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Karolinska Institutet, Stockholm, Sweden

CARDIOVASCULAR RISK FACTORS IN CHILDREN

by

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To my family

ABSTRACT

Non-communicable diseases, above all cardiovascular disease (CVD), are the most common causes of morbidity and mortality in Europe. CVD has been shown to have its roots in childhood, although the clinical manifestations do not become evident until several decades later. The adolescent CVD risk profile has been shown to predict the extent of the atherosclerotic process in adulthood, even if the nature of the effects of biological and life-style factors, and their interactions, on the CVD risk profile in children and adolescents are largely unknown. The same holds true for the secular trends.

The overall purpose of the present research was to increase our knowledge of physical activity, fitness, blood pressure, blood lipids, insulin and glucose, and the interactions between them, in children aged 9 and 15 years.

Data collection took place during the school year 1998/1999 in central Sweden, and the 1,137 subjects constitute the Swedish part of the European Youth Heart Study. The level and pattern of physical activity were objectively assessed with a uniaxial accelerometer, and cardiorespiratory fitness was estimated by a maximal ergometer bike test. Body composition was estimated from skinfold thicknesses, blood pressure was measured by an automatic oscillometric method, and fasting serum samples were analysed for insulin, glucose, triglycerides, total cholesterol and high density lipoprotein cholesterol.

The main outcomes in this population sample of healthy school children were: insulin was particularly influenced by body fat and total physical activity; data suggest a secular trend towards decreased cardiorespiratory fitness in 9-year-olds, but not in 15-year-olds; and a high fitness level was more important than high total physical activity level for a favourable CVD risk profile, but a gender difference was observed.

The practical implications of these findings are that fitness, and not only total physical activity, ought to be considered in longitudinal and future intervention studies, and that the gender perspective of these issues needs further attention.

Key words: Accelerometry, blood pressure, cardiorespiratory fitness, ergometer bike, glucose, insulin, lipids, physical activity.

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List of abbreviations

cpm	Counts per minute, level of total physical activity
CRF	Cardiorespiratory fitness
CVD	Cardiovascular disease
EYHS	European Youth Heart Study
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IPAQ	International Physical Activity Questionnaire
IR	Insulin Resistance
IRS	Insulin Resistance Syndrome
HDL cholesterol	High-density lipoprotein cholesterol
LDL cholesterol	Low-density lipoprotein cholesterol
MET	Metabolic energy turnover
MTI	Actigraph from Manufacturing Technology Inc.
PA	Physical activity
PEACH	Personal and Environmental Associations with Children's Health
SES	Socio-economic status
SSF	Sum of skinfolds
VO ₂	Oxygen consumption
VO _{2max}	Maximum oxygen consumption
VO _{2peak}	Highest oxygen consumption achieved during a test

1 INTRODUCTION

Non-communicable diseases, above all cardiovascular disease (CVD), are the most common causes of morbidity and mortality in Europe, despite important successes in the treatment of the overt disease (Socialstyrelsen, 2001; World Health Organization (b), 2003). In adults, it has been convincingly demonstrated that higher physical activity, or exercise, and higher physical fitness are associated with lower cardiovascular morbidity and mortality (Paffenbarger *et al.*, 1986; Blair *et al.*, 1989; US Department of Health and Human Services, 1996). The epidemiological evidences are mainly based on studies conducted in men, but similar health benefits of regular physical activity have been described in women (Oguma *et al.*, 2002). While data on relationships between physical activity, physical fitness, and risk factors for CVD are plentiful in adults, data on children are sparse. Thus, the present research was focused on young individuals, namely subjects of age groups 9-10 and 15-16 years, although much of the cited literature refers to findings in adults.

1.1 THE CHILDHOOD PERIOD OF LIFE

Human growth and development can be divided into different stages. These are commonly termed fetal, infancy, childhood, pubertal or adolescent, and adulthood (Karlberg *et al.*, 1987). Growth and development in the early stages are mainly determined by nutrition, and subsequently hormonal regulation also has an important role in somatic growth (Clayton & Gill, 2001). With the initiation of puberty, the growth spurt begins. The transition period from childhood into adulthood is frequently called the adolescent period, and is a time of rapid physical, emotional and psychological changes. The biological development can also be described in terms of growth and maturation. The term growth refers to an increase in size, which generally has a parallel pattern in different bodily functions, as represented by height, weight, heart volume, lung function, aerobic power and muscular strength (Malina & Bouchard, 1991). Maturation refers to stages of progress towards the mature biological state, and is most often viewed in the context of secondary sex characteristics or skeletal age (Malina, 1994). The most widely used model for assessment of sexual maturation is based on the five stages of secondary sex characteristics described by Tanner (Tanner, 1962). The terms children, adolescents, young people and youth are

often used interchangeably. In the present thesis the term *children* is used for both the studied subjects aged 9-10 years (hereafter referred to as 9-year-olds), and those aged 15-16 years (hereafter referred to as 15-year-olds).

1.2 ATHEROSCLEROSIS

Atherosclerosis constitutes the single most important contributor to cardiovascular disease. Atherosclerosis is characterized by accumulation of lipids and fibrous elements in the arterial wall. The atherosclerotic process is a progressive disease of the arteries that is initiated early in life, although in general the clinical outcome is not detected until several decades later (Ross, 1993; Libby *et al.*, 2002). The first reports of atherosclerotic lesions in young individuals who had died prematurely of non-cardiac causes came from autopsies on young soldiers who died in the Korean War (Enos *et al.*, 1955) and the Vietnam War (McNamara *et al.*, 1971). Similar findings were later associated with risk factors such as smoking and blood lipid levels in the study *Pathobiological Determinants of Atherosclerosis in Youth* (McGill *et al.*, 2000) and the *Bogalusa Heart Study* (Berenson *et al.*, 1998). Childhood levels of various risk factors have recently been shown to be predictive of adult values (Andersen & Haraldsdottir, 1993; Bao *et al.*, 1994; Eisenmann *et al.*, 2004). In the longitudinal study *Cardiovascular Risk in Young Finns*, Raitakari and co-workers found that the adolescent risk profile predicted the extent of the atherosclerosis process in adulthood, even independently of the current risk (Raitakari *et al.*, 2003). Those findings emphasize the importance of detecting subjects with an adverse CVD risk profile, and of early primary intervention.

1.3 RISK FACTORS

The primary modifiable factors associated with an increased risk of CVD include smoking, hypertension, high blood lipid levels, low fitness, physical inactivity, obesity, and insulin resistance or diabetes. Non-modifiable risk factors are advanced age, male gender, and other genetic variables (Wilmore & Costill, 2004). The physical activity perspective was included in 1992 (Fletcher *et al.*, 1992). The classical risk factors age, systolic blood pressure, serum cholesterol and smoking are still considered to be among the most important independent predictors of CVD (Menotti *et al.*, 1996).

The risk of acquiring clinical CVD increases with unfavourable values of these risk factors. Different “risk values” or “cut-off values” have been suggested on the basis of epidemiological findings. In children, such values are less clear-cut, and are not applicable to the same extent as in adults, as no “end-points” (e.g. diagnosed myocardial infarction) have been definable. The concept “risk factors for risk factors” or “risk indicators” has been suggested (Bergström, 1995) as a possibly better term in children. Only if the unfavourable value of the variable tracks from childhood into adulthood (i.e. persists in rank order over time, or has a strong correlation factor between subsequent measures), will it become a risk factor for CVD. In the present thesis, however, the term “risk factor” will be used.

1.3.1 Fitness

Physical fitness consists of a set of attributes that are either health- or skill-related (Caspersen *et al.*, 1985), and has several components (e.g. morphological fitness, muscular fitness, skeletal fitness, metabolic fitness and cardiorespiratory fitness). The single most important health-related fitness component is the ability to provide oxygen for the aerobic energy supply, which is the energetic basis of our existence in daily life activities (Brooks *et al.*, 2004). The maximum rate at which an individual is able to consume oxygen (oxygen consumption, VO_2 ; maximum oxygen consumption, VO_{2max}) is therefore an important determinant of the physical work capacity. The VO_{2max} depends on the capacity of the cardiovascular system, and the most commonly used way of assessing cardiovascular, aerobic or cardiorespiratory fitness (CRF) is to measure VO_{2max} or to estimate it from standardized ergometer tests, a work performance test. In laboratory settings VO_2 can be measured during indirect calorimetry (a measure of expired air volume per time unit and analysis of the expired air with regard to oxygen and carbon dioxide concentrations). This is rarely done in epidemiological studies, because of the need of advanced equipment. Instead, maximal or submaximal tests have been developed for calculation of VO_{2max} , based on the described relationship between oxygen consumption and workload (Åstrand *et al.*, 2003). Correlations between directly measured and indirect estimations of VO_{2max} have been found to be $r = 0.9$ for maximal tests and $r = 0.6$ for submaximal tests (Andersen *et al.*, 1987), but each test protocol has to be validated with reference to specific age and gender groups.

It has recently been reported that a decrease in physical performance took place in Swedish adolescents between 1974 and 1995, as measured by a running test, but the decrease in running performance was found to be statistically explained by a secular trend towards an increase in body dimensions (Westerståhl *et al.*, 2003). In another study no secular trend in CRF was observed among 16-year-old girls between 1987 and 2001, but a decrease was noted in 16-year-old boys (Ekblom *et al.*, 2004a). Tomkinson and co-workers reported a decline in aerobic performance between 1981 and 2000, mainly in Australian and European children (Tomkinson *et al.*, 2003), whereas children in the United States have been found to have a relatively stable aerobic fitness level (Eisenmann & Malina, 2002). Thus, despite the relatively large amount of fitness data, the conclusions drawn are not consistent with regard to a suggested secular decline in fitness level.

1.3.2 Physical activity

Physical activity is defined as “any bodily movement produced by skeletal muscles that results in energy expenditure above resting metabolic rate” (Caspersen *et al.*, 1985). To describe and analyse physical activity, four aspects need to be addressed, namely frequency, duration, intensity and type of activity. Recently, the domain, or arena, of physical activity has also been specified in the description of total physical activity.

Frequency and duration of physical activity. The frequency of physical activity is usually given in times per week, but can be referred to any unit of time (e.g. sessions per day or month). The duration of the activity is the length of time spent in each activity session.

Intensity of physical activity. The intensity of physical activity can be expressed in several ways. The absolute intensity of an activity can be expressed as the actual rate of oxygen required per time unit based on measurement of oxygen consumption ($l \times \text{min}^{-1}$ or $\text{ml} \times \text{min}^{-1} \times \text{kg body weight}^{-1}$). From the oxygen consumption, the energy expenditure ($\text{kcal} \times \text{min}^{-1}$ or $\text{kJ} \times \text{min}^{-1}$) can be calculated (McArdle *et al.*, 2001). To adjust for differences in resting metabolic rates related to sex, age and body composition, multiples of resting metabolic rate have been introduced as a descriptor of intensity; this is the Metabolic Energy Turnover (MET) classification of physical activities. One MET corresponds to the energy expenditure during rest, about 0.20 and 0.25 $l \text{ O}_2 \times \text{min}^{-1}$ in adult, normal-weight women and men, respectively, or 3.5 ml

$\text{O}_2 \times \text{min}^{-1} \times \text{kg}^{-1}$, or $1 \text{ kcal} \times \text{hour}^{-1} \times \text{square meter body surface area}^{-1}$ (McArdle *et al.*, 2001). In adults, more than 600 specific activities have been MET classified (Ainsworth *et al.*, 1993; Ainsworth *et al.*, 2000). The relative intensity of an aerobic activity also takes into account the aerobic fitness level of the subjects, thus describing the activity as the percentage of the maximal aerobic capacity for the specific activity (e.g. percentage of maximal oxygen consumption or percentage of maximal heart rate).

To describe the total physical activity from data obtained by self-reported methods the physical activity can be converted into METs and quantified and described as number of MET-minutes per day, or MET-hours per week (Ainsworth *et al.*, 1993; Ainsworth *et al.*, 2000). The total energy expenditure, consisting of the resting metabolic rate, diet-induced thermogenesis and activity energy expenditure, can then be calculated.

Most commonly, the intensity of physical activity is described as light, moderate, vigorous or hard, or very vigorous or strenuous. The MET cut-off values have been suggested for categorizing the intensity of physical activities in young individuals (Bouchard *et al.*, 1990b; Riddoch & Boreham, 1995; Pate, 1998).

Type of physical activity. The type or mode of activity can be classified according to the specific activity performed (e.g. sitting watching television, walking or swimming) and to the circumstances under which the activity is carried out (e.g. leisure time physical activity or occupational physical activity). Exercise is a subcategory of leisure time physical activity, defined as a subset of physical activity that is planned and structured, with repetitive bodily movements done to improve or maintain one or more components of physical fitness (Caspersen *et al.*, 1985).

Domains of physical activity. Physical activities for transportation are sometimes described separately, as are activities associated with household work. In the newly launched *International Physical Activity Questionnaire* (IPAQ), four domains, or arenas, of physical activity are addressed: physical activity during transportation, at work, during household and gardening tasks, and during leisure time, the latter including exercise and sport participation. This questionnaire was developed for surveillance and global comparison of subjects aged 18-65 years, and has been validated in different versions and settings (Craig *et al.*, 2003). It is now being validated also in 15-year-olds (www.ipaq.ki.se).

Assessment of physical activity. Various methods of assessing physical activity are available. Hitherto, the most commonly used methods have been self-reported methods (e.g. questionnaires, activity diaries, interviews). Objective measurements (e.g. doubly labelled water, heart rate monitoring, and motion sensors such as pedometers or accelerometers) have recently become common, but these require expensive equipment and are still in the methodological development stage with regard to validation and interpretation in a health care perspective.

There is general concern today about indications that levels of physical activity are low or decreasing. Scientific evidence for this is limited, however. This limitation is mainly due to a previous lack of objective assessment methods of physical activity. The methods previously used (e.g. questionnaires) were not validated against objective methods, and were generally difficult to apply in children (Boreham & Riddoch, 2001). Thus, there is a need of physical activity data based on objective methods, especially in children. These data are required for baseline descriptions and for studies on interrelationships.

1.3.3 Serum variables

Blood lipids

A high total cholesterol concentration was the first identified serum factor to be scientifically linked to CVD (Keys, 1951). High levels of plasma or serum triglycerides, total cholesterol, and low-density lipoprotein cholesterol (LDL cholesterol), and low levels of high-density lipoprotein cholesterol (HDL cholesterol) are generally recognized as traditional characteristics associated with a high risk of CVD (Wilmore & Costill, 2004). More recently, it has been suggested that fractions of lipoproteins may be even stronger indicators of a CVD risk than the traditional lipid profile (Kraus *et al.*, 2002), but in the present thesis only the established lipid risk factors are considered.

Oxidation of more lipids than carbohydrates in a variety of conditions seems to be a desirable metabolic characteristic from both a health and a performance perspective, and is one well described form of adaptation to regular physical activity in adults (Haskell, 1986; Durstine *et al.*, 2001).

Regular exercise is associated with a reduced risk of CVD in adults, despite the fact that exercise has only a minor effect on total cholesterol or LDL cholesterol. An

increase in HDL cholesterol and a decrease in triglycerides following periods of physical exercise are more frequently reported (Durstine *et al.*, 2001). In children, the effects of physical activity on the lipid profile remain to establish (Tolfrey *et al.*, 2004).

Glucose and insulin

Diabetes increases the risk of CVD 1.5 - 4.5-fold. A “pre-diabetic state” or “borderline diabetes” is also associated with an increased risk of CVD (Unwin *et al.*, 2002). The term impaired fasting glucose (IFG) is used for glucose concentrations above the fasting reference values, but not reaching the criteria used for diagnosing diabetes. Impaired glucose tolerance (IGT) is defined as an increased glucose concentration 2 hours after a defined glucose load (oral glucose tolerance test). Both IFG and IGT represent metabolic states that are intermediate between normal glucose homeostasis and diabetic hyperglycaemia, and are risk factors for CVD. The association between IGT and CVD has been found to be stronger than that between IFG and CVD (DECODE Study Group, 2001; Blake *et al.*, 2004).

Insulin decreases glucose concentrations by reducing hepatic gluconeogenesis and glycogenolysis. Insulin also enhances glucose consumption into skeletal muscles and adipocytes (Vander *et al.*, 2001). Insulin resistance (IR), or reduction in insulin sensitivity, is defined as a reduction in insulin-stimulated glucose metabolism. IR is tested for by a euglycaemic hyperinsulinaemic clamp technique, where insulin is infused at a steady rate (in an amount dependent on the body weight) and glucose is infused at a variable rate to maintain euglycaemia over a defined period of time (e.g. 40 min) (Ferrannini & Mari, 1998). Neither the glucose loading nor the clamp technique is feasible in epidemiological studies, especially not in children. Fasting glucose and insulin values can be used as surrogates for the more clinically established methods for IGT and IR testing (Alberti & Zimmet, 1998; Sinaiko *et al.*, 2001).

A fall in insulin sensitivity during puberty, i.e. pubertal insulin resistance, has been described, and is associated with a compensatory increase in insulin secretion (Caprio *et al.*, 1989). Insulin concentration is positively correlated with body mass index in children (Bergström *et al.*, 1996). Most studies on paediatric dyslipidaemia and insulin resistance have been carried out in obese children. Detailed data on serum lipid, glucose and insulin levels among healthy children in the Swedish population are largely lacking.

1.3.4 Body composition

Important health-related morphological factors are the body fat content and distribution. The percentage of body fat is positively correlated both with lipid and insulin levels and with blood pressure (Despres *et al.*, 1990). An unfavourable subcutaneous fat distribution is considered an important indicator of an enhanced risk for CVD and for non-insulin-dependent diabetes mellitus (Bouchard *et al.*, 1990a). The long-term impact of childhood overweight and obesity (Must *et al.*, 1992), the reported strong tracking into adulthood (Twisk *et al.*, 1997), and the increasing prevalence of overweight and obesity in Sweden as in many other countries (Petersen *et al.*, 2003), all emphasize the need for studying the body composition. Another important morphological health component, not included in this study, is bone density.

1.3.5 Blood pressure

A continuous positive relationship between blood pressure and vascular risk and mortality has been demonstrated in a large meta-analysis: The lower the pressure, even within normal ranges, the more substantial the decrease in CVD risk (Lewington *et al.*, 2002). Both the *Tecumseh Blood Pressure Study* (Julius *et al.*, 1990) and the *Bogalusa Heart Study* (Li *et al.*, 2004) have shown that even borderline hypertension at early ages calls for clinical attention. The understanding of factors related to blood pressure is therefore important.

1.3.6 The metabolic syndrome

Most individuals who develop CVD have multiple risk factors. The presence of insulin resistance together with dyslipidaemia, hypertension and overweight has been recognized as a common phenotype. This cluster of risk factors was initially called Syndrome X (Reaven, 1988), but it has since been referred to as insulin resistance syndrome (IRS) or metabolic syndrome. Components of the metabolic syndrome that are related to CVD according to the American National Heart, Lung, and Blood Institute/American Heart Association (Grundy *et al.*, 2004) have now been extended to:

- abdominal obesity
- dyslipidaemia (elevated triglycerides and low concentrations of HDL cholesterol)
- raised blood pressure

- insulin resistance (initially high insulin levels, later glucose intolerance which can evolve into diabetes-level hyperglycaemia or diabetes type II)
- pro-inflammatory state (elevation of C-reactive protein)
- pro-thrombotic state (increased plasma plasminogen activator inhibitor).

1.3.7 Other risk factors

Diet

Diet is a complex and important life-style component from all health aspects. Data on energy intake, nutrition and meal pattern for the present participants have been collected (24-hour recall; interview method), but are not included in this thesis.

Socio-economic status

In adults, measures of a low socio-economic position have been found to be associated with an increased risk for CVD (Kaplan & Keil, 1993; Wannamethee *et al.*, 1996; Mackenbach *et al.*, 2000). These associations were first described in men (Blane *et al.*, 1996; Wannamethee *et al.*, 1996), but more recently they have also been reported in women (Wamala *et al.*, 2001; Lawlor *et al.*, 2002). Health behaviour (e.g. eating and physical activity) has also been found to be related to the socio-economic status (SES) in children, although the adult socio-economic destination seems to be the best predictor of mortality, not the childhood origin per se (Diderichsen, 1990; Lynch *et al.*, 1994; Lynch *et al.*, 1997). A gender difference is described in a Spanish study where men were found to be more susceptible to adverse childhood circumstances than women (Regidor *et al.*, 2004). The impact of childhood socio-economic status on CVD in adulthood is probably dependent upon particular historical, political and cultural settings, which vary between countries and periods of time.

Smoking

Smoking was one of the first identified factors that were found to be associated with CVD. Findings from autopsies in the *Bogalusa Heart Study* showed that both the prevalence and extent of atherosclerosis were higher in smokers than in non-smokers, even among young adults (≤ 39 years old) with a relatively short period of smoking (Berenson *et al.*, 1998).

1.4 RATIONALE FOR THIS RESEARCH

Data on the risk factors presented above are largely missing for children. Descriptive data of such values are needed for planning and evaluation of public health activities, and for clinical use, and need to be population-specific. Furthermore, data are needed for studies of secular trends, interrelationships and, when longitudinal, causal relations. In the present studies physical activity was for the first time assessed by the objective accelerometer method in a substantial number of children, providing an opportunity to elucidate relationships with physical activity more accurately.

2 PURPOSES

The overall purpose of the investigations summarized in this thesis was to increase our knowledge of the levels and patterns of life-style and biological factors and their associations with other risk factors for cardiovascular disease in children; and through that knowledge to contribute to the scientific rationale for health promotion activities targeting cardiovascular health in young individuals.

The specific aims of the separate studies were as follows:

- to evaluate the sampling procedure and the representativeness of the sample (Paper I);
- to present baseline data on the traditional risk factors for CVD in the specific age (9-year-olds and 15-year-olds) and gender groups (Paper II);
- to study the secular trend of cardiorespiratory fitness by comparing the cardiorespiratory fitness results obtained in this study with data from earlier studies (Paper III);
- to analyse the combined and separate contributions of physical activity and fitness to their interactions with other CVD risk factors (Paper IV).

3 MATERIALS AND METHODS

3.1 ETHICS

The local ethical committees approved the study protocols (Örebro City Council case no. 690/98 and Huddinge University Hospital case no. 474/98), and the study was carried out in accordance with the Helsinki Declaration (World Medical Association, 2002) and national guidelines (Vetenskapsrådet, 2002). The boards of all collaborating schools approved the use of school localities during school hours. Conducting the study during school hours and at the school campuses guaranteed that the participants were insured through the comprehensive school insurance system. The children and their families received written information about the purpose and the content of the study, and written consent was obtained from one of the parents or a legal guardian for the 9-year-olds, and from one of the parents or a legal guardian and the child for the 15-year-olds.

Special attention was paid to treating the participants as children and not solely as study subjects. If they hesitated to undergo venipuncture, they were encouraged to take part in the other parts of the study, and venipuncture was attempted only once. At the end of the test session they were also reminded about the possible 6-year follow-up.

3.2 THE EUROPEAN YOUTH HEART STUDY (EYHS)

EYHS is a cross-sectional, school-based study of risk factors for future cardiovascular disease in children aged 9 or 15 years, including both the traditional serum cardiovascular risk factors (total cholesterol, triglycerides, high density lipoprotein), and a number of other biological and life-style factors.

EYHS was designed by a Scientific Committee of senior researchers. Central original members of the committee were Drs. Chris Riddoch (UK), Michael Sjöström (Sweden), Karsten Froberg (Denmark) and Dawn Edwards (UK), but scientific co-workers have changed during the course of the project.

EYHS data have been collected in five countries: Denmark (Odense, 1997/1998), Sweden (Örebro and Stockholm, 1998/1999), Estonia (Tartu, 1998/1999), Portugal (Madeira, 1998/1999) and Norway (Oslo, 1999/2000). All participating EYHS countries followed the protocol manual, and to attain comparable results between the

countries educational meetings were held for new groups and one central laboratory was used for serum analysis. One member of the original scientific committee (Dr Dawn Edwards) was responsible for quality control of the study, and visited Sweden in November 1999 to supervise the Swedish team's rehearsal of the protocols.

3.2.1 Study design

The study was a school-based, cross-sectional study of healthy children in school year 3 (aged 9-10 years) and school year 9 (aged 15-16 years). Data were collected between the beginning of September 1998 and the end of May 1999 (one school year). The sampling frame was all schools in Örebro and the South region of Stockholm (Central Sweden). The sample constitutes the Swedish part of the European Youth Heart Study.

3.2.2 Study population

The municipality of Örebro had 160,000 inhabitants, of whom there were about 1,500 pupils in each of the EYHS age groups, i.e. inhabitants born in 1983 or 1989. The South region of Stockholm, defined here as the municipalities of Botkyrka, Haninge, Huddinge, Nynäshamn, Salem, Södertälje and Tyresö, had about 370,000 inhabitants, of whom there were about 5,000 pupils in each of the two age groups.

3.2.3 Sampling procedure

All public schools (i.e. non-private schools) with more than 20 children in the age groups in question were initially included. The schools were stratified with regard to school grade and to the mean income level in their catchment areas (below or above the mean in their municipality). A random sampling procedure from each stratum was carried out, and the sampled schools were invited to participate in the study.

From the complete lists of pupils in the collaborating schools, groups of pupils proportional to the sizes of the respective schools were randomly selected with the aim of obtaining 250 participants in each age and gender group (totally 1,000 participants). Fifty per cent oversampling was performed on the basis of experiences from a pilot study. Two additional school units were sampled as reserve schools. The sampling procedure on an individual level was organized as follows: i) Sampled pupils received a letter with information, and a written informed consent form. The pupils were asked to return the completed form to the class teacher in an enclosed envelope within a week.

ii) A reminder was distributed in the same way to non-responding families ten days after the first letter had been sent. iii) For those who agreed to participate, a third letter with practical information was distributed one week before the day of the test. iv) Lists of participants were distributed to the teachers in order to ensure that the pupils would be allowed, and reminded, to undergo the tests during school hours.

3.2.4 Description of a typical test day

The day of the test started with short information and application of EMLA® local analgesic cream for those who wanted this before the venipuncture. The blood sampling then started with those who not had chosen the cream, as the analgesic cream had to be allowed to take effect for 45-60 minutes. After the blood sampling, breakfast was served. Usually eight to ten 9-year-olds, or ten to twelve 15-year-olds were tested during one day. After breakfast, half of the group were sent back to the classroom to take part in the lessons until lunch break and were asked to return to the test localities again after lunch. The other half remained in the test localities and underwent a series of procedures: resting blood pressure recording, ergometer bike test, anthropometry, dietary interview and computerized questionnaire. The resting blood pressure was always recorded before the bike test; otherwise the different procedures were carried out in random order (according to the subject's choice of what to do first or last, or to which test station was not occupied). After their tests, the tested group had lunch and then went back to their lessons.

The accelerometers were usually distributed to the subjects before the day of the tests, when the study team was visiting the school for distribution/collection of information letters or for arranging the test localities. If so, the accelerometer was handed back and downloaded by a computer interface to an Excel file. If a subject had not already received the accelerometers, this was arranged during the day of the tests and the accelerometer was then handed back to the class teacher and picked up later by the test team.

3.3 CARDIORESPIRATORY FITNESS

3.3.1 Bike test protocol

The test protocol and the calculations were as described by Hansen et al. (1989). The subjects cycled at 50-70 revolutions per minute in a sitting position to exhaustion on an electronically braked Monark ergometer (model 829E, Varberg, Sweden). The continuous workload stages had a duration of 3 minutes, and both the initial and incremental workloads were age- and gender- dependent (e.g. for 9-year-olds <30 kg in body weight: initial work rate 20 W and an increase by 20 W every 3 minutes; for 9-year-olds >30 kg in body weight: initial work rate 25 W and an increase by 25 W every 3 minutes; for 15-year-old girls: initial work rate 40 W and an increase by 40 W every 3 minutes; and for 15-year-old boys: initial work rate 50 W and an increase by 50 W every 3 minutes). The test was terminated when the subjects could no longer maintain the power output despite verbal encouragement. A true exhaustive effort was considered to have been achieved if the subject had a heart rate (HR) exceeding 185 beats per minute and at the same time showed clear signs of fatigue (the test leader's subjective opinion). HR was monitored continuously by telemetry (POLAR Vantage NV, Kempele, Finland). Power output was calculated as $= W1 + (W2 \times t/180)$, where $W1$ = workload (W) at fully completed stage, $W2$ = workload increment (W) at final incomplete stage, and t = time (s) at final incomplete stage. The "Hansen formula" for calculated VO_2 ($ml \times min^{-1}$) = $12 \times$ calculated power output + $5 \times$ body weight in kg (Hansen *et al.*, 1989). The ergometer bike was calibrated electronically each test day, and mechanically after being moved. All calculations of interrelationships with CRF in the present studies are based on the W/kg expression, as these values were most close to the original data (i.e. power output and body weight).

3.3.2 Validation study

The Hansen formula for calculation of oxygen consumption was developed in 11-year-old children. To validate the formula for age groups in the European Youth Heart Study (EYHS), see below, 53 subjects were tested by indirect calorimetry with analysis of expired air. The protocol of Hansen et al (1989) was used in the validation study, with an additional sampling of expired air through a mouthpiece during the ergometer bike test. A group of twenty-one 9-year-old children (7 girls and 14 boys) underwent an ergometer bike test with indirect calorimetry measurements at the Norwegian

University of Sport and Physical Education, Oslo, Norway (CPX system, Medical Graphics Corporation, USA), and a group of 32 adolescents (12 girls and 20 boys) was tested at Örebro University Hospital (CPX system, Medical Graphics Corporation, USA). The subjects in the validation study were not participants in the main study.

3.4 PHYSICAL ACTIVITY

The habitual physical activity was assessed by a motion sensor, an accelerometer, which detects single plane (vertical) accelerations. The Computer Science Application (CSA, Shalimar, Florida) accelerometer model 7164, now also known as the MTI actigraph (Manufacturing Technology Inc., Fort Walton Beach, FL, USA), was used. The CSA is a uniaxial piezoelectric accelerometer with a dynamic range of $\pm 2.13g$ and a frequency-dependent bandwidth filter, which filters out unphysiological values. The CSA samples accelerations at 10 Hz and integrates the accelerations over the user-defined period of time, or epoch. Here, we used a 1-minute epoch, thus an integration of 600 measurements for each data point. The small monitor (4.5×3.5×1.0 cm, 43 g) is worn in an elastic belt around the waist, during all time awake, except during water activities. The subjects were instructed to wear the accelerometer for four consecutive days. The registration was included in the analysis if the registration covered more than 10 hours of activity data per day for at least three days. Time periods of zero registration of 10 minutes or longer were interpreted as missing values (e.g. CSA not worn), and excluded from the registered time. The data output is expressed as counts per minute (cpm). As a measure of total physical activity, the total number of counts during the complete registration was divided by the total registered time, yielding counts per minute (cpm). The total physical activity (cpm) was always used in the present studies, unless different intensity levels of physical activity (e.g. moderate or high intensity) were being addressed. When so, the cut-off values suggested by Trost were used (Trost *et al.*, 2002).

Precision data for the instrument are as follows: Intra-instrument CV = 1.3% and 1.4% for fast and medium speeds of movements, respectively, and inter-instrument CV = 4.6% and 5.0% for fast and medium speeds of movements, respectively (Metcalf *et al.*, 2002). The result is given in counts per chosen time interval (e.g. minute), and can be used for measures of total amount of physical activity, for describing the intensity level of activities (Janz, 1994) and for estimation of activity energy expenditure (Ekelund *et al.*, 2001).

3.5 BLOOD SAMPLES

3.5.1 Blood sampling

All subjects were offered EMLA cream® for local analgesia. About 30% of the 9-year-old subjects chose to have the cream applied. The fasting status was considered confirmed if the question “When was the last time you ate something?” was satisfactorily answered (i.e. more than 8 hours ago). Blood samples were taken by venipuncture with a minimum of stasis, with the subject in a supine position. Vacuum tubes (Vacuette, Greiner Lab Technologies Inc) were used. Venipuncture was attempted only once per subject. If it was successful, three tubes of 6 ml were taken. First the tube for obtaining serum was filled. Thereafter a tri-sodium citrate tube and an EDTA tube were filled. Serum was obtained after clotting for 30 to 60 minutes at room temperature and centrifuging for 10 minutes at 2000 g, and the plasma tubes were treated similarly. The serum and plasma were then portioned into 0.5 ml cryo vials. The cells from the EDTA and tri-sodium citrate tubes were stored in separate cryo vials. All samples were immediately put in a refrigerator (+4°C), and transferred to a freezer (-80°C) the same day, where they were stored.

3.5.2 Serum analyses

Serum lipid and glucose analyses were performed at Bristol Royal Infirmary, UK. The laboratory is accredited by the Clinical Pathology Accreditation (UK) Limited. The following analytes were measured on an Olympus AU600 autoanalyser (Olympus Diagnostica GmbH, Hamburg, Germany): Total cholesterol using the esterase/oxidase enzymatic method, triglycerides using the lipase/glycerol kinase/glycerol phosphate oxidase enzymatic method, HDL cholesterol using the homogeneous polyanion/cholesterol esterase/oxidase enzymatic method, and glucose using the hexokinase method. Insulin analysis was performed at Huddinge University Hospital, Sweden, using an immunometric method on a Modular Analytics Modul E (Elecsys, Roche Diagnostics GmbH, Mannheim); CV was 5.0% (at 12.3 mU/L). Single-point measurement was performed for all analytes.

3.6 BLOOD PRESSURE

The resting systolic and diastolic blood pressures were measured with an automatic oscillometric method (Dinamap model XL Critikron, Inc., Tampa, Florida.). The equipment has been validated in children (Park & Menard, 1987). An appropriate cuff size was chosen in accordance with the manufacturer's recommendation after measuring the arm circumference, and recordings were made from the left arm. The subjects were in a sitting, relaxed position, and the blood pressure was taken every 2 minutes for at least 10 minutes with the aim of obtaining a set of systolic recordings that did not vary by more than 5 mm Hg. The mean value of the last three recordings was used as the resting systolic and diastolic blood pressures, in mm Hg. A technical check of the automatic blood pressure equipment (Dinamap) against known compressor pressure was performed at Örebro University Hospital, Dept. of Bioengineering, before and after the study period.

3.7 ANTHROPOMETRY

Body weight was measured to the nearest 0.1 kg (SECA digital balance beam, calibrated after transportation) and height to the nearest 0.5 cm (Harpenden transportable stadiometer), with the subjects clad only in their underwear. Body mass index (BMI) was calculated as weight/height^2 (kg/m²). Skinfold thicknesses were measured using a Harpenden caliper (Durnin & Rahaman, 1967). The left side was used for measurements over the m. biceps brachii, m. triceps brachii, m. triceps surae, subscapularly, and superior to the spina iliaca anterior superior (the oblique fold) (Lohman *et al.*, 1988). Two measurements were taken at each anatomical site, and if the difference between the two values was more than 2 mm, a third measurement was taken. The mean of the two best corresponding values was used for further calculations. In these studies the sum of the skinfold thicknesses at the five sites has been used as an indicator of body fat. The relative body fat mass (body fat in per cent of total body weight) was calculated from the triceps and subscapular skinfolds (Slaughter *et al.*, 1988). Fat-free body mass was calculated by subtracting fat mass from body weight. The waist circumference was measured midway between the lower rib margin and the iliac crest, at the end of a gentle expiration. The hip circumference was measured midway between the symphysis level and the level of the spina iliaca anterior superior. Circumferences were measured in duplicate with a metal anthropometric tape, and the mean of the two values was used for further calculations. Pubertal stage, or sexual

maturation, (Tanner I-V) was identified by a researcher of the same gender as the subject (Tanner, 1962). Breast development in girls, and genital development in boys, was used for pubertal classification.

Quality controls were conducted once daily by a double check of skinfold measurements (two different research team members measured the same subject) and no significant inter-tester difference was found (data not shown).

3.8 QUESTIONNAIRES

Three different questionnaires were used in the EYHS:

Parents' questionnaire

The parents reported the genetic background and health status of the subjects, the socio-economic status of the family (i.e. educational level, profession and annual income) and their own smoking and physical activity habits.

Children's questionnaire

The computer-based questionnaire Personal and Environmental Associations with Children's Health (PEACH) was used to provide self-reported information on physical activity related issues. The PEACH questionnaire was developed at the University of Bristol specifically for the EYHS, but is based on previously published questionnaires. The questions used were mainly drawn from *The Child and Adolescent Trial for Cardiovascular Health study* (Edmundson *et al.*, 1996). The questionnaire had questions regarding attitudes and behaviours in the areas of physical activity, diet, smoking, alcohol usage and psychology (e.g. self esteem and attitudes). The questionnaire contains approximately one hundred questions, depending on the alternatives chosen (e.g. if a subject answered "yes" to smoking, there were three following questions).

Non-participant questionnaire

For the follow-up study, 38 of the questions in the PEACH questionnaire were chosen, focusing on physical activity and diet.

3.9 STATISTICS

The software SPSS versions 10.0 to 12.0 were used for all analyses (SPSS Inc., Chicago, IL). Basic descriptive statistics were applied for each age and gender group (Papers I, II, III and IV). Nominal or ordinal data were tested by χ^2 - test (questionnaire data in studies I and II, and test of proportions in study IV). The two-sample t-test was used to compare mean differences in continuous variables between groups (written marks in participants versus non-participants in study I). The paired t-test for testing within-group differences (measured versus calculated oxygen consumption results in the validation study described in Paper III). Analyses of correlation coefficients were performed in study III (measured versus calculated oxygen consumption results in the validation study) and in study IV (the physical activity and cardiorespiratory variables versus the selected CVD risk variables). Relationships between continuous variables were tested by linear regression in study II (physical activity versus serum variables). Analysis of variance was used to test for main effects and interactions between factors in one-, two- or three-way ANOVA. After studying the residuals, appropriate transformations were made (e.g. ln-transformation of triglycerides in study II). All factors used were entered as fixed factors (e.g. age group, gender and socio-economic background group in study II, and age group, gender and study in study III). Post hoc tests were performed as described by Tukey. Holm's multiple test procedure for correction of mass significant effects was applied in studies I and IV (Holm, 1979). Canonical correlation between two sets of variables was done in study IV (Hotelling, 1936). All variables included were standardized (z-transformed) in this analysis. In all analyses, the significance level was set at $\alpha = 0.05$.

4 RESULTS AND GENERAL DISCUSSION

4.1 REPRESENTATIVENESS OF THE SWEDISH EYHS SAMPLE (PAPER I)

The participation rates were 62 per cent among the 9-year-olds and 41 per cent among the 15-year-olds. In both age groups, girls had a higher participation rate than boys (Paper I). These rates may be considered rather low, and might give rise to criticism against the use of this sample as representative of children in central Sweden.

The participation rate *per se* does not say much about the representativeness of the samples, unless it is 100 per cent. What matters most is how the sampling procedure is conducted and whether non-participants differ from participants. Non-response and self-selection are known general problems in any epidemiological study, and have been discussed previously (Nathanson, 1977; Berg *et al.*, 1998; Klesges *et al.*, 1999; Lundström & Särndal, 2001). The risk of losing the most unfit group in studies specifically addressing fitness, has also been considered previously (Grimby *et al.*, 1970). The interest in participation depends partly on how much the subjects have to do in the study, or contribute to it, but may also depend on local traditions, and attitudes of schoolteachers and parents. In Odense, the Danish study location, there is a tradition of similar studies, and perhaps therefore greater awareness of and trust in this kind of activity there (Hansen *et al.*, 1990). In the Danish EYHS, 83 per cent of the 9-year-olds and 67 per cent of the 15-year-olds participated (Wedderkopp, 2001). The participation rates in the Swedish EYHS are similar to those in the Norwegian EYHS, where 75 per cent of the 9-year-olds and 42 per cent of the 15-year-olds participated (Klasson-Heggebø, 2003). The participation rates in the Portuguese and Estonian EYHS were more than 90 per cent (unpublished data). In similar studies including teenagers, participation rates of about 75 per cent have been reported (Hansen *et al.*, 1990; Boreham *et al.*, 1993).

In the extended follow-up study undertaken to compare participants and non-participants, it was found that the data for the chosen Swedish EYHS municipalities corresponded well to national official data regarding socio-economic status (i.e. income, education and profession). High- and low-income catchment areas were distributed to the same extent in participating as in non-participating areas within the respective municipalities. Thus, there was no sampling bias on the regional or school level. Non-manually working parents were slightly over-represented among parents of

participating children. Non-participating subjects corresponded in most respects to participants, with a few exceptions. The primary finding from the questionnaire handed out to all pupils in the follow-up study was that participants and non-participants reported similar attitudes and behaviours in most areas covered by the questionnaire. Of the 38 questions, only the answers to a few questions showed differences between participants and non-participants in the specific age and gender groups: In 9-year-old girls, no difference between participants and non-participants was found; in 9-year-old boys the answers to three questions differed; in 15-year-old girls one answer differed; and in 15-year-old boys two answers differed. The differences mainly implied greater interest in physical exercise among participants than among non-participants. This pattern was also confirmed in the comparison of written marks in the compulsory school subject Physical Education & Health, where the participants had a higher mean value than the non-participants.

Thus, the main finding from the non-participation follow-up study was that the non-participating subjects corresponded in most respects to the participants. Furthermore, the previously described socio-economic effect on propensity to respond was confirmed, i.e. an expected over-representation of children from higher socio-economic families was observed in the participating groups. Our data suggest that in view of the propensity to respond phenomenon, oversampling of disadvantaged groups is required to achieve a representative sample. Taken together, this selection bias might have led to a distribution skewed towards more physically active children, resulting in possible overestimation of values for variables such as physical activity and CRF and possible underestimation of values for anthropometrical measures. As we share this situation with many studies in which participation is truly voluntary, we consider our results to be comparable to those of other population studies in this area in which a maximum fitness test and blood sampling are required.

4.2 DESCRIPTIVE DATA (PAPERS II AND III)

4.2.1 Missing values

In total 1,137 subjects participated in the study, but the number of results differs in almost every single variable. In one school we were not permitted to do skinfold measurements, because of recent cases of anorexia at the school. Four subjects fainted or became uneasy after the blood sampling, and did not take part during the rest of the

test session. A few subjects who were expected to be in the afternoon test group did not show up after lunch. The number of valid recordings and tests performed, the numbers of questionnaires answered, and comments on missing values are given in Table 1.

Table 1. Numbers of valid recordings and tests and numbers of questionnaires answered in the subjects of the Swedish EYHS 1998/1999. N = 1,137.

	n	%	Comments on missing values
Height	1131	99.5	
Weight	1130	99.4	
Skinfold thickness	1072	94.3	No permission from one school. Some partially missing values.
Pubertal status (Tanner score)	1038	91.3	No permission from one school.
Ergometer bike test	935	82.2	Heart rate <185 beats/min (Paper III).
Accelerometer recordings	802	70.5	Technical problems, too short recording time, forgotten or lost accelerometer.
Blood sampling	969	85.2	Failed venipuncture. Some partially missing values. (Paper II).
Blood pressure	1128	99.2	
Childrens' questionnaire	1135	99.8	
Parents' questionnaire	1228		
Non-participants' questionnaire	2360		

4.2.2 Basic characteristics

The basic characteristics are presented in Tables 2a (9-year-old) and 2b (15-year-olds). Eighty-eight per cent of the subjects were reported (in parents' questionnaires) to be Caucasians and 12 per cent were reported to have another genetic background. Since no sub-group exceeded a proportion of 2 per cent, except Caucasians, the subjects were regrouped into the dichotomization "Caucasian" and "Other" genetic background. On average, according to demographic statistics, about 15 per cent of the inhabitants in the included municipalities were first- or second-generation immigrants (Statistics Sweden, 2000). Caucasian boys tended to be taller than boys of other genetic background in both age groups (+2.1 cm, $p=0.160$ and +3.8 cm, $p=0.092$ for 9- and 15-year-old boys,

respectively). In 15-year-old girls, Caucasians were taller (+3.7 cm, $p < 0.001$) than those of other genetic background, while no difference in height was found in 9-year-old girls of different genetic background. No difference was found between the two genetic groups in the serum variables (Paper II). Caucasian 15-year-olds had higher CRF than 15-year-olds of other genetic background (+2.3 and +4.0 $\text{ml} \times \text{min}^{-1} \times \text{kg}^{-1}$ in girls and boys, respectively). In 9-year-olds, no such difference was observed. No difference in total physical activity was noted between the two genetic groups. The only variable found to be influenced by the socio-economic background (unstratified for genetic background) was height, with the largest difference in 15-year-old boys, boys born of university-educated mothers being taller than boys of mothers with only compulsory schooling, by 5 cm, $p = 0.002$ (Paper II).

The prevalence of overweight in the Swedish EYHS sample was 12 per cent as calculated from the age- and gender-specific BMI cut-off points proposed by Cole (Cole *et al.*, 2000). These cut-offs are linked from the adult cut-off centiles, which correspond to BMI =25 or BMI =30 (cut-offs for overweight and obesity, respectively, in adults), to the same centiles in children of different ages and gender. The prevalence of overweight was highest in 9-year-old girls, by about 15 per cent, and in the other groups it was about 11 per cent. The prevalence of obesity in the Swedish EYHS sample was 2 per cent. The 9-year-old boys had the highest prevalence of obesity, i.e. about 4 per cent, and in the other groups it was 1 to 2 per cent. Similar figures for overweight and obesity were found in the recently reported *COMPASS* study of 15-year-olds in Stockholm (Samhällsmedicin *et al.*, 2004). In recent Swedish studies specifically addressing body weight, the prevalence rates of overweight and obesity have been found to be slightly higher than in the Swedish EYHS sample (Berg *et al.*, 2001; Ekblom *et al.*, 2004b).

4.2.3 Smoking

Of the 9-year-old pupils, one boy claimed to smoke regularly, while 8 per cent (38 of 446 subjects) claimed to have “tried a cigarette or a few puffs”. Among the 15-year-old subjects, 12 per cent (56 of 516) claimed to smoke regularly. There was no difference in history of smoking with regard to genetic background ($p = 0.225$) or gender ($p = 0.725$), but SES, assessed as the educational level of the mother, was negatively associated with smoking ($p = 0.039$). No difference at all was found between non-smokers and smokers in the serum variables or in anthropometrical measures. Smoking

prevalence in adults (18-84 years) has recently been reported to be 19 per cent among women and 14 per cent among men, with more smokers among low-SES groups than among middle- or high-SES groups (Statens folkhälsoinstitut, 2004). Thus, the influence of SES on smoking prevalence is similar in children and adults.

Table 2a. Basic characteristics of the 9-year-old subjects, girls and boys, of the Swedish EYHS 1998/1999.

	Mean	SD	Min	Max
Girls, n = 290				
Age (yr)	9.5	0.4	8.5	10.3
Height (cm)	138.9	6.5	122.9	161.0
Weight (kg)	33.6	6.6	21.9	65.3
BMI	17.3	2.4	12.5	29.1
Sum of 5 skinfolds (mm)	50.0	20.5	19.3	157.6
Waist circumference (cm)	60.2	6.1	49.5	93.0
Hip circumference (cm)	72.4	7.0	57.0	97.0
Waist-hip ratio	0.83	0.04	0.71	0.98
Pubertal status, %				
Tanner I	58			
Tanner II	40			
Tanner III	4			
Tanner IV	-			
Tanner V	-			
Boys, n = 272				
Age (yr)	9.5	0.3	8.7	10.3
Height (cm)	139.2	5.8	121.0	154.8
Weight (kg)	33.5	6.2	21.3	61.2
BMI	17.2	2.4	12.9	26.9
Sum of 5 skinfolds (mm)	39.8	18.7	18.4	125.3
Waist circumference (cm)	60.8	6.0	49.7	91.4
Hip circumference (cm)	71.7	6.0	60.1	94.3
Waist-hip ratio	0.85	0.04	0.74	0.97
Pubertal status, %				
Tanner I	99			
Tanner II	1			
Tanner III	-			
Tanner IV	-			
Tanner V	-			

Table 2b. Basic characteristics of the 15-year-old subjects, girls and boys, of the Swedish EYHS 1998/1999.

	Mean	SD	Min	Max
Girls, n = 317				
Age (yr)	15.5	0.4	14.7	16.4
Height (cm)	165.3	6.3	146.9	181.5
Weight (kg)	57.8	8.8	38.9	98.5
BMI	21.2	2.7	15.3	32.2
Sum of 5 skinfolds (mm)	65.2	22.8	26.8	165.7
Waist circumference (cm)	70.0	6.7	54.5	96.5
Hip circumference (cm)	92.3	7.4	64.0	122.0
Waist-hip ratio	0.76	0.05	0.62	0.93
Pubertal status, %				
Tanner I	-			
Tanner II	-			
Tanner III	5			
Tanner IV	45			
Tanner V	50			
Boys, n = 258				
Age (yr)	15.6	0.4	14.8	16.5
Height (cm)	175.6	7.5	152.5	195.8
Weight (kg)	64.0	10.7	38.0	109.0
BMI	20.7	2.8	15.0	32.5
Sum of 5 skinfolds (mm)	41.3	19.4	22.1	153.2
Waist circumference (cm)	73.8	7.1	60.5	109.0
Hip circumference (cm)	91.0	6.9	75.0	116.1
Waist-hip ratio	0.81	0.04	0.73	0.97
Pubertal status, %				
Tanner I	0.5			
Tanner II	0.5			
Tanner III	3			
Tanner IV	17			
Tanner V	79			

4.2.4 Cardiorespiratory fitness

The results of the validation study showed that the Hansen formula, developed for 11-year-olds, was valid for calculation of VO_2 in 9-year-olds and 15-year-olds. The correlation coefficient for measured relative VO_2 versus estimated, or calculated, relative VO_2 was 0.913 ($p < 0.01$) for the 9-year-olds and 0.930 ($p < 0.001$) for the 15-year-olds. The calculated VO_2 was non-significantly underestimated by the Hansen formula compared to the measured VO_2 by about $1 \text{ ml} \times \text{min}^{-1} \times \text{kg}^{-1}$ in 9-year-olds and 15-year-old boys, but overestimated in 15-year-old girls by $3 \text{ ml} \times \text{min}^{-1} \times \text{kg}^{-1}$ ($p = 0.002$). The subjects of the validation study had higher power output than the EYHS subjects, especially the 15-year-old girls, which detracted from the results of the validation study. The Hansen formula was used in the Swedish EYHS without modification. It is important to stress, however, that in the validation study it was not the Hansen protocol that was compared with other protocols, but only the formula for calculation of VO_2 from the calculated power output.

The estimated relative oxygen consumption ($\text{ml} \times \text{min}^{-1} \times \text{kg}^{-1}$, mean \pm SD) was 37.3 ± 5.5 in 9-year-old girls; 42.8 ± 6.6 in 9-year-old boys; 40.4 ± 5.6 in 15-year-old girls; and 51.5 ± 6.5 in 15-year-old boys (Paper III). These values are to be regarded as the peak VO_2 ($\text{VO}_{2\text{peak}}$) reached during the Hansen bike test, and not the maximal oxygen consumption, as the test protocol, as used in the Swedish EYHS, has been considered not to yield a maximum result, especially not in the 9-year-olds (Paper III). The boys had higher $\text{VO}_{2\text{peak}}$ than the girls, by 14 and 27 per cent in 9-year-olds and 15-year-olds, respectively. In the 15-year-olds, this difference is similar to that reported from other studies (Krahenbuhl *et al.*, 1985; Armstrong & Welsman, 1994; McMurray *et al.*, 2002). In the 9-year-olds, the difference is similar to contemporary results (McMurray *et al.*, 2002), but greater than reported previously (Krahenbuhl *et al.*, 1985; Armstrong & Welsman, 1994). The suggested mechanisms for these gender differences include a lower muscle mass, lower stroke volumes and lower postpubertal haemoglobin concentrations in girls (Rowland *et al.*, 2000). The tendency towards a larger difference in $\text{VO}_{2\text{max}}$ between girls and boys already in the 9-year-olds, compared with previous reports, could be due to the earlier onset of female puberty (Liu *et al.*, 2000)

The Swedish EYHS results are in line with the Danish (Wedderkopp, 2001) and Norwegian (Klasson-Heggebø, 2003) EYHS results. In the 9-year-old Swedish EYHS subjects the $\text{VO}_{2\text{peak}}$ values are lower than in previously reported Nordic studies

(Åstrand, 1952; Andersen *et al.*, 1974). In the 15-year-olds the results are similar to previously reported Nordic findings (Åstrand, 1952; Jonsson & Berggren, 1979; Andersen *et al.*, 1987; Jansson & Hedberg, 1991; Ekblom *et al.*, 2004a).

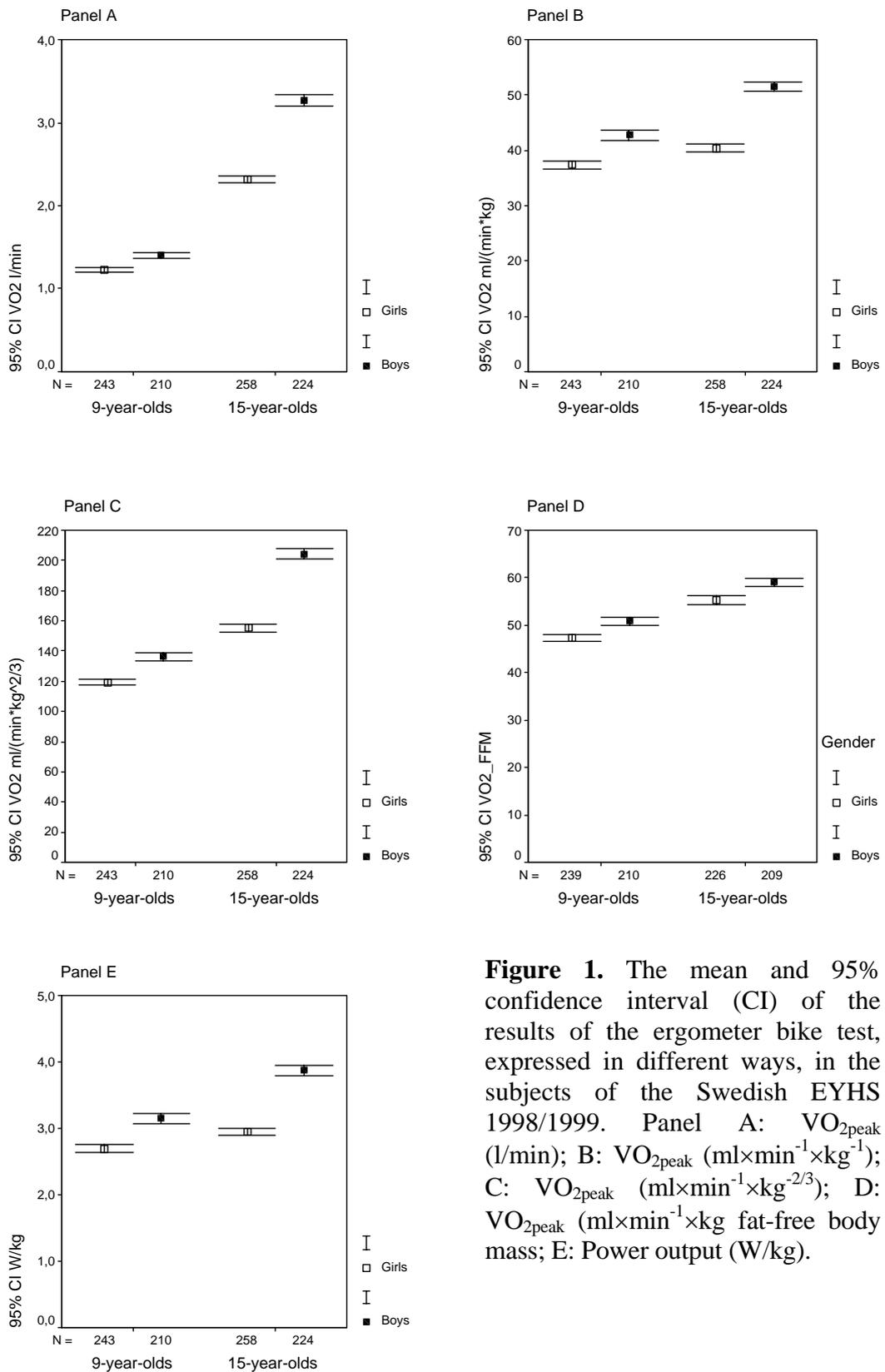


Figure 1. The mean and 95% confidence interval (CI) of the results of the ergometer bike test, expressed in different ways, in the subjects of the Swedish EYHS 1998/1999. Panel A: $\text{VO}_{2\text{peak}}$ (l/min); B: $\text{VO}_{2\text{peak}}$ ($\text{ml} \times \text{min}^{-1} \times \text{kg}^{-1}$); C: $\text{VO}_{2\text{peak}}$ ($\text{ml} \times \text{min}^{-1} \times \text{kg}^{-2/3}$); D: $\text{VO}_{2\text{peak}}$ ($\text{ml} \times \text{min}^{-1} \times \text{kg}$ fat-free body mass); E: Power output (W/kg).

There are several ways of expressing oxygen consumption. Cardiorespiratory fitness can be expressed as VO_{2max} in absolute values, $\text{litres} \times \text{min}^{-1}$, or in relation to body weight, e.g. as $\text{ml} \times \text{min}^{-1} \times \text{kg}^{-1}$. To adjust for differences in body composition, the relation to fat-free body mass was suggested at an early date, as also was adjustment for differences in body size with expression of the values as $\text{ml} \times \text{min}^{-1} \times \text{kg}^{-2/3}$ (von Döbeln, 1956). Measurement as $\text{ml} \times \text{min}^{-1} \times \text{kg}^{-0.75}$ has also been suggested (Svedenhag, 1995), but is less common. The use of allometric scaling factors, which yield the best fit exponent for the specific study population, impedes comparisons between studies. Application of other ratio standards besides body weight may be useful when addressing specific scientific questions. Lean body mass has been shown to correlate with cardiac functional reserve (Rowland *et al.*, 2000), but as a fitness indicator it is not useful for estimation of work capacity, at least not in weight-bearing activities (Rowland, 1996).

The Swedish EYHS results follow the age- and gender-associated variation in VO_{2max} during growth and maturation. A gradual increase in VO_{2max} in relation to chronological age has been reported in boys aged 8-16 years. In girls, a levelling-off or even fall in VO_{2max} has been observed after the age of 13-15 years (Krahenbuhl *et al.*, 1985; Armstrong & Welsman, 1994; Armstrong & Welsman, 2001; McMurray *et al.*, 2002).

Different ways of expressing the oxygen consumption and physical work capacity in the Swedish subjects are presented in Figure 1. The general pattern, seen in all graphs, was that boys of both age groups had higher VO_{2peak} (or power output) than girls of the same age, irrespective of the way in which it was related to body size. Within each gender, the 15-year-olds had higher VO_{2peak} (or power output) values than the 9-year-olds. This consistent pattern, independent of the mode of adjustment for differences in body size, illustrates that the unit of CRF is not crucial when CRF is included in statistical models, as long as it is used consistently. All calculations of interrelationships with CRF in the present studies were based on the W/kg expression, since this was most close to the original data (i.e. power output and body weight).

Secular trends

Judging from Swedish EYHS data, with methodological considerations, data suggest a secular trend towards decreased cardiorespiratory fitness in 9-year-olds, but not in 15-

year-olds (Paper III). In adolescents, this has also been the main finding in other Swedish studies (Westerståhl *et al.*, 2003; Ekblom *et al.*, 2004a). Earlier data on 9-year-olds are very limited and therefore more difficult to interpret (Paper III). In Denmark, no secular change in average CRF was found, but there was a greater difference between the lowest CRF decedile and the highest CRF decedile, comparing data from 1986 with data from 1997 (Wedderkopp *et al.*, 2004). In American girls (over 14 years) a 20 per cent decline in aerobic fitness during the last decades has been reported, while CRF remained relatively stable among boys and younger girls (Eisenmann & Malina, 2002).

4.2.5 Physical activity

In both age groups, boys were more physically active than girls ($p < 0.001$); further, the 9-year-olds were more physically active than the 15-year-olds ($p < 0.001$) (Tab. 3). This is consistent with findings in other studies, both with regard to the gender difference and the decline in total physical activity with increasing age (Caspersen *et al.*, 2000; Riddoch *et al.*, 2004). The same pattern was observed when accelerometer data were categorized into time spent in at least moderate physical activity (>3 METs). Boys of both age groups spent more time in physical activities of moderate or high intensity levels than girls ($p < 0.001$). For time spent in inactivity, no difference was observed ($p = 0.089$ in 9-year-olds and $p = 0.105$ in 15-year-olds); (Fig. 2).

The physical activity level has repeatedly been shown to decline substantially during adolescence (Riddoch & Boreham, 1995; Armstrong & van Mechelen, 1998; Caspersen *et al.*, 2000; Kimm *et al.*, 2000; Sallis *et al.*, 2000; van Mechelen *et al.*, 2000; Trost *et al.*, 2002), and this was also the case both in the Swedish EYHS and in the other four EYHS countries (Riddoch *et al.*, 2004). It has been suggested that a biological explanation for the decline with age might involve the central dopamine system (Ingram, 2000). Gender differences, with girls less physically active than boys, have been reported previously, both from observational (Manios *et al.*, 1999) and from accelerometer studies (Trost *et al.*, 2002). To capture the habitual level of physical activity, it is proposed that monitoring protocols covering seven consecutive days will provide reliable estimates in children and adolescents (Trost *et al.*, 2000). Because of high day-to-day variation, monitoring for more than three days has been suggested for studies in children (Janz, 1994), while three days of monitoring may be sufficient in adults (Tudor-Locke *et al.*, 2005).

Table 3. Results from the accelerometer recordings in the Swedish EYHS 1998/1999 sample in each of the age- and gender groups.

	Mean	SD	Min	Max
9-year-old girls, n = 237				
Total physical activity (cpm)	669	188	258	1321
Moderate+high PA (minutes >3 METs)	183	52	61	337
Inactivity (minutes <100 cpm)	289	63	108	570
9-year-old boys, n = 201				
Total physical activity (cpm)	797	266	206	1755
Moderate+high PA (minutes >3 METs)	217	67	29	411
Inactivity (minutes <100 cpm)	278	73	101	487
15-year-old girls, n = 208				
Total physical activity (cpm)	488	154	151	1081
Moderate+high PA (minutes >3 METs)	69	28	14	149
Inactivity (minutes <100 cpm)	437	63	260	613
15-year-old boys, n = 156				
Total physical activity (cpm)	561	199	212	1214
Moderate+high PA (minutes >3 METs)	82	38	18	268
Inactivity (minutes <100 cpm)	425	84	217	620

cpm, counts per minute (mean counts per minute during total recorded time); PA, physical activity; MET, metabolic energy turnover

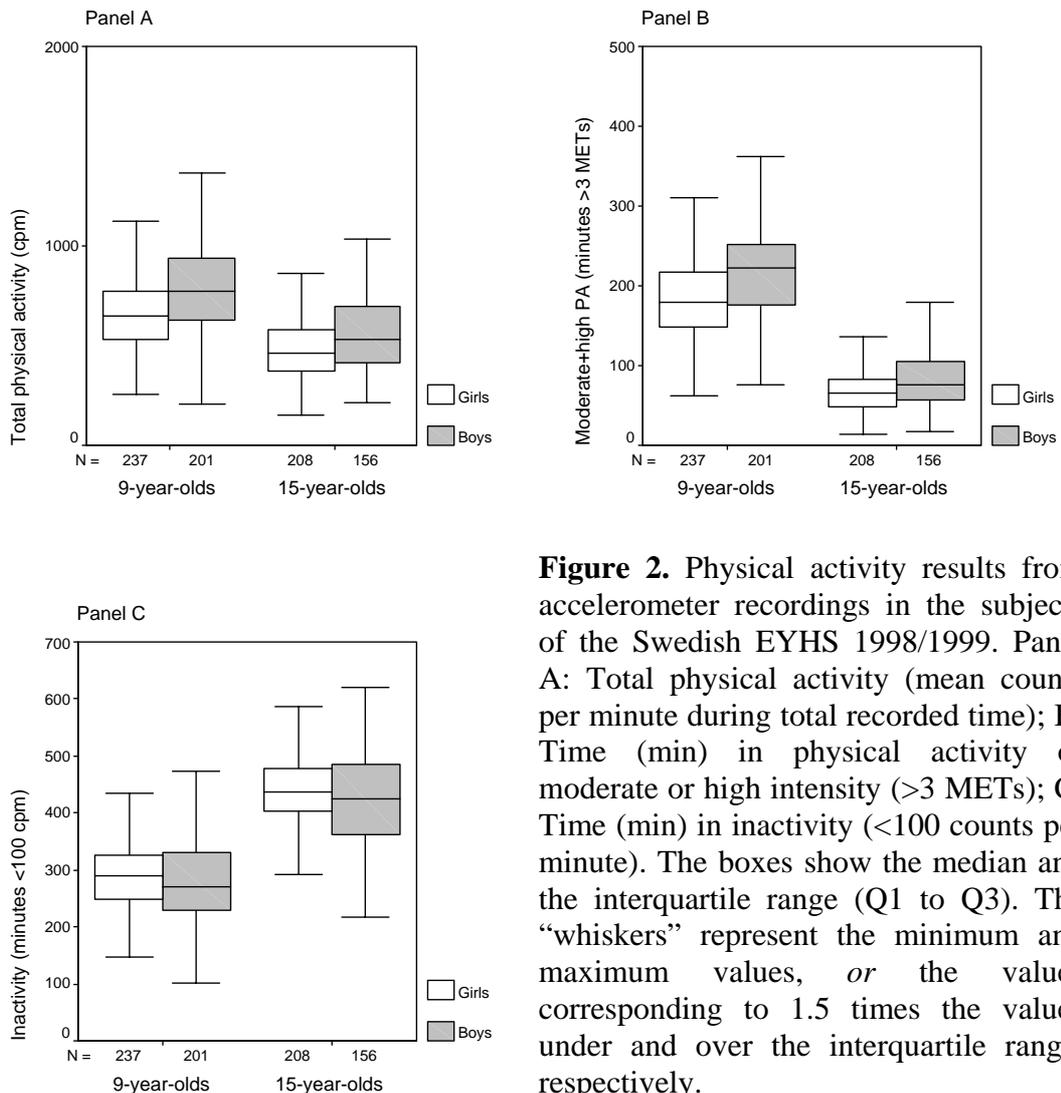


Figure 2. Physical activity results from accelerometer recordings in the subjects of the Swedish EYHS 1998/1999. Panel A: Total physical activity (mean counts per minute during total recorded time); B: Time (min) in physical activity of moderate or high intensity (>3 METs); C: Time (min) in inactivity (<100 counts per minute). The boxes show the median and the interquartile range (Q1 to Q3). The “whiskers” represent the minimum and maximum values, or the values corresponding to 1.5 times the values under and over the interquartile range, respectively.

4.2.6 Serum analysis

Glucose and insulin

The glucose values showed only minor variations in the Swedish EYHS subjects, but nevertheless a main effect of gender and sexual maturation was observed (Paper II). An approximately 30 per cent decrease in insulin sensitivity during the pubertal transition from Tanner I to III has been demonstrated in a longitudinal study (Goran MI 2001). This was also seen in the Swedish EYHS study (Fig. 3).

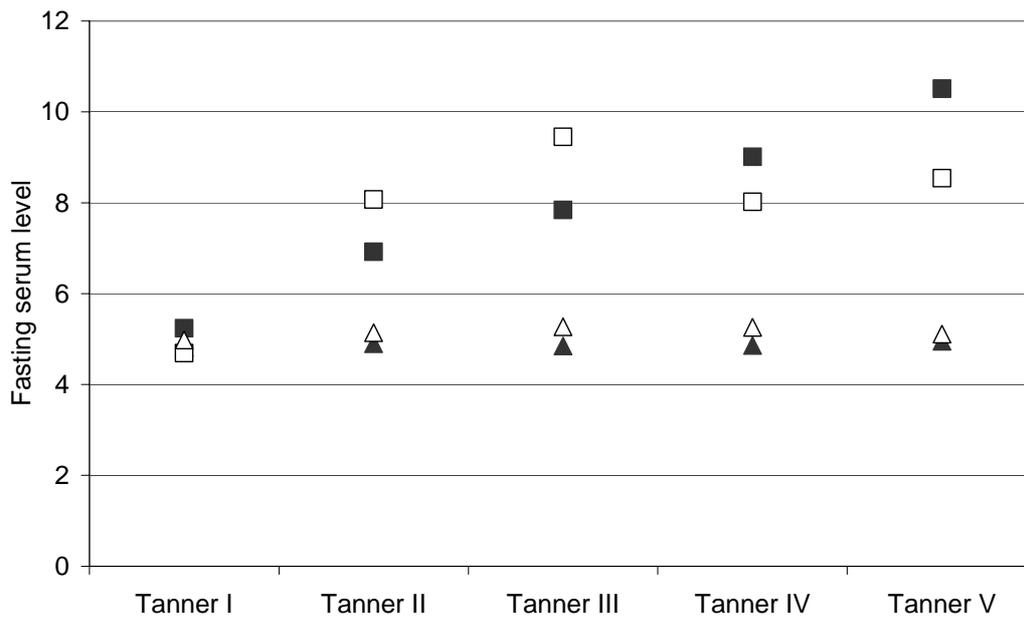


Figure 3. Fasting serum levels of insulin (mU/l) and glucose (mmol/l) in the subjects of the Swedish EYHS 1998/1999, divided into sexual maturation stage groups according to Tanner (1962).

■ = Girls insulin, mU/l (ELECSYS) ▲ = Girls glucose, mmol/l
 □ = Boys insulin, mU/l (ELECSYS) △ = Boys glucose, mmol/l

It is interesting to note that the suggested Nordic upper reference glucose value, 6.3 mmol/l in plasma (Rustad *et al.*, 2004), is clearly above the diagnostic threshold for impaired glucose regulation, now designated as impaired fasting glucose according to the American Diabetes Association: IFG is defined as fasting plasma glucose between 5.6 and 6.9 mmol/l (American Diabetes Association, 2004). When the 5.6 mmol/l threshold was used, 30 Swedish EYHS subjects were found to have IFG, but none had glucose value >6.3 mmol/l.

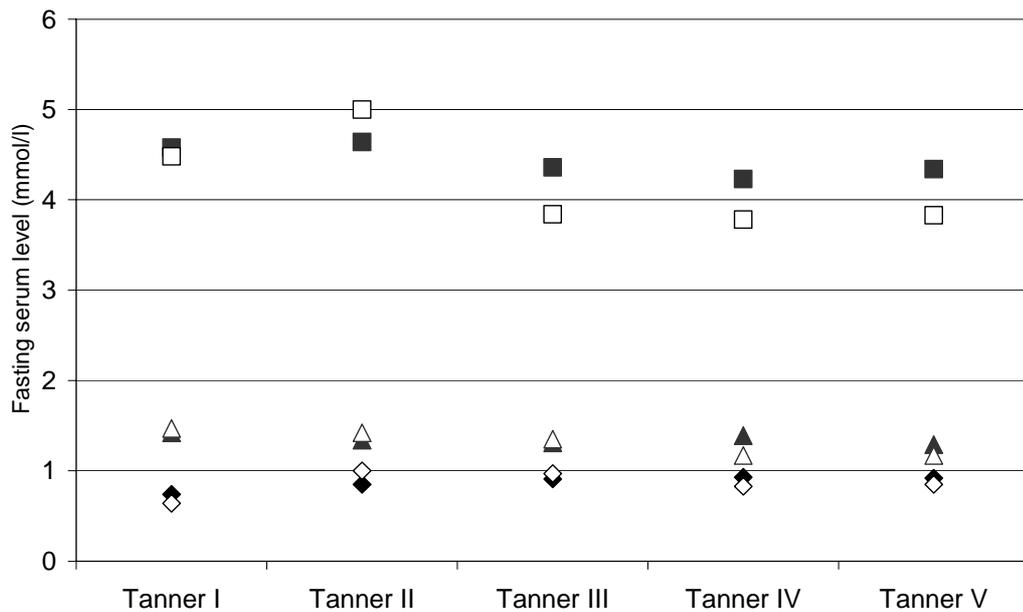


Figure 4. Fasting serum levels (mmol/l) of triglycerides, total cholesterol and HDL cholesterol in the subjects of the Swedish EYHS 1998/1999, divided into sexual maturation stage groups according to Tanner (1962).

■ = Girls triglycerides ▲ = Girls total cholesterol ◆ = Girls HDL cholesterol
□ = Boys triglycerides △ = Boys total cholesterol ◇ = Boys HDL cholesterol

Lipids

The levels of triglycerides, total cholesterol and HDL cholesterol in the Swedish EYHS subjects are in line with findings in the Danish EYHS (Wedderkopp, 2001) and in other studies (Hickman *et al.*, 1998; Samuelson *et al.*, 2001). There was a general pattern, however, of approximately 10 per cent lower total lipid values in the Swedish EYHS sample compared to studies conducted in Northern Sweden (Larsson *et al.*, 1991; Bergström *et al.*, 1995). Since the time of these northern studies (1987-1990), a general decline in mean cholesterol levels has been reported (Porkka *et al.*, 1997; Evans *et al.*, 2001), so whether the tentative difference is the result of a secular trend or is a difference between north and central Sweden, is not known. The distribution of triglycerides, total cholesterol and HDL cholesterol across the sexual maturation groups is shown in Figure 4.

The methodological problem of insulin analysis

Initially, an enzyme immunoassay, microtitre plate format, was used for insulin analyses at Bristol Royal Infirmary, UK (DAKO Diagnostics Ltd., Ely, England). The coefficient of variation reported from the laboratory was 6.9 per cent (at 15.9 mU/l) or 5.9 per cent (at 51.3 mU/l).

On the DAKO result list there were 40 individuals (4 per cent) with an insulin value of >26 mU/L, in combination with a glucose level of <6.0 mmol/L. These individuals made us very observant of the insulin results. Their other variables of the subjects did not indicate a metabolic syndrome profile (e.g. the subjects had low to average weight, average to high fitness). *Graphically*, the insulin point of 26 mU/l was identified as a cut-off in the positive tail of the histogram, which suggested the data points as possible outliers. These subjects could have been regarded as not being in a fasting state, but as insulin levels may fluctuate widely during puberty (Cook *et al.*, 1993; Caprio, 1999), these subjects could not be excluded from further analysis of the data. Moreover, a medical follow-up was needed and arranged for. Of the subgroup (n = 12) who attended the medical follow-up at the Department of Pediatrics, Örebro University Hospital, all showed a lower glucose level than in the first sample (median values 5.0 and 4.2 mmol/l in the first and second sample, respectively), and all except one subject had fasting serum insulin <20 mU/l (median=11.2 mU/l) at the follow-up (chemiluminescence immunoassay, Advia Centaur, Bayer, FRG): Thus, none of the subjects in this subgroup had manifest insulin resistance. Whether the previous results were due to a non-fasting state on the EYHS test occasion, to timing of the peak of pubertal insulin resistance or to a methodological problem was still not known, and remained an important issue for the decision on how to handle the data analysis – whether to exclude them or include them ?

Theoretically, the influence of food intake on the insulin level lasts longer than that on the on glucose level. While the glucose level normalizes within 60-90 minutes, the insulin response to food intake persists for about 3 hours (Sundell *et al.*, 1989). If this is applied to the test situation, food intake before coming to the test, despite oral confirmation of a fasting state, would seem the most plausible explanation of high insulin values in combination with normal glucose values. The timetable for the test situation coincided with the differences in response times for the two variables. This suggested that the above data should be excluded.

Statistically. All statistical analyses were made both with inclusion and exclusion of these individuals. Excluding them made the increase in insulin with increasing maturity become more evident, especially in boys, and made relations to studied predictor variables marginally stronger, but did not change any relationship from non-significant to significant. These effects did not support the graphically based suggestion that the subjects should be excluded. Misclassifications, in general, decrease the strength of relationships. At this stage we suggested that all insulin results should be included.

Insulin re-analysis. Finally, all DAKO insulin samples >20 mU/l ($n=90$) and, randomly selected, an equal number of DAKO samples <20 mU/l were analysed at Huddinge University Hospital, Sweden, using the ELECSYS, a method considered to be clinically relevant on an individual level (Elecsys, Roche Diagnostics GmbH, Mannheim). The correlation coefficient between the DAKO >20 mU/l and ELECSYS values was low ($r = 0.112$, $p=0.352$), while the correlation coefficient between DAKO samples <20 mU/l and ELECSYS was high ($r = 0.754$, $p<0.001$). A regression factor adjustment of DAKO results was discussed, but rejected. It was graphically obvious that DAKO >20 mU/l not only gave a higher mean value, but also in some cases very large differences between the two methods (Fig. 5). All serum samples were then analysed with the ELECSYS methods, and only these results are used in the reporting of Swedish EYHS results. For comparisons within the international EYHS, however, the DAKO results are used, but with the samples with an insulin level of >26 mU/l excluded.

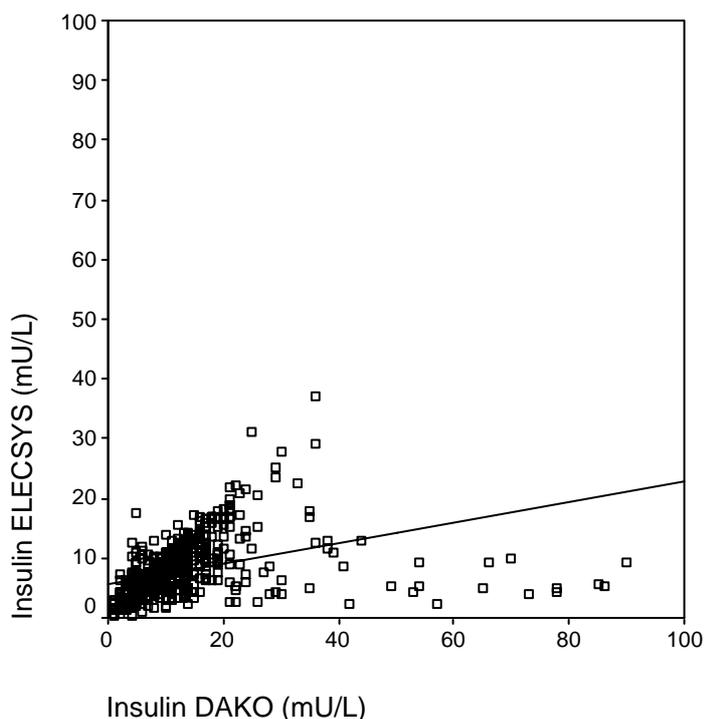


Figure 5. Scatter plot of the insulin levels measured by two different methods of assay in the Swedish EYHS 1998/1999 subjects. Regression equation, $y = 0.17x + 5.64$

4.2.7 Blood pressure

Systolic blood pressure (mm Hg; mean \pm SD) was 104 ± 7 in 9-year-old girls, 104 ± 6 in 9-year-old boys, 109 ± 8 in 15-year-old girls and 116 ± 11 in 15-year-old boys. The 9-year-olds had lower systolic pressure than the 15-year-olds ($p < 0.001$). A gender difference was found only in the 15-year-old group, where the boys had higher systolic blood pressure than the girls ($p < 0.001$). Diastolic blood pressure (mm Hg; mean \pm SD) was 62 ± 6 in 9-year-old girls, 62 ± 6 in 9-year-old boys, 64 ± 6 in 15-year-old girls and 63 ± 6 in 15-year-old boys. The 9-year-olds had lower diastolic pressure than the 15-year-olds ($p = 0.003$). No gender difference was found. The distributions are illustrated in Figure 6. Suggested cut-off values for high systolic blood pressure in children are 126 mm Hg for 9-year-olds and 136 mm Hg for 15-year-olds (Boreham *et al.*, 1993). Application of these cut-offs in the present study resulted in only one 9-year-old boy and two 15-year-old girls with hypertension. Among the 15-year-old boys, eight (3 per cent) had hypertension. The blood pressure levels are in accordance with the results in the Norwegian EYHS, and with the pooled EYHS results from Norway, Denmark, Estonia and Portugal (Klasson-Heggebø, 2003) and also with other pooled international

data (Brotons *et al.*, 1998), but are lower than blood pressure levels reported from the *Northern Ireland Young Hearts Project* (Boreham *et al.*, 1993).

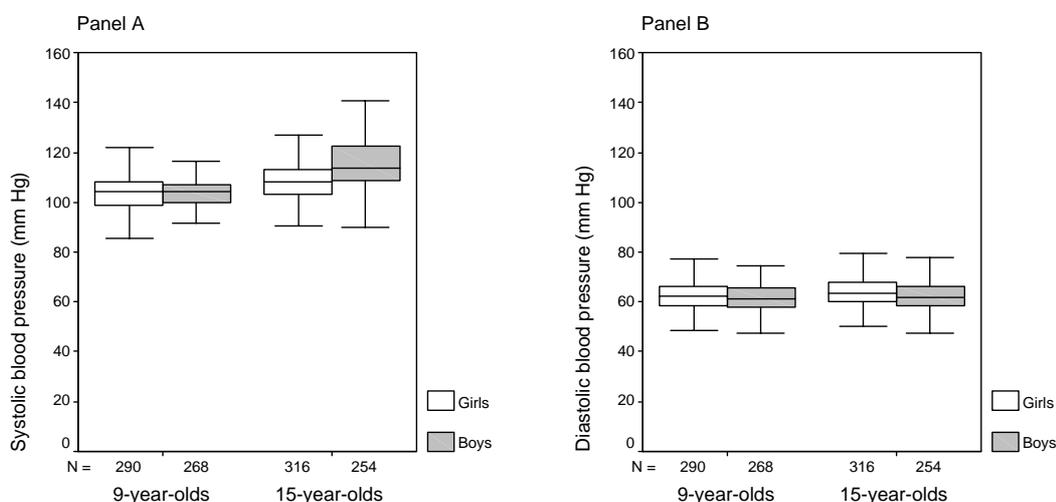


Figure 6. Blood pressure results in the subjects of the Swedish EYHS 1998/1999. Panel A: Systolic blood pressure (mm Hg). Panel B: Diastolic blood pressure (mm Hg). The boxes show the median and the interquartile range (Q1 to Q3). The “whiskers” represent the minimum and maximum values, *or* the values corresponding to 1.5 times the values under and over the interquartile range, respectively.

4.3 INTERRELATIONSHIPS (PAPERS II AND IV)

Previous studies on CVD risk factors have shown relationships of physical activity (PA), fitness (CRF) and fatness (sum of skinfolds; SSF) to other CVD risk factors. In the present statistical analysis of the relationships between the studied CVD risk variables, CRF, PA and SSF were mainly used as predictors, and the other variables (serum variables and blood pressure) as response variables. The standardized regression coefficients between the predictors and the response variables are presented in Table 4.

4.3.1 Associations between the predictor variables

The relationships between the predictor variables are also important to know for an understanding of the results in Table 4. The standardized regression coefficients between CRF and PA ranged from 0.116 to 0.276 across the age and gender groups. The explanatory power, $R^2(\text{adj})$, of the association between CRF and PA ranged between 1 and 7 per cent across the age and gender groups. Thus, the variations in CRF

were only to a limited extent explained by variance of PA (or vice versa), with higher values in the girls than in the boys (Fig. 7).

The standardized regression coefficients between CRF and SSF ranged from -0.502 to -0.633 across the age and gender groups. The explanatory power, $R^2(\text{adj})$, of the association between CRF and SSF ranged between 25 per cent and 44 per cent; thus the variations in CRF were to a high extent explained by the variations in SSF (or vice versa). Finally, the standardized regression coefficients between PA and SSF ranged from -0.059 to -0.153 across the age and gender groups and the explanatory powers, $R^2(\text{adj})$, of the association between PA and SSF were all close to zero; thus the variations in SSF were not explained by the variations in PA (or vice versa).

Table 4. Standardized regression coefficients between the predictor variables cardiorespiratory fitness (CRF), total physical activity (PA) and sum of skinfolds (SSF), and the serum and blood pressure response variables, in the Swedish EYHS 1998/1999 subjects.

		Insulin	Glucose	Triglyc	Tot chol	HDL	Systolic	Diastolic
9-year-olds								
Girls	CRF	-0.265**	-0.177*	-0.247**	-0.104	0.195**	-0.104	-0.029
	PA	-0.092	0.010	-0.111	-0.000	0.158	0.013	-0.034
	SSF	0.422**	0.169**	0.291**	0.048	-0.265**	0.213	-0.046
Boys	CRF	-0.326**	-0.085	-0.174*	-0.033	0.119	0.047	0.101
	PA	-0.058	-0.060	-0.131	-0.007	0.064	0.061	-0.045
	SSF	0.273**	0.107	0.103	0.132	-0.147*	-0.081	-0.073
15-year-olds								
Girls	CRF	-0.342**	-0.133*	-0.046	-0.086	0.165*	-0.055	0.020
	PA	-0.190*	-0.132	-0.066	-0.235**	-0.024	-0.129	-0.118
	SSF	0.251**	0.069	-0.004	0.055	-0.142	0.138*	0.012
Boys	CRF	-0.406**	-0.074	-0.335**	-0.082	0.235**	-0.111	-0.207**
	PA	-0.175*	-0.093	0.003	0.055	-0.011	-0.040	-0.124
	SSF	0.616**	0.158**	0.424**	0.098	-0.267**	0.184**	0.177**

* p<0.05; ** p<0.01

Triglyc, triglycerides; Tot chol, total cholesterol; HDL, HDL cholesterol; Systolic, systolic blood pressure; Diastolic, diastolic blood pressure.

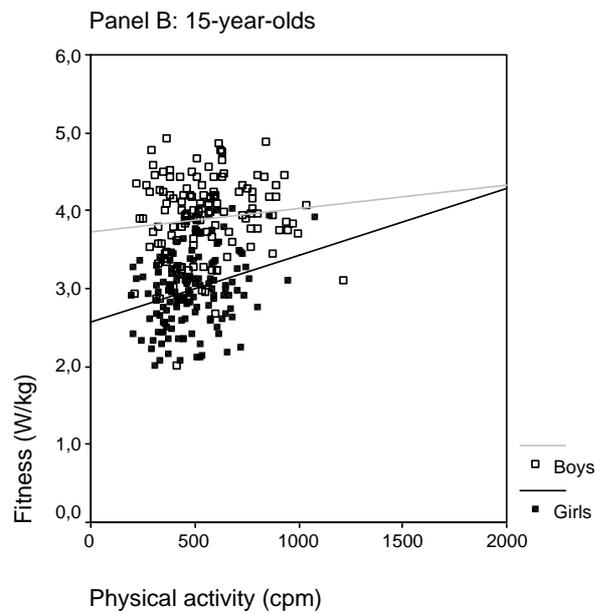
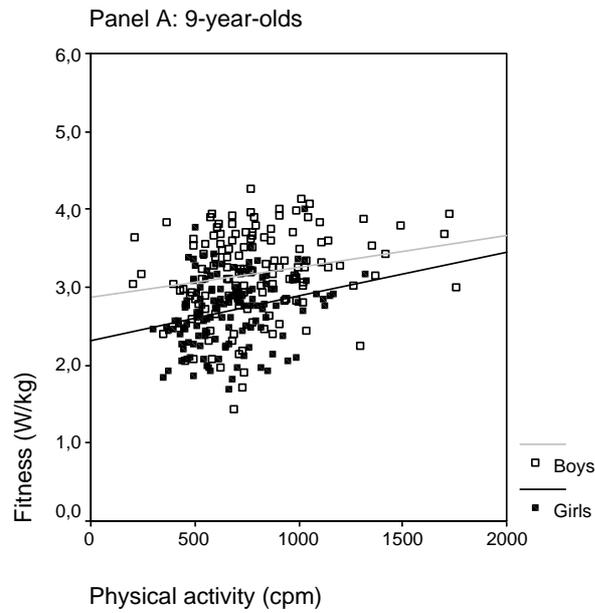


Figure 7. Association between cardiorespiratory fitness (W/kg) and total physical activity (mean counts per minute, cpm, during total recording period) in the Swedish EYHS sample 1998/1999. Panel A: 9-year-old girls (regression equation, $y = 0.0006x + 2.32$) and boys (regression equation, $y = 0.0005x + 2.86$); Panel B: 15-year-old girls (regression equation, $y = 0.0009x + 2.57$) and boys (regression equation, $y = 0.0003x + 3.74$).

4.3.2 Fitness v. serum variables and blood pressure

Cardiorespiratory fitness (W/kg) was generally negatively associated with all response variables, except with HDL cholesterol, to which it was positively associated, as expected. The explanatory power, $R^2(\text{adj})$, of the associations was generally <10 per cent. The highest $R^2(\text{adj})$ values were found for insulin in 15-year-old girls and boys, where they were 11 and 16 per cent, respectively. CRF was weakly associated with the blood pressure measures. The highest $R^2(\text{adj})$ was found for diastolic blood pressure in 15-year-old boys (4 per cent).

A higher level of fitness, independently of body fatness, has consistently been associated with a favourable CVD risk profile in adults (Blair *et al.*, 1995). This is also mainly the case in studies in children (McMurray *et al.*, 2000; Eisenmann, 2004), but several studies have indicated that such relationships may be mediated by body fatness rather than by CRF per se (Tell & Vellar, 1988; Bergström *et al.*, 1997; Boreham *et al.*, 2001). From the longitudinal *Muscatine Study*, it has been reported that decreases in fitness precede adiposity outcomes, thus suggesting a cause-effect model (Janz *et al.*, 2002). When fitness is, conventionally, expressed in relation to body weight (e.g. watt per kg or oxygen consumption per kg) it shows by definition an inverse relationship to body size, or body weight, and may obscure the separate influence of body composition and fitness per se. Twisk and co-workers have reported findings at a re-analysis of fitness values in which maximal oxygen consumption divided by body mass was compared to maximal oxygen consumption corrected for body fatness. The strength of the relationships found between fitness and CVD risk variables was attenuated when maximal oxygen consumption was corrected for body fatness (Twisk *et al.*, 2002).

4.3.3 Physical activity v. serum variables and blood pressure

Total physical activity (cpm) accounted for a limited part of the variance in the response variables. Values of $R^2(\text{adj})$ were generally below 2 per cent. The two highest $R^2(\text{adj})$ for physical activity as a predictor were found for insulin and total cholesterol in 15-year-old girls, where they were 3 and 5 per cent, respectively.

In studies in adults, the terms fitness and physical activity are often used interchangeably, and the fitness level of an adult is considered to depend mostly on the physical activity (Bouchard & Pérusse, 1994). The relationship between physical activity and cardiorespiratory fitness has been found to be weaker in children than in

adults. In a review of studies in children (Morrow & Freedson, 1994), median correlation coefficient of $r = 0.17$ was reported, thus a weak relationship in general, and our results are similar. It has been suggested that growth and maturation explain the major part of the variance in fitness in children, as this variation follows a pattern similar to that of increases in height and weight (Malina, 1994). This association was also observed in our study.

The influence of physical activity on body weight in children and adolescents is controversial (Rowlands *et al.*, 2000). Future studies of the influence of physical activity on body weight require investigation of more details of energy expenditure and energy intake.

Physical activity has also been found previously to be weakly associated with lipid levels (Armstrong & Simons-Morton, 1994) and blood pressure (Casperson *et al.*, 1998). In addition, longitudinal studies have indicated that childhood levels of physical activity are weakly associated with adult levels of CVD risk factors (Kemper *et al.*, 1990; Lefevre *et al.*, 2002). In the *Northern Ireland Hearts Projects*, adolescent physical activity has been reported to be unrelated to the later CVD risk status (Boreham *et al.*, 2002). The most frequently observed weaker dose-response gradient for PA versus other CVD risk factors than for CRF versus other CVD risk factors, has been suggested to be due to greater misclassification of self-reported PA, compared to the more objectively measured CRF. Using objectively measured PA and multivariate statistical analyses (studies in adults), Wareham and co-workers have shown habitual energy expenditure to be a stronger than fitness as an independent predictor of blood pressure (Wareham *et al.*, 2000b), glucose tolerance (Wareham *et al.*, 2000a), fasting concentration of non-esterified fatty acids (Franks *et al.*, 2002), and an index of the metabolic syndrome (Franks *et al.*, 2004). On the other hand, in a doubly labelled water study, Dvorak and co-workers found that higher levels of CRF had greater cardioprotective effects than higher levels of PA in older individuals (Dvorak *et al.*, 2000).

4.3.4 Sum of skinfolds v. serum variables and blood pressure

Sum of skinfolds (mm) was in general positively associated with all response variables, except with HDL cholesterol, with which it was negatively associated, as expected. Among the predictor variables, SSF had generally higher $R^2(\text{adj})$ values compared to CRF and physical activity. SSF accounted for 6 to 38 per cent of the variance in insulin,

with the highest $R^2(\text{adj})$ in 15-year-old boys. In the same group, SSF also accounted for 18 per cent of the variance in triglycerides. This influence of body fat on CVD risk factors is well established in the literature (Freedman *et al.*, 1999; Twisk *et al.*, 2000; Caprio, 2002; Goran *et al.*, 2003).

Among the predictors, we found the strongest associations between SSF and the CVD risk variables. The dependent structures between the predictor variables SSF and CRF, however, make the interpretation difficult. Body weight and variables including body weight are closely related to all variables depending on growth and maturation. This suggests that investigations of the influence of body weight on response variables in children require a study design which allows this collinearity problem to be addressed (e.g. matched control study).

4.3.5 Focus on insulin

As shown in Table 4, insulin was the variable most frequently significantly associated with the predictor variables, and a focus on insulin was therefore considered relevant.

Insulin versus physical activity

There was a covariation between seasonal changes in PA and mean serum levels of insulin in both age groups, as observed in study II. Insulin was lowest during May, although it was only significantly lower than in February (winter period), which was the month of the second lowest PA ($p < 0.05$). Standardized regression coefficients for the relations between insulin and PA during the period January-May were -0.166 ($p < 0.05$) in 9-year-olds (girls and boys together), and -0.353 ($p < 0.001$) in 15-year-olds (girls and boys together). During all months of testing these coefficients were -0.116 ($p < 0.05$) in 9-year-olds, and -0.180 ($p < 0.01$) in 15-year-olds. Seasonal effects were not observed in any other studied variable (Paper II).

In hyperinsulinaemic subjects, an insulin-lowering effect of an increase in physical activity of low intensity has been demonstrated (Laaksonen *et al.*, 2002). Life-style interventions, including physical activity, have been shown to reduce the incidence of diabetes by more than half in persons with impaired glucose tolerance (Tuomilehto *et al.*, 2001), but also in a Finnish population study of healthy subjects (Hu *et al.*, 2003). The complete biological mechanisms by which insulin sensitivity is enhanced by physical activity are still under investigation. Enhanced glucose transport into muscle

cells through GLUT 4 is described (Richter *et al.*, 2001; Zierath, 2002). Other suggested mechanisms are increased post-exercise synthesis of muscle glycogen (Perseghin *g* 1996). Skeletal muscle is the principal site of glucose uptake (DeFronzo *et al.*, 1981). A higher percentage of oxidative type muscle fibres and higher capillary density in skeletal muscles is associated with improved insulin sensitivity (Hedman *et al.*, 2002). These characteristics correspond to the adaptation of muscle fibres to aerobic exercise described previously (Gollnick *et al.*, 1972; Eriksson *et al.*, 1974). A relationship between physical activity and indices of insulin resistance has been observed in studies with objectively measured (Brage *et al.*, 2004a; Brage *et al.*, 2004b) and self-reported (Schmitz *et al.*, 2002) physical activity.

Insulin versus body composition

Body composition, expressed as SSF in Table 4, was significantly associated with insulin in all age and gender groups. In study II, an extended analysis of the relationship between insulin and body composition, using BMI as indicator of body composition, showed that only subjects in the highest BMI groups (in each gender and age group) had statistically significantly higher insulin levels than all other subjects. This was also seen when SSF was used as an indicator of body composition (Fig. 8). The adverse effect of body fatness on the insulin level was limited to children within the highest SSF groups. In study II, BMI was used because of the more easily applied BMI cut-offs for an increased risk of high insulin levels in growing children, as skinfold measurements are rarely used clinically.

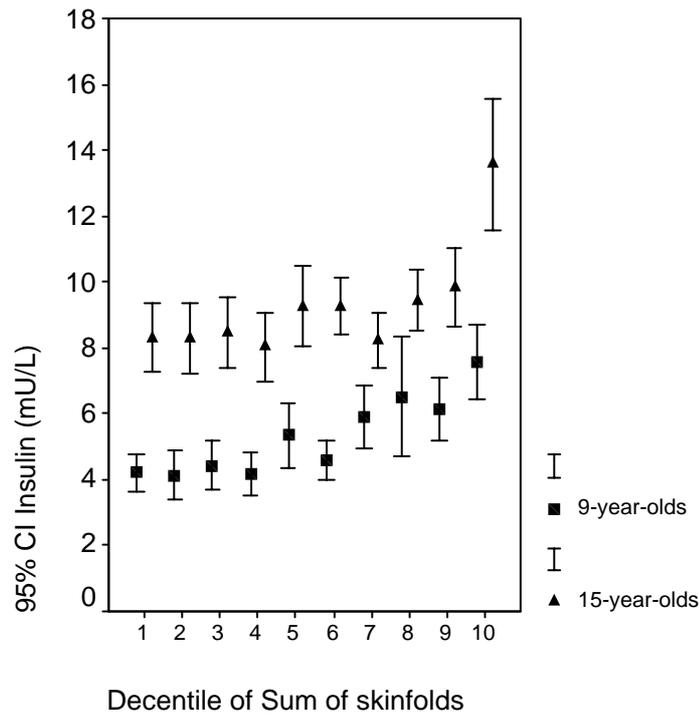


Figure 8. Means and 95% confidence intervals (CI) of insulin per decentile of sum of skinfolds are shown for the two age groups in the Swedish EYHS 1998/1999.

4.3.6 Fitness versus physical activity

In attempts to understand the separate influence of fitness and physical activity on each variable, numerous figures have been constructed. The visual impression in the present study was that the lowest fitness quartiles, in general, yielded an unfavourable level of the response variable compared to the higher fitness quartiles. No such consistent pattern was observed for the physical activity quartile group. The pattern was most clear for the insulin variable (Fig. 9a).

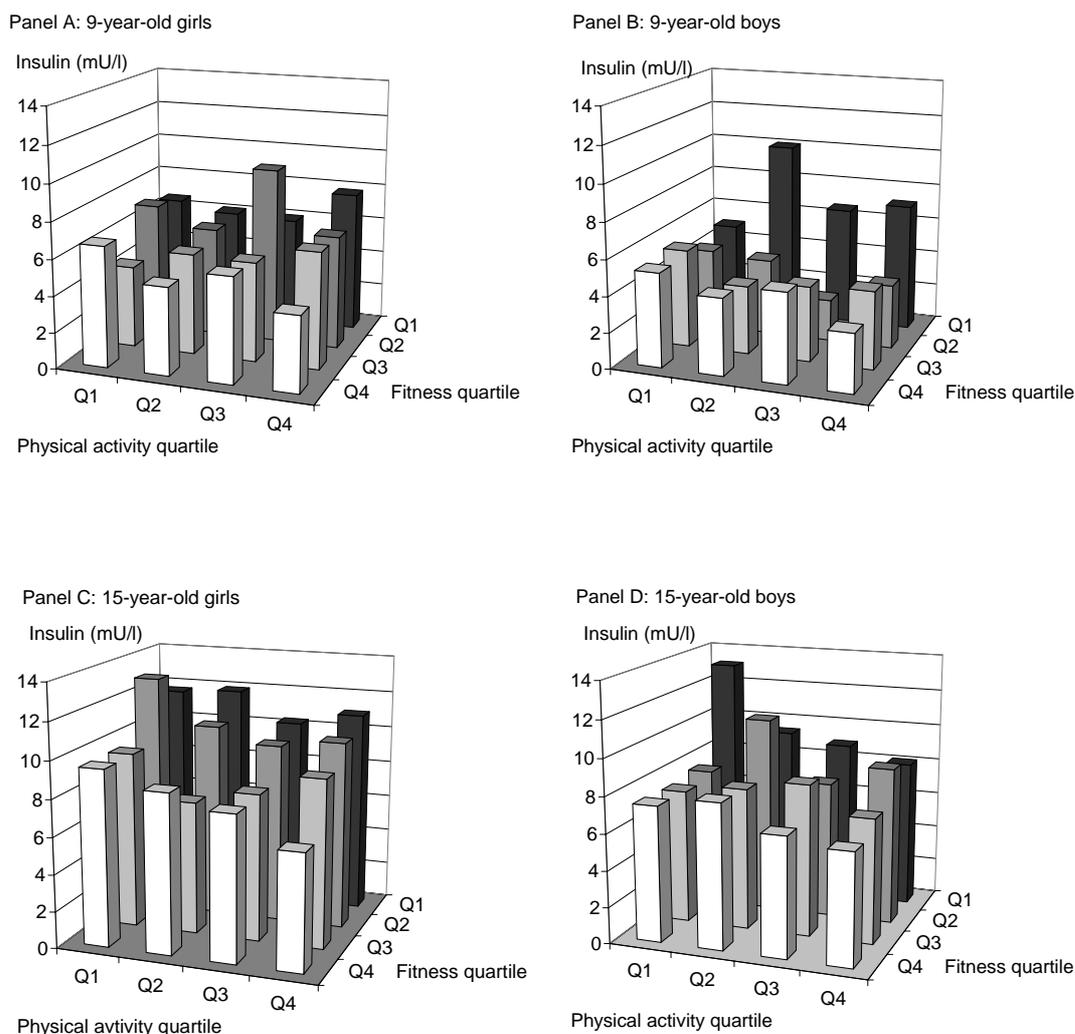
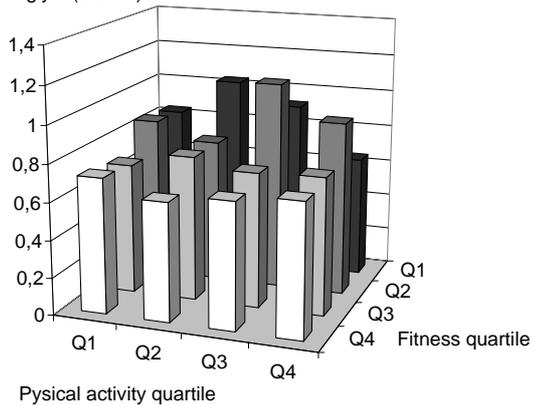


Figure 9a. The interactive effect of physical activity (cpm) and fitness (W/kg) on fasting level of insulin (mU/l) in the subjects of the Swedish EYHS 1998/1999.

The “response surface” patterns in Figure 9a clearly indicated that in 15-year-olds both physical activity and fitness contributed to the fasting insulin levels. In the case of triglycerides (Fig. 9b), the fitness quartile contributed more than did the physical activity quartile. The “response surface” pattern for HDL cholesterol (Fig. 9c) showed only minor differences between the quartile groups, and generally indicated that fitness was more influential than physical activity. This is in contrast to findings in adults, where an increase in HDL is the most commonly reported activity-induced lipid change (NIH Consensus Development Panel, 1993).

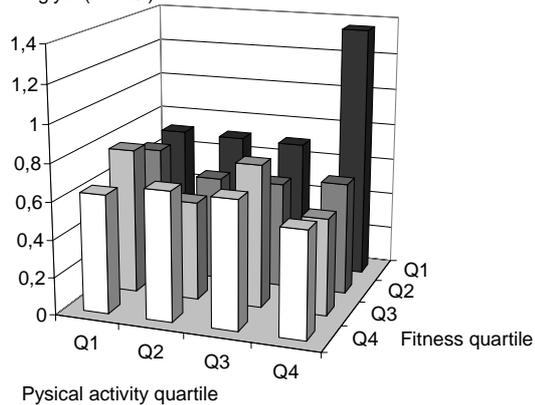
Panel A: 9-year-old girls

Triglyc. (mmol/l)



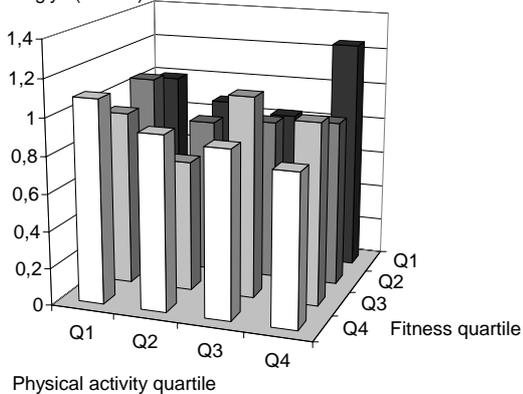
Panel B: 9-year-old boys

Triglyc. (mmol/l)



Panel C: 15-year-old girls

Triglyc. (mmol/l)



Panel D: 15-year-old boys

Triglyc. (mmol/l)

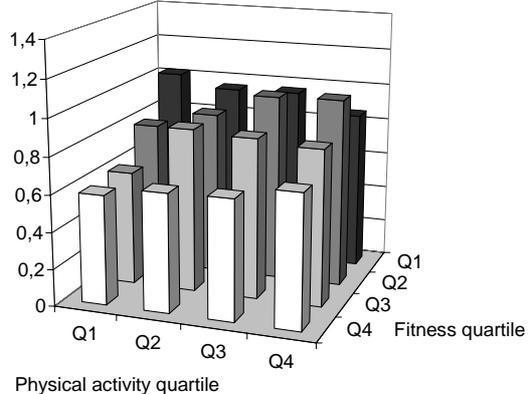
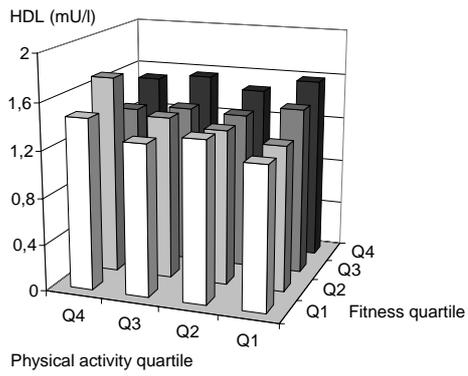
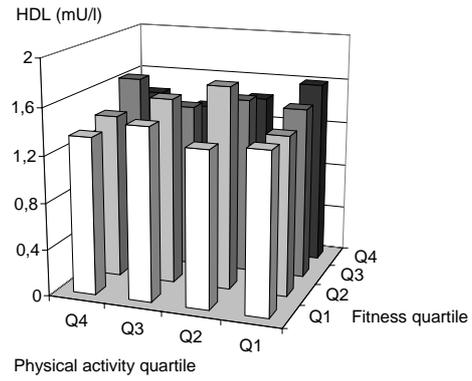


Figure 9b. The interactive effect of physical activity (cpm) and fitness (W/kg) on fasting level of triglycerides (mmol/l) in the subjects of the Swedish EYHS 1998/1999.

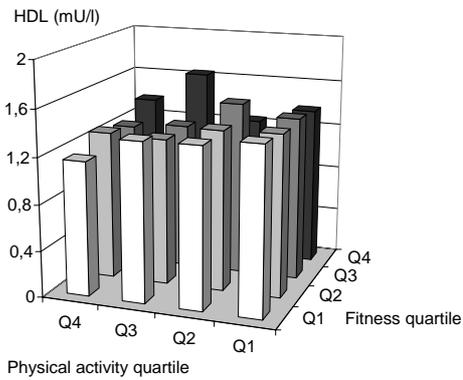
Panel A: 9-year-old girls



Panel B: 9-year-old boys



Panel C: 15-year-old girls



Panel D: 15-year-old boys

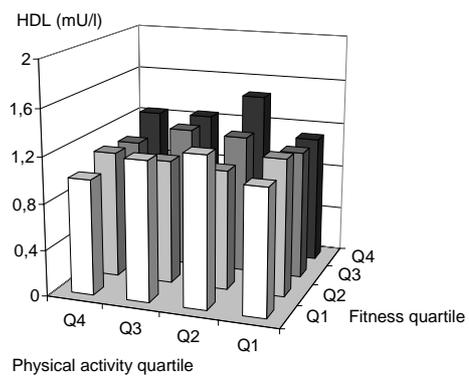


Figure 9c. The interactive effect of physical activity (cpm) and fitness (W/kg) on fasting level of HDL cholesterol (mmol/l) in the subjects of the Swedish EYHS 1998/1999.

As a result of the age- and gender-specific levels of all risk factors (Paper II), the main disadvantage of handling data in this way is the decreasing group sizes when the age and gender groups are split into 16 other subgroups (4 fitness quartiles \times 4 physical activity quartiles). The advantage is that an age- and gender-specific pattern, if any, can be detected. To increase group sizes, z-values ($z = [\text{value} - \text{mean}]/\text{SD}$) were calculated within each age and gender group. The age and gender groups could thereby be combined (Fig. 10a-c). The age and gender perspective was then lost, as well as the picture of what the differences meant in absolute values (e.g. mmol/l of triglycerides).

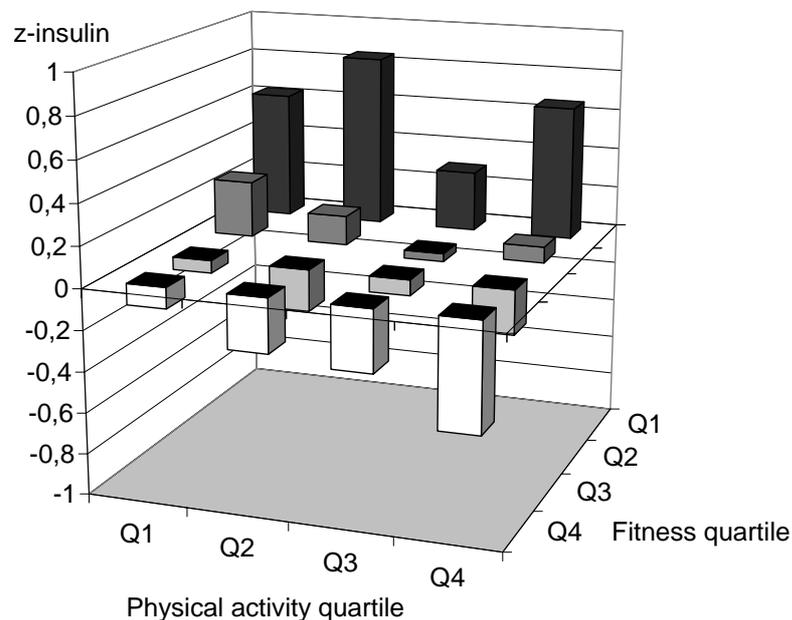


Figure 10a. The interactive effect of physical activity (cpm) and fitness (W/kg) on fasting level of insulin (mU/l) in the subjects of the Swedish EYHS 1998/1999. The z-values ($z = [\text{value} - \text{mean}]/\text{SD}$) were calculated in each age and gender group separately, and thereafter combined in the figure.

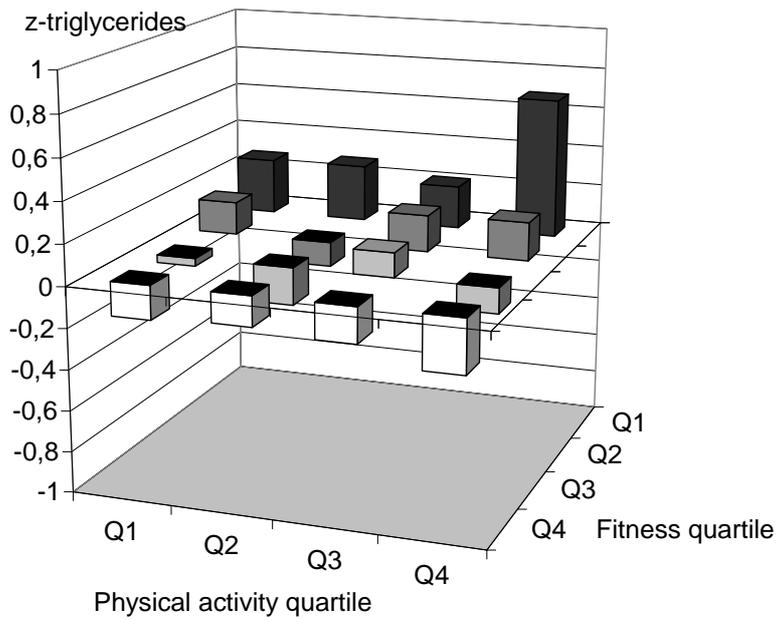


Figure 10b. The interactive effect of physical activity (cpm) and fitness (W/kg) on fasting level of triglycerides (mmol/l) in the subjects of the Swedish EYHS 1998/1999. The z-values ($z = [\text{value minus mean}]/\text{SD}$) were calculated in each age and gender group separately, and thereafter combined in the figure.

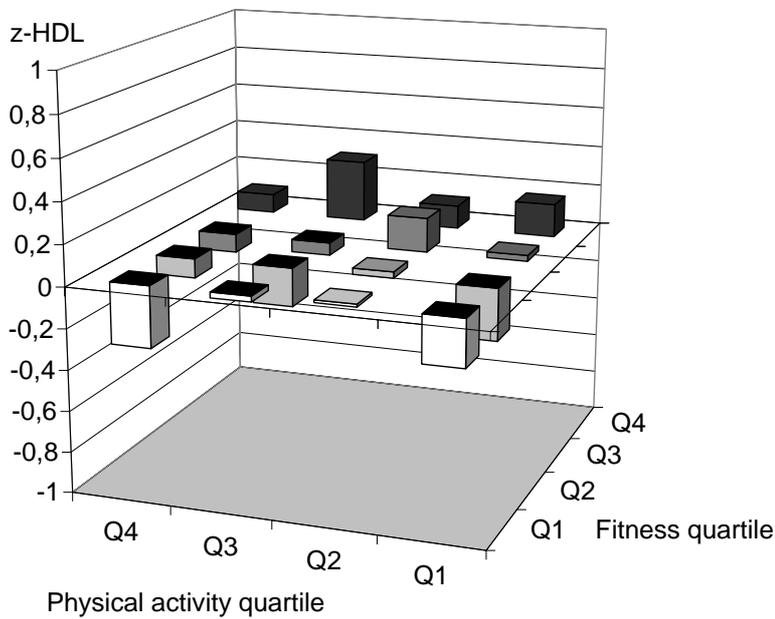


Figure 10c. The interactive effect of physical activity (cpm) and fitness (W/kg) on fasting level of HDL cholesterol (mmol/l) in the subjects of the Swedish EYHS 1998/1999. The z-values ($z = [\text{value minus mean}]/\text{SD}$) were calculated in each age and gender group separately, and thereafter combined in the figure.

4.3.7 Aggregation of risk factors: A risk score ?

Construction of a risk score could be a way of illustrating a risk profile. It is also another way, as an alternative to combining groups by using z-scores, of increasing statistical power. In the present study, a score based on the quartile values (i.e. “1” for lowest quartile, “2” for second quartile, “3” for third quartile and “4” for fourth quartile) was constructed. Quartile affiliation was age and gender group-specific. The risk score was calculated as follows: quartile of insulin + quartile of glucose + quartile of total cholesterol + quartile of triglycerides + quartile of sum of skinfolds + quartile of (systolic blood pressure + diastolic blood pressure/2) – quartile of HDL cholesterol. The risk score used is a modification of a risk score used in adults (Franks *et al.*, 2004). The use of the score made it possible to combine the age and gender groups, as in the graphical presentations in Figure 11. The influence of total physical activity (below or above the median value) on the risk score varied across the fitness-quartile groups. In contrast, in every quartile of total physical activity, the highest risk score was observed without exception in the least fit median group. When the graphs were split into quartile groups of both physical activity and fitness (Fig. 12), a consistently lower risk score across the physical activity quartile groups was seen in the subjects in the highest fitness quartile, but generally the fitness quartile had a steeper gradient across all physical activity quartile groups.

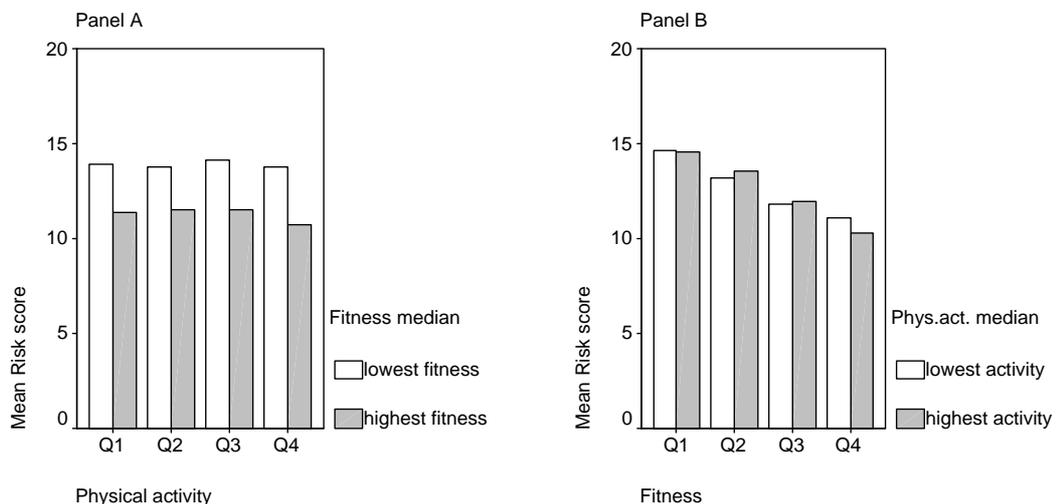


Figure 11. Relationships between physical activity (cpm), fitness (W/kg) and a calculated risk score in the subjects of the Swedish EYHS 1998/1999. Panel A: Risk score distribution between quartiles (Q1-Q4) of physical activity and a calculated risk score, stratified by cardiorespiratory fitness (below and above the median). Panel B: Risk score distribution between quartiles (Q1-Q4) of cardiorespiratory fitness and a calculated risk score, stratified by physical activity (below and above the median).

The main disadvantage of constructing a risk score is that all included variables are given the same importance and have the same influence on the score. The comparability between studies is also restricted, as there is no consensus on how to define the “most predictive” risk score. Furthermore, the use of relative risk groups, based on the observed distribution (i.e. the most unfavourable quartile), in the absence of clear cut-off values in children, reduces the physiological information provided by the results. To be in the most unfavourable quartile does not necessarily mean that the subjects have risk values compared to those of other samples or to reference values. Despite the methodological weaknesses, an indicator of aggregation, or clustering, of CVD risk factors can be of importance. The impact of aggregation of CVD risk factors in children has been demonstrated in the *Bogalusa Heart Study*. Antemortem levels of CVD risk factors correlated positively with postmortem atherosclerotic lesions found at autopsy (Berenson *et al.*, 1998).

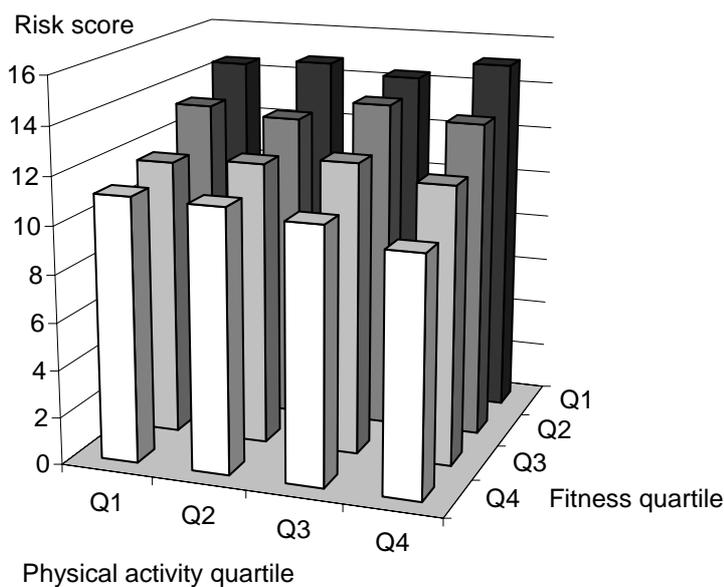


Figure 12. The interactive effects of physical activity (cpm) and fitness (W/kg) on a calculated risk score in the subjects of the Swedish EYHS 1998/1999. The risk score, as well as the physical activity and fitness quartile affiliation, was calculated in each age and gender group separately, and thereafter combined in the figure.

Previous studies of clustering of risk factors have mainly shown that subjects with low fitness and/or increased body weight more frequently have multiple risk factors

compared to more fit and/or leaner subjects (Bao *et al.*, 1994; Chu *et al.*, 1998; Teixeira *et al.*, 2001; Andersen *et al.*, 2003; Carnethon *et al.*, 2003)]

Results concerning the gender perspective with reference to clustering of risk factors, if addressed, has shown inconsistent results. In Estonia, boys more frequently had multiple risk factors than girls (Grunberg & Thetloff, 1998), and so was the case in Finland (Raitakari *et al.*, 1994). In Portugal, girls had a higher percentage of multiple risk factors than boys (Ribeiro *et al.*, 2004). In the Danish EYHS children, the clustering of risk factors was equally distributed between the age and gender groups (Andersen *et al.*, 2003). The Danish EYHS sample has also been studied with a computed metabolic syndrome risk score. It was found that physical activity was related to this score, but that the relationship was possibly modulated by fitness level (Brage *et al.*, 2004a).

4.3.8 A multivariate approach (Paper IV)

To study the relationship between two sets of variables, the physical activity and fitness variables in one set and the other CVD risk factors in the other set, and to find out which of fitness and physical activity contributes most to the other CVD risk factors, canonical correlation analysis was performed (Hotelling, 1936; Johnson & Wichern, 2002). This multivariate statistical model has been used in one previous study in children, the *Quebec Family Study* (Katzmarzyk *et al.*, 1998; Katzmarzyk *et al.*, 1999).

To focus on our question whether fitness or physical activity is the more important in influencing other CVD risk factors, we constructed the two sets of variables as follows:

- The physical activity and fitness variables comprised one set (hereafter called the PA&CRF set) and consisted of time (min) spent in inactivity, time (min) spent in physical activity of moderate to high intensity, total physical activity (cpm), and cardiorespiratory fitness in W/kg.
- The other CVD risk factors comprising the other set of variables (hereafter called the CVD set) consisted of insulin, glucose, triglycerides, total cholesterol, HDL cholesterol, sum of five skinfolds, systolic blood pressure and diastolic blood pressure

A canonical variate is a linear combination of standardized variables belonging to a certain set of variables; here the PA&CRF and CVD sets. In canonical correlation analysis the correlation between the canonical variates is maximized by a proper

choice, i.e. by mathematical selection, of the weights in each linear combination. The correlation coefficient between these two canonical variates, the first canonical correlation, is a measure of the strength of association between the PA&CRF and the CVD sets of variables. The weights within a canonical variate are scaled so that the largest weight is numerically scaled to one, and weights can be compared by their ratios (Johnson & Wichern, 2002).

Both Swedish and Estonian EYHS subjects were included in the canonical correlation analysis in order to increase the statistical power (Paper IV). The first canonical correlations, r_c , between the two canonical variates were significant in all age and gender groups. In girls aged 9 years, $r_c = 0.46$, $p < 0.0001$; in boys aged 9 years, $r_c = 0.61$, $p < 0.0001$; in girls aged 15 years, $r_c = 0.48$, $p < 0.0001$; and in boys aged 15 years, $r_c = 0.54$, $p < 0.0001$. Thus, from the squared canonical correlation coefficients it was estimated that between 20 and 37 % of the variation in the CVD risk canonical variate was explained by the PA&CRF canonical variate. Figure 13 presents the correlations between the standardized PA, CRF and CVD variables and their respective canonical variate in the 15-year-olds (Paper IV shows all groups). The correlation coefficients can be interpreted as the influence or weight of each variable on the canonical variate to which it belongs. In 15-year-old girls, the canonical variate for the PA&CRF set places nearly 2.5 times as much weight on CRF than on total physical activity and time spent in physical activity of moderate or high intensity level. Our statistical model shows a clear dominance of positive CRF weight on the PA&CRF canonical variate, compared to the loadings of the PA variables (Paper IV). In girls of both age groups, a positive weight of moderate to high intensity physical activity and total physical activity was also seen, while this was not the case in boys of either age group.

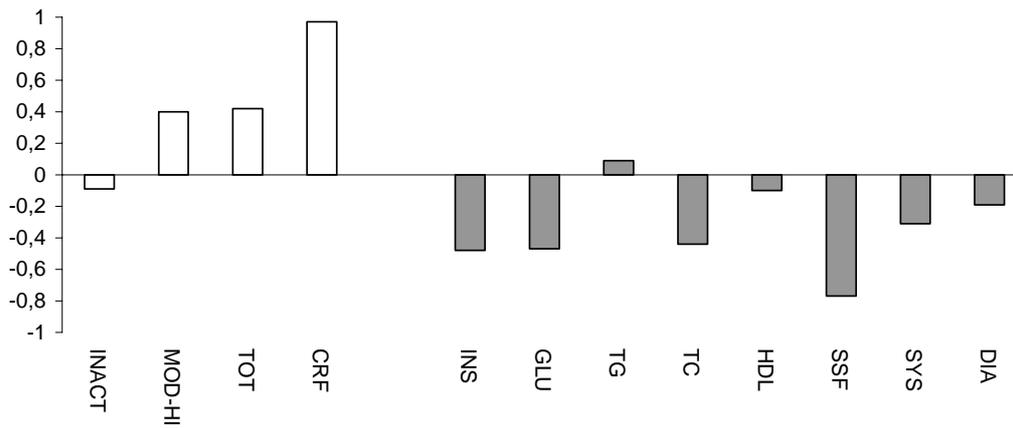
In the *Quebec Family Study*, a slightly different approach was used with regard to the construction of the canonical variates. In that study, the sum of six skinfolds was entered as a morphological fitness variable in one canonical variate, together with other indicators of health-related fitness variables (e.g. leg muscle strength, physical working capacity and number of sit-ups). The canonical correlations were calculated separately for these fitness variables versus response variables (e.g. serum lipids and blood pressure), and for physical activity variables (e.g. energy expenditure and TV time) versus the response variables. Thus, we used SSF as an outcome of fitness or physical activity, while the Canadians used it as a determinant of the response variables. Despite the different approaches in the statistical models and the different methods used for

fitness and physical activity estimations, the variations in the CVD risk profile were explained in a similar pattern in the two studies: Fitness-related variables explained more than physical activity-related variables in the *Quebec Family Study*, also.

Gender differences in the associations between physical activity and other risk factors were not reported from the *Quebec Family Study* (Katzmarzyk *et al.*, 1998). A positive correlation between physical activity and physical performance (9- minute running test) has been found in girls, but not in boys, and such a gender difference, correlation in girls but not in boys, was also found in the levels of muscle enzyme activities in the same study (Jansson & Hedberg, 1991). A gender difference was found in a Finnish study in 4- to 7-year-old children, in that physically active girls had lower serum lipids than inactive girls, while the effects of physical activity were smaller in boys (Saakslähti *et al.*, 2004). In the longitudinal *Danish Youth and Sport Study*, a gender difference in responses to changes in fitness and activity were observed. Whereas weak relationships were found in women, stronger ones were found in men (Hasselstrøm *et al.*, 2002).

It has been suggested that the level of CRF may have an impact on the association between physical activity and an index of the metabolic syndrome in adults. Stronger associations between physical activity and risk profile have been found in low-fitness subjects (Franks *et al.*, 2004). As girls have lower CRF than boys, and the total physical activity clearly has higher weight on the CRF&PA canonical variate in girls than in boys, the findings in the Swedish EYHS are compatible with those reported by Frank and co-workers. The gender perspective was not addressed in the British study, but only the fitness and physical activity aspects.

Panel A: 15-year-old girls



Panel B: 15-year-old boys

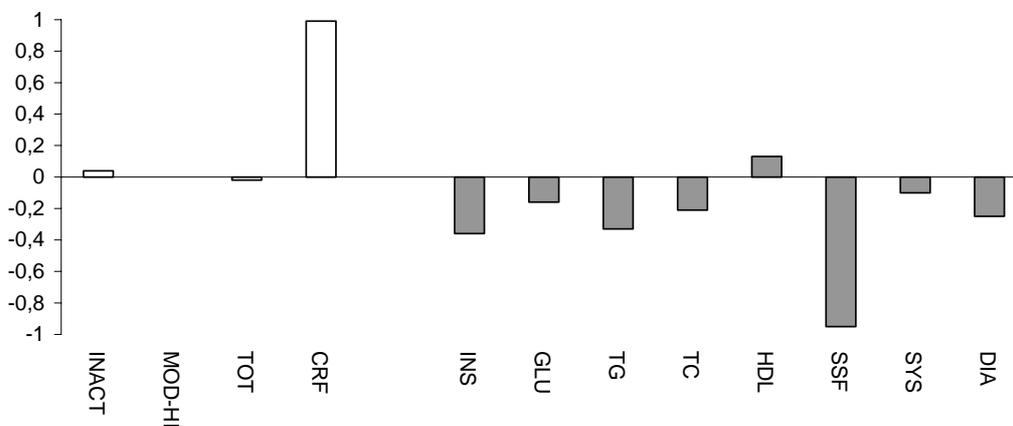


Figure 13. Pearson correlations among the variables included in the canonical variates in the 15-year-old girls and boys of the Swedish EYHS 1998/1999. White bars illustrate the PA&CRF canonical variate; Grey bars illustrate the CVD risk canonical variate. Each correlation reflects the “loading” or the contribution of the original variable to its canonical variate. INACT, time (min) in inactivity; MOD-HI, time (min) in physical activity of moderate or high intensity level (>3 METs); TOT, total physical activity (mean counts per minute during total recorded time); CRF, cardiorespiratory fitness (W/kg); INS, insulin; GLU, glucose; TG, triglycerides; TC, total cholesterol; HDL, HDL cholesterol; SSF, sum of skinfolds; SYS, systolic blood pressure; DIA, diastolic blood pressure.

4.3.9 Public health recommendations

Since it is generally accepted that the onset of CVD risk factors takes place in early childhood, preventive strategies should start as early in life as possible (Berenson *et al.*, 1998). The public recommendations for healthy levels of physical activity in young

people (Sallis & Patrick, 1994; Biddle *et al.*, 1998), have been criticized for lack of scientific rationale, and one of the main reasons for this has been the imprecise methods for assessment of physical activity (Twisk, 2001; Ridloch *et al.*, 2004).

Previously, the public health advice for adults included vigorous physical activities (at least 20 minutes 3 times per week), but there has been a shift in the public health recommendations towards moderate physical activities (accumulation of at least 30 minutes on most, preferably all, days of the week) (Pate *et al.*, 1995; World Health Organization, 2003). The scientific rationale for the change was based on the finding that the greatest health benefit was gained when moving from “sedentary” to “moderately active” (Blair *et al.*, 1989), but there was also a social incitement to achieve greater adherence to the recommendations.

The basic recommendations for children have been similar to those in adults, namely accumulation of at least 30 minutes of moderate physical activity, with an additional recommendation to engage in three 20-minute sessions of moderate to vigorous activities (Sallis & Patrick, 1994; Biddle *et al.*, 1998). Addressing in addition the maintenance of body weight, more recent international consensus statements, however, recommend moderate activity for at least 60 minutes (Cavill *et al.*, 2001; World Health Organization, 2003). Swedish authorities advocate engagement in physical activity of at least moderate intensity for at least 30 minutes per day in adults and 60 minutes per day in children (Statens folkhälsoinstitut, 2003).

Our data suggest that the recommendation for children ought to place emphasis on vigorous physical activities so as to achieve higher CRF, as our results indicate that CRF has the strongest association with the CVD risk indicator profile in all EYHS age groups.

4.4 METHODOLOGICAL CONSIDERATIONS

The relationships between biological, life-style and environmental factors are complex. Studies on children require additional considerations regarding study protocols and ethics. Furthermore, the results may be concealed by the general growth and development during childhood, and the absence of CVD outcomes precludes conventional risk estimations based on morbidity and mortality. Despite these difficulties, studies of risk factors in children are of major importance for following secular trends and detecting individuals or groups at high risk of developing CVD.

The scientific committee's choices of methods were based on long experience and a good sense of what could be relevant to do under field-like conditions when visiting all the different schools. Almost every method has its equal or superior alternative, but considering all circumstances (costs, convenience for the subjects, transportability etc) the methods used are among the best available. They are all established and evaluated methods and are discussed in the different sections of Materials and Methods.

In general, the methods chosen by the scientific committee appear reasonable under the circumstances of running the project (as an ambulatory lab). The only part of the study that easily could have been conducted differently was the bike test protocol. Use of a different work rate profile, aiming to reach exhaustion within a shorter time, could possibly have increased the CRF result in 9-year-olds, without increases in risk, costs or other important features of the study. If the participants had been tested in an ordinary laboratory environment, other methods could have been used (e.g. a treadmill test). The bike test protocol may be questioned (Paper III), despite the high correlation coefficients in the validation study. Primarily, it is the long period of time required to reach exhaustion that may cause a validity problem. Not all children can stay focused during a prolonged testing period, especially if the test environment is not optimal. Opening of doors, classmates passing by, and so on, happen more often under field-like conditions than in a stationary laboratory setting. The validation study was performed in a stationary laboratory, and the participants were not EYHS subjects, and were not put under the same "total pressure" with blood sampling, etc, as the EYHS participants. It could therefore be assumed that the validation study subjects would be able to focus better during their very limited task than could the EYHS participants.

In the Swedish EYHS II, a 6-year follow-up undertaken during the present school year (2004/2005), seven days of accelerometer recording is performed to improve the quality of the physical activity data, but so many days of recording were not feasible during the EYHS 1998/1999. Furthermore, questions about the menstrual cycle and the use of contraceptives are now included in the questionnaire, to elucidate the variations in the female lipoprotein lipids caused by hormonal fluctuations.

To draw conclusions about the influence of determinants on the risk factors, and finally on CVD outcomes, longitudinal and intervention studies need to be conducted. Only from such findings can age- and gender-specific health promotion recommendations be further developed. Other factors affecting the CVD risk profile than those included in this thesis, such as diet and genetics, are still to be analysed.

5 GENERAL CONCLUSIONS

- The sample used in this study is representative of the source population, and the study is comparable to other population studies in children despite the relatively low participation rate. This conclusion is based on the findings from the extended non-participant follow-up study, where participants and non-participants were found to correspond well in most respects.
- The fasting serum insulin concentration is particularly influenced by indicators of body fat and by the total physical activity level in healthy children. Sexual maturation (biological age) has a significant influence on the studied serum variables and ought to be considered, rather than chronological age, when serum results are interpreted in children.
- Data suggest a secular trend towards decreased cardiorespiratory fitness in 9-year-olds, but not in 15-year-olds.
- Cardiorespiratory fitness had a greater impact than physical activity on cardiovascular risk factors in this random sample of healthy school children. A gender difference in the influence of physical activity was observed: In girls of both age groups not only cardiorespiratory fitness but also physical activity had inverse relations with the CVD risk factors, while this was not the case in boys. Physical activity has a stronger influence on fasting serum insulin than on the other studied variables.

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