the EYHS and "Danish Youth and Sport Study". But the difference was still significant after adjustment for age and obesity. The adolescent girls could be looked upon as childhood representatives of the late eighties, who has continued their positive trend in PF up through the nineties as adolescents, thus emphasising the difference between children of the nineties and children of the eighties. However only time will show if this negative trend in PF will be continued. PF only tracks moderately and a change is possible. A follow up in due time can show if the trend is persistent.
The low fitness of the children is set into perspective by the fact, that the PF level of the children of the lowest deciles in this study is as low as the fitness of blind children, who because of their handicap do not engage in any vigorous physical activity, and therefore have a very low PF level [162].
No up to date study regarding secular trends in fitness of adolescents was found. A search in the databases Medline and Sports Discus, with the terms fitness and adolescents together with trends, tendency, tendencies and evolution was performed. This emphasises the necessity of this type of study, because it describes the trends and developments in society, whereas longitudinal studies describes the developments

inside persons which is also important. But as society changes, habit changes and risk factors changes. This is best described by studying secular trends of the risk factors in repeated cross-sectional studies over time. Thus this type of studies will indicate, what kind of preventive strategies should be applied and in which population groups they should be implemented.

9.6.2 Secular trends in obesity:
The measure of obesity used in the secular trend analysis of this study, has been validated by the Bogalusa Heart Study for use in longitudinal studies and trend studies. It has been shown that the ponderal index relates better to future levels of risk factors than the body mass index (kg/m^2) [3;34;163]. The increase in prevalence and degree of obesity have been found in other countries too [48;164] and have been suggested to be a growing problem [56]. Obesity tracks and obese children and adolescents run a greater risk of being obese adults than non obese children and adolescents [56;165]. In addition the increase in obesity follows and correlates highly with the increase in the number and magnitude of ACVD-risk factors [166].
Thus the high correlation of obesity with ACVD [167], and the clustering of risk factors in the obese [15;122;139], makes it imperative to follow, to attempt to control and lower the number of obese subjects and the degree of obesity in the obese. Especially because of the increase in both the degree of obesity and the number of obese in both Denmark and other countries [51;52;123]. In this study both a significantly larger number of obese children and adolescents than previously were found and a significant increase in degree of obesity (fig 8a-8d). This is in agreement with previous findings in Danish adults [51;52].

9.7 Importance of obesity and low physical fitness as risk factors for ACVD:
Obesity and low PF are important ACVD risk factors [6;8;15;122]. PF has been used as a proxy of physical activity in children [46], but PF might by it self be an important independent factor associated with ACVD-risk factors in both children and adults [168;169]. This relationship has been shown in adults, where there is a clear negative relationship between PF and ACVD regardless of body composition. It has even been suggested that being fit may reduce or totally remove the hazards of obesity [170-172].
Blair et al also describes overweight and other risk factors adjusted by fitness in adult men as; "men who maintained or improved adequate PF were less likely to die from all causes and from cardiovascular disease during follow-up than persistently unfit men."[42;172;173]. In the present study fitness was the factor, which had the highest association with other ACVD-risk factors in children and the factor with highest correlation to obesity in both children and adolescents. In addition children and adolescents with low fitness had highly increased risk of having clustering of risk factors. These findings are in accordance with the findings of Blair et al in adults [174].
Obesity tracks highly and fitness tracks moderately [33;49], further new data suggests that obesity in adulthood that became established in childhood may be more harmful than obesity that has appeared in adulthood. The possible mechanism could be that the continuos obesity from childhood to adulthood generates prolonged insulin resistance, with the result of clustering of risk factors for the metabolic syndrome and ACVD[20].

9.8 The role of improving PF:
It seems as if high fitness protects against ACVD in adults [170].[172;175;176] In the present study PF was associated with cardiovascular risk factors in both children and adolescents (table 6). This suggests that PF is a parameter, that should be monitored during childhood in order to apply preventive strategies if the fitness level is low.
Previous studies have shown that controlled exercise such as physical aerobic training in untrained children improves PF [154;177] and lowers the BP in adolescents and childhood boys [177;178]. High intensity physical activity

increases the fitness by increasing VO_2 [179] and total physical activity has been shown to reduce obesity [150;180]. One of the effects of exercise and PF on BP is probably mediated through a lowering of the activity of the sympathetic nervous system independent of any weight loss [23;181]. However it could be speculated, that if fitness is a proxy of regular training or a certain amount of habitual high intensity physical activity, then the explanation of the increase in obesity, in number of obese subjects, subjects with NIDDM and metabolic syndrome, is a low or non-existing amount of high intensity physical activity in these subjects. This is supported by the findings in adults by Dela et al [148]. They found that "physical training increases insulin sensitivity in skeletal muscle and increases extraction of insulin in everyday life, resulting in a lower plasma insulin concentration[148]. These findings are achieved in both normal subjects and in NIDDM patients[148]. Dela et al. concludes, that "physical training does not cure the disease (NIDDM), but it should be an integral part of the treatment" [148]. However it is necessary to continue the high intensity physical activity regularly, because the effect of it seems to disappear within a week in NIDDM subjects and shortly after a week in normal subjects [148].
In the "normal" subject moderate intensity training (jogging, biking or brisk walking) increases the capillarisation of the muscle and the metabolic capacity of the muscle [100]. This has shown to increase the HDL/LDL ratio [100]. The increased capillarisation of the muscles induced by exercise has also been shown to bring about a fall in BP [147]. This is in accordance with the study by Hansen et al in children [177].
Rowland et al suggests that “a value of VO_2max per kilogram is as likely to provide insight regarding a child’s body composition as his cardiovascular fitness” [182]. If VO_2max per kilogram is as much a measure of body composition as of cardiovascular fitness in all people, children, adolescents and adults. This, together with a higher insulin sensitivity and a decreased action of the sympathetic nervous system, would explain why PF is associated with nearly all ACVD risk factors in children, as well as it would explain the same findings in adults [13;42].
The PF level of the children and adolescents with low fitness is comparable to that of blind children and adolescents [162]. One of the reasons of the increase in obesity and the decline in PF in some children might be the "American paradox", "a reduced fat and calorie intake and frequent use of low-calorie food products associated with a paradoxical increase in the prevalence of obesity. These diverging trends suggest a dramatic decrease in total physical activity related energy expenditure" [183]. Therefore the engagement in any kind of physical activity, would probably increase the PF of the children and adolescents with the lowest PF level and reduce their obesity.
By controlled exercise with high physical activity, it would be possible to increase the PF level, and lower the levels of risk factors such as obesity and blood pressure in the children and adolescents at risk [150;177].

9.9 Inherited risk, educational level and smoking association with risk factors:
9.9.1 The educational level, association with risk factors and physical fitness:
The educational levels of the parents were associated to major risk factors and clustering of these risk factors in this study, and these findings are supported by studies from other countries. Most studies have assessed the ACVD risk factor profile of adults, although some studies have analysed the relation to parental socio-economic status in children and adolescents [17;64;184].
People with low level of education, low income and living in lower socio-economic areas, often have the most unfavourable risk factor profiles, and they have an increased vulnerability to ACVD [185;186], and a higher total mortality [63;187]. However people with low socio-economic status also have a high risk life style [62;188], and in young adults the influence of parental education is still connected to levels of risk in young female adults [188]. The meta-

bolic syndrome is also strongly correlated to socio-economic status [189], which could explain some of the social inequalities in ACVD risk. When considering children and adolescents, then ACVD risk factors have been found to cluster in adolescent girls from families with low socio-economic status [17], and found to be associated to a range of ACVD risk factors [17;64;184].

9.9.2 Smoking:
No children smoked, but 39 (17.8%) adolescent girls and 25 (12.4%) smoked. Smoking was only significantly associated to one risk factor (S-triglycerid). The odds ratio for the smokers to have three or more ACVD risk factors was 1.4, showing a tendency of clustering of risk factors in smokers, even though the odds ratio was not significant. This could be a type B error, to few subjects. The number of adolescents smoking is lower than earlier reported. In 1983 39% of the male and 32 % of the female adolescents smoked [33], compared to 12% and 18% today. This fall in number of smokers generally was expected. However a change from a higher proportion of the smokers being males towards a higher proportion of the smokers being female are evident. This change could be one of the factors responsible for an increasing percentage of the subjects getting ACVD in the future being women..

9.9.3. Inherited risk:
One-hundred and eleven male and 119 female parents had had some kind of ACVD diagnosed. Several studies have found associations between parental disease and childhood risk factor levels and later morbidity [73;190]. In this present study parental disease was significantly related to several risk factors in children but not in adolescents. In addition a significant odds ratio for having 3 or more risk factors was also found in children but not in adolescents. These findings that no single parental disease was significantly related to risk factor levels in adolescents could be because of too few participants. A larger epidemiological study or a case control study with children of parents with a disease as cases and children of healthy parents as controls would be a better approach to detect differences between such two groups. None of children and adolescents in this study came from families with known genetic disorders e.g. familial hypercholestrolaemia, or combined hyperlipidemia. These rare monogenic genetic disorders are related to very high incidences of ACVD in affected subjects. The majority of individuals with increased levels of ACVD risk factors are poly-genitically affected in combination with environmental contributions such as low physical activity and high energy diet.

10. Comparable studies:
Even though no significant clustering of risk factors was found in the total population in this study, the results of other studies, e.g. Bogalusa Heart Study, Cardiovascular Risk in Young Finns Study, Umeå Heart study, and a recent local study, Odense Schoolchild Study, contradicts this [17;18;38;122;142]. Even though the same strategy for defining clustering was used by the above cited studies, other combinations and number of risk factors were used. This could explain why the other studies found significant clustering of risk factors in children and adolescents, which was not found in the total population of the present study. However other studies did find an interrelationship between the risk factors [38;39] and fasting S-insulin has also been found to precede the development of a potentially atherogenic risk factor profile [24].
This indirectly supports the results of this study. The clustering of risk factors and unfavourable ACVD risk factor levels in subjects with low PF, indicates that it is low physical activity acting through low PF, obesity and decreased insulin sensitivity, which has an impact on ACVD.
The multivariate relationship between PF and ACVD risk factors have not been shown in children before. The bivariate clustering of risk factors, the high multivariate odds ratios of having clustering of risk factors and increased levels of biological ACVD risk factors in the

children and adolescents with low fitness have not been shown before. Previous studies either have not been large enough to show the relationship when adjusting for confounders [44;45],or they have used either sub-maximal fitness tests [44;45] or fitness tests that were not reproducible [160;161;191]. In addition most studies focused on one or two risk factors, usually obesity and BP and not a range of risk factors [46;169]. Though some studies do support the protective effect of PF and physical activity on some risk factors in children and adolescents [177], none have shown the highly increased odds of having clustering of unfavourable levels of biological ACVD risk factors. No doubt about the effect of PF and physical activity on biological ACVD risk factors in adults exist [172;176;192;193].

The increased obesity in children and adolescents today compared to earlier have been shown by other international studies [48;56;126], though no Danish studies have looked at the problem coming from the eighties into the late nineties. The increasing obesity and increasing number of obese children and adolescents in Denmark suggesting a quickly growing obesity problem will have to be confirmed in future studies.

The secular trends in PF has not been shown by any other Danish study, and the only international study by Dollman et al [48] showing a secular trend in children used a test with very low reproducibility and low association with VO_2max. Even so they found results similar to the present study. In adolescents no previous studies have been found.

11. Conclusion and recommendations:

The major findings of this study were that in children and adolescents, an association between physical fitness and ACVD risk factors exists, and that subjects with low fitness have a high risk of clustering of ACVD risk factors. Furthermore, children with low physical fitness of today have a lower physical fitness than children 12 years previously and a greater difference between children in good shape and children in bad shape has emerged. There is also an increasing number of obese children, and a tendency for the obese children to be more obese today than 12 years earlier. This negative trend in and polarisation of physical fitness and obesity in Danish children suggest a subsequent adult generation with a higher degree of ACVD-risk, and it might turn the positive development that has been seen in ACVD morbidity and mortality during the last two decades. Inverse associations between parental educational level and some ACVD risk factors were found in both children and adolescents. Associations between parental disease and some ACVD risk factors in children were also found. Smoking was only found to be significantly associated with an increased S-triglyceride level in adolescents.

These major findings imply that intervention toward healthy living should start at very young age.

Recommendations for the future:

1. Childhood should be considered a vital period for primary ACVD prevention programmes.
2. Further investigations are needed, and especially secular trend studies are important.
3. A focus should be placed on:
    a. Obese children.
    b. Children with poor physical fitness.
    c. Children coming from families of low socio-economic status.
    d. Children coming from families with known ACVD disease.
    e. A combination of the above.

This would provide possibilities for aiming and pinpointing future preventive campaigns at groups with high risk of future ACVD in order to prevent or delay the atherosclerotic lesions and ultimately prevent or delay the appearance of ACVD.

SUMMARIES:

ENGLISH SUMMARY:

This study was performed at the Institute of Sport Science and Clinical Biomechanics, Faculty of Health Science, University of Southern Denmark. Main campus: Odense University.
Study Design: A cross-sectional survey of 1020 subjects in Odense, Denmark, consisting of children and adolescents aged 8-10 and 14-16 years, obtained through two-stage cluster sampling from schools stratified according to school type, location and socio-economic character of uptake area.
Objectives: In Danish children and adolescents to:

1. Investigate whether the phenomenon of clustering of atherogenic cardiovascular (ACVD) risk factors is observable.
2. Investigate the influence of physical fitness on ACVD risk factors and clustering of risk factors.
3. Describe and discus the implications of the secular trends of physical fitness and obesity

Summary of Background Data: Studies performed in other countries have shown clustering of cardiovascular risk factors to be present in adolescents and have suggested its presence in children too. The data on the association between physical fitness and these risk factors are uncertain, because no clear information is available. Only one study has reported on secular trends of physical fitness. This study used a sub-maximal physical fitness test and a sub-maximal fitness test is not as valid as a maximal fitness test. Secular trends studies on obesity have been performed previously but not in Danish children. All other reported studies in western countries have shown an increase in number of obese subjects and an increased level of obesity in the obese subjects.
Methods: Cross sectional study of cardiovascular risk factors in 1020 randomly selected children and adolescents, with special focus on physical fitness, obesity and blood lipids. In addition the physical fitness and obesity data were analysed, and compared with data from Odense Schoolchild Study and Danish Youth Sport Study to assess the secular trends of physical fitness and obesity. Parental educational levels were assessed and compared to levels of the total Danish population.

Results: It was found that

1. Clustering tendencies were evident in children and adolescents with low physical fitness.
2. An inverse relationship was found between physical fitness and cardiovascular risk factors in children and adolescents.
3. Physical fitness was the variable with the highest and most consistent association with the cardiovascular risk factors in children.
4. During the last decade the physical fitness of children and adolescents with low physical fitness has become lower than previously. The children and adolescents with high physical fitness had either the same or a higher level fitness than previously measured.
5. The difference between the children with high and low physical fitness respectively has increased. An increased polarisation in physical fitness level was found.
6. During the last decade the obese children and adolescents have become even more obese and they have increased in number.
7. An inverse relationship was found between parental educational level and a number of risk factors.
8. A relationship was found between parental disease and ACVD risk factors in children, but not in adolescents.

Conclusion:
A group of children and adolescents with a lower physical fitness and increased obesity is starting to emerge. This negative trend in and polarisation of physical fitness and obesity in a group of Danish children suggests a subsequent adult generation with a higher prevalence of cardiovascular disease, metabolic syndrome and type 2 diabetes. An inverse association between parental educational and income level and some ACVD risk factors were found. This implies that intervention strategies toward a healthy life style should be encouraged at a young age. Childhood should be considered as a vital period for primary prevention strategies and programmes, to prevent or delay the atherosclerotic lesions and ultimately prevent or delay the appearance of cardiovascular disease.

Dansk Resume:

Kardiovaskulære risikofaktorer hos børn og unge:

En undersøgelse af fysisk form, anthropometri, blodtryk og Kardiovaskulære risikomarkører i serum hos 9-10 og 15-16 årige danske skolebørn.
Delprojekt af "European Youth Heart Study"
Ph.d.-afhandlingen er skrevet på baggrund af et studie udført under min ansættelse på Institut for Idræt og Biomekanik ved Syddansk Universitet i Odense.

Baggrund: Det er veldokumenteret, at både livsstils og genetiske risikofaktorer har stor indflydelse på forekomsten af Kardiovaskulære sygdomme hos voksne.
Flere studier har kunnet påvise disse faktorer hos børn og unge. Mindst en af livsstils faktorerne blev fundet i op til 70% af børnene i en årgang af 12 årige skoleelever. Både livsstils faktorerne og konsekvenserne heraf som f.eks. fedme, dyslipidæmi, hypertension og dårlig fysisk form, har en tendens til at blive bevaret hos det enkelte individ gennem barndommen og ind i voksenlivet .
Der er derfor stigende bekymring for at en livsstil med en tiltagende fysisk inaktivitet, nedsat fysisk form og overvægt, vil medføre øget risiko for kardiovaskulære sygdomme i voksenalderen.
De kliniske manifestationer og konsekvenserne af risikofaktorerne viser sig typisk først i voksenlivet, men autopsi studier har vist, at aterosklerotiske læsioner kan identificeres hos børn. Stumme kliniske manifestationer som forhøjet blodtryk, forstørrelse af venstre ventrikel og forhøjet serumkolesterol er fundet hos børn med nedsat fysisk aktivitet, nedsat fysisk form og overvægt.

Undersøgelses design: Tværsnitsstudie af kardiovaskulærer risikofaktorer hos børn og unge, med speciel fokus på fysik form, overvægt og blod lipider samt deres indbyrdes sammenhænge. Endvidere blev der foretaget sammenligninger af overvægt og fysisk form med store undersøgelser foretaget i 80erne (Odense Schoolchild Study og Danish Youth Sport Study). Herved blev det muligt at udtale sig om den tidsmæssige udvikling af disse parametrer. Fordelingen af forældrenes uddannelses niveauer blev undersøgt og sammenlignet med fordelingen af uddannelsesniveauet hos den danske befolkning.

Resultater: Undersøgelsen viste, at

1. Det blev fundet at børn og unge med lav fysisk form har stor risiko for at have ophobning af risikofaktorer.
2. Der var er negativ korrelation mellem fysisk form og kardiovaskulærer risikofaktorer hos børn og unge.
3. Fysisk form var den af de undersøgte faktorer, som bedst relaterede sig til kardiovaskulære risikofaktorer hos børn.
4. I slutningen af 90erne var børns fysiske form ringere end den var i 80erne. Der var ingen forskel mellem teenage drengene i 90erne og teenage drengene i 80erne, hvorimod teenage pigerne i 90erne var i bedre fysisk form end teenage pigerne i 80erne.
5. En større grad af polarisering har udviklet sig, således at der i slutningen af 90erne var en større forskel mellem de børn og unge som var i dårlig fysisk form og de som var i god fysisk form end der var midt i 80erne.
6. De overvægtige børn og unge var mere overvægtige i slutningen af 90erne end de var i 80erne.
7. Der var en invers sammenhæng mellem forældrenes uddannelsesniveau og nogle af risikofaktorerne.
8. Der var sammenhæng mellem aterosklerotisk sygdom hos forældrene og aterosklerotisk risiko status hos børnene, men ikke hos teenagerne.

Konklusion:
En relativ stor gruppe af børn og unge, var mere overvægtige og i dårligere fysisk form end børn og unge i 80erne. Dette betyder, at de har større risiko for at få livsstils sygdomme, samt at de vil få disse sygdomme tidligere og derfor også må forventes at få en kortere levetid. Barndommen må derfor i dag betragtes, som den livsperiode hvor forebyggelse af livsstilssygdomme bør starte. Det er vigtigt, at børn hjemmefra og i skolen bliver påvirket til at få hensigtsmæssige motions og spisevaner, da disse livsstilsfaktorer har stor betydning for udviklingen af livsstilssygdomme.

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