Cardiovascular Risk Indicators in Adolescents
The Umeå Youth Study

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av
Erik Bergström

Umeå 1995
Abstract

Cardiovascular Risk Indicators in Adolescents: The Umeå Youth Study

Erik Bergström, Department of Epidemiology and Public Health and Department of Paediatrics, Umeå University, S–901 85 Umeå, Sweden.

Atherosclerotic cardiovascular diseases (CVD), particularly coronary heart disease (CHD) and cerebrovascular disease, are today major causes of death in the industrialised parts of the world. There are evidence to suggest that the atherosclerotic process starts in childhood, implying that preventive measures should be implemented already in children and adolescents.

The aim of this study was to examine CVD risk indicators and their determinants in healthy Swedish adolescents. The study population comprised 14- and 17-year-old boys and girls (n=1032), in the city and surroundings of Umeå in northern Sweden.

Biochemical, anthropometric, and physiological parameters associated to CVD (s-lipoproteins and s-apolipoproteins, s-insulin, s-ferritin, anthropometric measurements, blood pressure, and physical fitness) were evaluated in relation to family history of CVD, weight and length at birth, infant feeding regimen, physical growth during infancy and childhood, current diet, physical activity, smoking, and educational level and occupation of the parents.

The main findings of the study were that, on average, total serum cholesterol (TC) values in boys and girls were at the same level as reported from other European countries. A family history of CVD, short duration of breast feeding, low attained height during infancy and childhood, high body mass index (BMI), and low physical fitness were all associated with an unfavourable serum lipid profile. The findings also showed that features typical of the insulin resistance syndrome are present already in adolescents. In boys, iron stores, estimated by serum ferritin, were related to BMI and physical fitness, in a similar way as well established CVD risk indicators. Compared to previous dietary studies in Sweden, mean relative (energy %) fat intake had decreased substantially although the mean relative intake of saturated fat was still rather high. For both boys and girls, reported relative energy intake (energy intake/estimated energy expenditure) decreased with increasing level of BMI. Furthermore, daily smoking was more common among adolescents from families with low socio-economic status (SES) but was most strongly associated to smoking in peers. Tobacco use was considerably higher among adolescents attending vocational programs at secondary high school as compared to theoretical programs. Daily smokers had a more unfavourable serum lipid profile compared to non-smokers. Low socio-economic status of the parents was related to higher BMI and low educational level to higher dietary fat intake in both boys and girls.

In conclusion, the findings of the study show that parameters linked to adult CVD when examined in adolescents, are related to family history, infant nutrition, previous physical growth, current body composition, physical fitness, physical activity, smoking, and social status and educational level of the parents.

Key words: Cardiovascular risk factors, adolescents, serum lipids, serum insulin, serum ferritin, anthropometry, blood pressure, physical fitness, physical activity, diet, smoking, socio-economic status
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"The childhood shows the man. As the morning shows the day".

John Milton

To my family
Abstract

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Key words: Cardiovascular risk factors, adolescents, serum lipids, serum insulin, serum ferritin, anthropometry, blood pressure, physical fitness, physical activity, diet, smoking, socioeconomic status
Abbreviations

Apo A–I Apolipoprotein A–I
Apo B Apolipoprotein B
BMI Body mass index (kg/m²)
BMR Basic metabolic rate
BP Blood pressure
BW Body weight
CHD Coronary heart disease
CI Confidence interval
CL Confidence limits
CVD Cardiovascular diseases
DBP Diastolic blood pressure
E% Percentage of total energy intake
Fam Family history of CVD
HDL-C High-density lipoprotein cholesterol
LDL-C Low-density lipoprotein cholesterol
Lp(a) Lipoprotein (a)
MUFA Mono-unsaturated fatty acids
NIDDM Non-insulin-dependent diabetes mellitus
OR Odds ratio
PUFA Poly-unsaturated fatty acids
RDA Recommended Dietary Allowances
SES Socio-economic status
SBP Systolic blood pressure
SF Serum ferritin
SFA Saturated fatty acids
SNR Swedish Nutrition Recommendations
TC Total cholesterol
TG Triglycerides
VLDL-C Very low-density lipoprotein cholesterol
Original papers

This thesis is based on the following papers, which will be referred to by their respective Roman numerals:


V Bergström E, Hernell O, Persson LÅ. Physically fit adolescent boys have lower BMI, a more favourable serum lipid profile, lower serum insulin, and lower serum ferritin values (submitted).

VI Bergström E, Hernell O, Persson LÅ. Cardiovascular risk indicators cluster in girls from families of low socio-economic status (submitted).
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Introduction

From poverty to affluence

Some generations ago child health care in Sweden faced the same health problems as are today prevalent in developing countries. Infectious diseases, such as tuberculosis and measles, as well as dietary deficiencies contributed to a major part of morbidity and mortality. Nutritional deficiencies causing iron deficiency anaemia and rickets, were common [1]. During the second half of this century the picture has changed completely. Today increasing concern of health hazards related to affluence and dietary excess instead of dietary insufficiency has evolved. What are the long term health consequences of a more sedentary lifestyle in children and adolescent, i.e. a lifestyle characterized by low physical activity, high-energy diet rich in saturated fat, and prevalent cigarette smoking? These factors are regarded as some of the major determinants behind many of today's common public health problems in adults, e.g. cardiovascular diseases (CVD), non-insulin-dependent diabetes mellitus (NIDDM), cancer, and osteoporosis [2,3].

The emergence of cardiovascular diseases

"A fat poet after a hearty meal and much orating, climbed a flight of stairs, was seized by great discomfort in his chest and died within a few minutes. At autopsy he was found to have such narrowed coronary arteries that is was impossible to insert even the end of a needle into them."

Bonetus, 1700

It was not until the last century that CVD, with atherosclerotic-thrombotic changes in the coronary, cerebral and other arteries, became major public health problems. Myocardial infarction (MI) and stroke are since the 1950's the leading causes of death in most industrialised countries, including Sweden [2,4]. There are large differences in CVD mortality rates between countries, as well as between regions within countries [5,6,7]. In Sweden, the mortality is higher in the northern compared to the southern parts of the country [6]. During the last decades, however, the mortality rates from CVD has declined in some countries, e.g. the United States, Western European countries, while other countries show increasing trends, for example in Eastern European countries [8].

The epidemiological data revealing differences and changes in CVD mortality rates between countries point at the causal role of environmental factors. This is further supported by the variation in rates by ethnicity, socioeconomic status, and the shift in CVD rates seen in migrant populations [3]. One hypothesis to explain the epidemics of life-style related diseases, e.g. CVD and NIDDM, is the "thrifty genotype". As stated by Neel - "genes and combinations of genes which were at some time an asset may in the face of environmental change become a liability" [9]. To have an "energy- and iron saving genotype" would be an advantage in an environment with shortage of food and high prevalence of iron consuming diseases and frequent blood losses, but not when confronted with "western" life-style and society.
This hypothesis bear some support by the absence of CVD in native populations who have remained unaffected by western lifestyle [3,10,11]. Hence, these epidemiological data showing variation in CVD prevalence between and within populations are a basis for the view that CVD is a preventable condition, given of course, that these environmental causes are, or become, known and modifiable.

The natural history of cardiovascular diseases

Despite intense research in the last decades the natural history of CVD is yet not fully understood. The clinical manifestations of CVD are, with few exceptions, not apparent before 40 years of age. The first evidence that atherosclerotic coronary heart disease (CHD) may start much earlier in life, and thus might also be of concern for paediatricians, came from pathologists. The early development of atherosclerosis, particular in the aorta was recognized early during this century [12–16]. In the 1950's these observations were "rediscovered" by Enos and co-workers [17], reporting atherosclerotic changes in the coronary arteries of young American soldiers who were killed in the Korean war, a finding later confirmed in 1971 by Macnamara et al in autopsy studies on soldiers killed in Vietnam [18]. Intimal thickening, foam cell formation, and fatty streaks, which are thought to be precursor lesions for atherosclerosis [19], have in later studies been found in the arterial vessels of children as early as at six years of age [20] (Figure 1). Fully developed atherosclerosis is found in children as six years of age with the homozygote form of familial hypercholesterolaemia as young as six years of age [21, 22].
Although evidence is accumulating that atherosclerosis is a silent, continuous process starting early in life with clinical manifestations in middle age there is still no conclusive longitudinal evidence available confirming this hypothesis [23]. Fatty streaks in the arteries are known to be reversible and the relationship of fatty streaks to the formation of fibrous plaque, which is the most characteristic lesions of advancing atherosclerosis, has also been questioned [24].

**Risk factors**

"...diseases of the heart arise, not from single causes only but from conspiracies of causes. It is not the seed alone that matters but also the soil and the weather... the hope of advances lies in prevention rather than cure."

Coombs, 1926

The term "risk factor" in relation to CVD, and particularly CHD, was used for the first time in a report from the Framingham study [25]. Risk factors are often used as a general concept denoting statistical correlation to increased morbidity and/or mortality in CVD, thus indicating, but not proving, a causal relationship. From epidemiological and clinical studies in adults a large number of factors have been proposed as risk factors for CVD [26]. The three most established CVD risk factors in adults are dyslipoproteinaemia, hypertension, and smoking [2]. Risk factor trends, particularly in diet and smoking, are regarded as the most important explanation of the diverging trends in mortality in different countries [3,8].

For the understanding of how different CVD risk factors interact and contribute to the disease process it is essential to distinguish between different kinds of risk factors, and also to explore how and when these risk factors operate. Some factors may predispose, or be a prerequisite, for the disease, e.g. a genetically determined metabolic disturbance or susceptibility. A "risk environment" or "risk habit" may initiate, promote, enhance, or "trigger" the disease process. The term risk factors is also commonly used for biochemical, physiological, and anthropometric markers indicating early metabolic disturbances (e.g. hypercholesterolaemia), or early stages of CVD, i.e. "disease precursors" (e.g. hypertension, angina).

Different models trying to describe the relationship between different causative risk factors and CVD have been suggested. One that is often used is the "web of causation" model [27]. Another is, the "sufficient cause" model by Rothman, stressing the importance of separating between "necessary" and "contributing" factors and that different combinations of necessary and contributing factors form sufficient causes for the disease [28].

When preventive strategies towards CVD are discussed it is also important to distinguish between CVD risk factors which are not modifiable, e.g. male sex, genetic susceptibility, or advancing age, and risk factors that are modifiable, either by medical therapy, behavioural or environmental changes or, e.g. hypertension, or hypercholesterolaemia, smoking, unhealthy diet, physical inactivity, obesity, stress, lack of
Social support, socio-economic disadvantages, unemployment.

**Risk factors in childhood and adolescence for cardiovascular disease in adult life**

Based on the evidence that atherosclerosis starts in childhood it has been suggested that primary prevention of adult CVD should start with children and adolescents [2, 23, 29–31]. Paediatricians in the United States were the first to suggest that prevention of adult CVD should be a concern also for paediatricians [32]. This was the starting point for a number of epidemiological studies in the United States focusing parameters shown to be associated to CVD in adults. Two of the major studies in the United States are; the Muscatine Study [33] and the Bogalusa Heart Study [34]. These studies have been followed by studies in Norway [35], Finland [36], and Denmark [37]. Until now there has been no similar studies in Sweden.

**Early metabolic programming**

Although there is much evidence to say that the causes of CVD are predominantly related to affluence in adulthood it has been hypothesized that poverty in early life, with poor prenatal and infant nutrition resulting in growth retardation, could make the child more predisposed to development of atherosclerosis and other metabolic disturbances such as insulin resistance and NIDDM, later in life. This hypothesis has also been named the "thrifty phenotype hypothesis" [38].

The effect of early life experiences is hypothesized to be mediated through a "metabolic programming" and is suggested to be one explanation behind the regional differences in CVD [39, 40]. The quality of infant feeding, e.g. breast milk and different compositions of formula, and its long-term effects on growth, development, and disease development, has also been a focus of interest [41–43]. It is, however, difficult to separate possible biological harmful effects during foetal life or infancy from confounding effects by associated social and nutritional factors operating through childhood into adult life [44–47]. If foetal malnutrition may result in increased risk of CVD, the low occurrence of adult CVD in developing countries with concurrent malnutrition and high rates of growth retardation in new-borns, may look paradoxical. This could, however, partly be explained by a shorter life span in developing countries. It has been suggested that the shift from nutritional insufficiencies to nutritional overload may be particularly harmful [40]. To conclude, the "programming hypothesis" suggests that experiences in early life may permanently affect the risk of developing CVD in adulthood. The hypothesis does, however, not exclude that the risk may be affected also by environmental factors, or life-style later in life.
Tracking of "risk values" or "risk habits" from childhood to adulthood

In adults the risk of getting clinical CVD increases with increasing values of certain biochemical, physiological, and anthropometric variables, e.g. serum cholesterol, blood pressure, and obesity. Based on this relationship and as a tool to identify individuals with increased risk of having CVD, specific "risk values" or "cut-off values" have been developed. As the association between "risk values" in childhood or adolescence and subsequent clinical CVD in adulthood is not known, such adult cut-off values are not immediately applicable for predicting the risk of future CVD in children and adolescents. Rather, the rationale for identifying CVD "risk values" in children and adolescents relies on the concept of "tracking", or persistence, of high values or certain "risk habits" from childhood into adulthood. If these values or habits persist, they will eventually become risk factors of CVD, i.e. when identified in children and adolescents they may be regarded as "risk factors for risk factors" (Figure 2). We have therefore in the following decided to use the concept "cardiovascular risk indicators".

Indices of tracking may be based on either the correlation coefficients between absolute values of paired observations over time, or on the persistence of relative values or percentile ranks in a given population. Another approach to describe the development of risk factors over time is serial observations of absolute values in individual subjects by repeated measurements over a period of time. This last approach is referred to as the development of "growth curves" of risk factors [48].

![Figure 2. "Risk factors for risk factors".](image-url)
Available data on tracking of blood pressure and serum cholesterol values from childhood into young adult life show that less than half of those being classified as having high values in childhood (upper percentiles of the distribution) remain in the same percentile [49-54]. These data have been interpreted both as in favour [52,55] of and against [31,56-58] general screening for "risk individuals" in childhood and adolescence. It should also be noted that the majority of adults who die from CVD have not had a high serum cholesterol or a high blood pressure prior to the myocardial infarction or the stroke event [2,58,59].

It has also been suggested that behavioural risk indicators, or "risk habits", e.g. unhealthy diet, low physical activity, and tobacco use track from adolescence to adulthood. However, there is no convincing and indisputable evidence available that they do. Contrary, there are reasons to believe that tracking of behavioural risk indicators is even lower than tracking of biochemical or physiological parameters. In conclusion, this imply that the predictive value for adult CVD of "risk values" or "risk habits" found in childhood may in fact be very poor. However, more evidence is needed before this conclusion is settled, or contradicted.
The research project

Background

The Umeå Youth Study

Although Sweden has a relatively high CVD morbidity and mortality in adults, especially in the northern parts of the country [6,7], there is limited information on cardiovascular risk indicators in Swedish children and adolescents. This thesis is based on data from the first cross-sectional study within the Umeå Youth Study, a school based prospective study on life-style and health of Swedish adolescents with special reference to risk indicators of future CVD in adulthood. The design of the study is shown in Figure 3.

Aims

The aims of this thesis were to describe the distributions and co-variation of biochemical, physiological, and anthropometric parameters which have been related to CVD in adulthood, and to evaluate how these parameters are associated to family history of CVD, socio-economic status, previous physical growth, infant nutrition, current dietary intake, physical activity, and tobacco use.

<table>
<thead>
<tr>
<th>Year</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989/90</td>
<td>14 years 17 years</td>
</tr>
<tr>
<td>1992/93</td>
<td>22 years</td>
</tr>
<tr>
<td>1997/98</td>
<td>25 years</td>
</tr>
</tbody>
</table>

Figure 3. Design of the Umeå Youth Study, shaded areas denotes the part included in this thesis.
Study population

Study area

The study was carried out in the municipality of Umeå, situated at the Gulf of Bothnia in the County of Västerbotten in northern Sweden, 400 km south of the Arctic Circle (Figure 4). At the time of the data collection in 1989 the municipality of Umeå had around 90,000 inhabitants of whom 60,000 lived in the city.

Subjects

Two groups of adolescent students, 14 and 17 years of age, from four different schools in the city of Umeå, representing about 45% of the total number of students in these age groups in the municipality, were invited to participate in the study. The group of 14-year-old students (mean age 14.4 years, range 13.5–15.7 years) consisted of all students (n=439,225 boys and 214 girls) in grade eight in three primary schools. The group of 17-year-old students (mean age 16.8 years, range 15.9–18.2 years) consisted of all students (n=523,304 boys and 219 girls) in grade one in one of two senior high schools. All 17-year-old adolescents (n=70, 38 boys and 32 girls) that did not attend any secondary high school at the time of the study were also invited. The primary schools were chosen as representative of different types of living areas (city, suburb, countryside). The senior high school included received students both from the city and from municipalities outside Umeå (25%) and offered both theoretical and vocational study programs.

Figure 4. Map of Scandinavia, indicating the study area.
Methods

In this study we used a combination of questionnaires, diaries, clinical and biochemical investigations, physiological tests, and register information (Table 1). The research project was discussed and planned with representatives of the school authorities of the municipality and the principles and teachers of the respective schools. All study activities were performed at school.

Data collection

During spring 1989 the questionnaires and the 7-day dietary recording were tested in a pilot study in three grade eight classes and the questions were adjusted and further developed according to the experience obtained. The data in the main study were collected from September through December 1989 (14-year-olds) and from January through June 1990 (17-year-olds).

Questionnaires

The adolescents answered their questionnaires at school and the parents received a mailed questionnaire [III, V, VI]. Confidentiality was emphasized and the parents and the adolescents returned their questionnaires in a closed envelope.

Clinical examination, blood samples, laboratory analyses

The clinical examination and fasting venopuncture blood samples were conducted by two specially trained research nurses using standardized technique [I]. The blood samples were analysed at three different laboratories; Umeå University Hospital; total cholesterol (TC), high-density cholesterol (HDL–C), triglycerides (TG) [I], Department of Geriatrics, Uppsala University Hospital; serum insulin (s-insulin), serum apolipoproteins A-I (Apo A-I) and B (Apo B), and lipoprotein (a) (Lp(a)) [I,II], Department of Nutrition, University of California, Davies, California, USA; serum ferritin (s-ferritin), serum iron, total iron binding capacity (TIBC), and serum transferrin receptors (Tf-receptors) [III].

Physical fitness test

Physical fitness was assessed with a 3 km running test (time in minutes) using standardized procedures. The test was conducted by specially instructed physical education teachers and was performed during regular physical education classes (V).

7-day recording of dietary intake and physical activity

The 7-day-records consisted of a parallel self-reported recording of dietary intake [I] and physical activity [III,V]. In the dietary recording household measures and photocopy models of various foods were used to quantify portion size.
Registry study

Data on weight and length at birth were collected from the Medical Birth Registry of the National Board of Health and Welfare. Information on weights and heights from infancy and childhood were excerpted from medical records of the Child Welfare Clinics (CWC) and the School Health Services (SHS). From the health records at CWC, feeding regimen (breast feeding and formula feeding) during the first year of life was also collected [I].

Table 1. Study variables and methods.

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BIOCHEMICAL PARAMETERS</strong></td>
<td></td>
</tr>
<tr>
<td>Serum lipids</td>
<td>Serum analyses (I–III)</td>
</tr>
<tr>
<td>TC, LDL–C, TG, Apo A–I, Apo B, Lp(a)</td>
<td></td>
</tr>
<tr>
<td>Serum insulin</td>
<td></td>
</tr>
<tr>
<td><strong>SERUM IRON STATUS</strong></td>
<td></td>
</tr>
<tr>
<td>s–ferritin, s–iron, s–TIBC, Tf–receptors</td>
<td></td>
</tr>
<tr>
<td><strong>PHYSIOLOGICAL PARAMETERS</strong></td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td>Clinical examination (II)</td>
</tr>
<tr>
<td>Physical fitness</td>
<td>Running test (V)</td>
</tr>
<tr>
<td><strong>ANTHROPOMETRIC PARAMETERS AND MATURATION</strong></td>
<td></td>
</tr>
<tr>
<td>Height, weight</td>
<td>Clinical examination (II)</td>
</tr>
<tr>
<td>Circumference</td>
<td>Clinical examination (II)</td>
</tr>
<tr>
<td>arm, waist, hip</td>
<td></td>
</tr>
<tr>
<td>Skinfolds</td>
<td></td>
</tr>
<tr>
<td>biceps, triceps, subscapular, suprailiac</td>
<td></td>
</tr>
<tr>
<td><strong>PREVIOUS PHYSICAL GROWTH</strong></td>
<td>Swedish MBR(^a)(I)</td>
</tr>
<tr>
<td>weight and height at birth</td>
<td>Medical records CWC(^b), SHS(^c) (I)</td>
</tr>
<tr>
<td>weights and heights during infancy/childhood</td>
<td></td>
</tr>
<tr>
<td><strong>PUBERTAL STAGE</strong></td>
<td>Clinical examination (I)</td>
</tr>
<tr>
<td>According to Tanner(^d)</td>
<td>7–day–records (IV)</td>
</tr>
<tr>
<td><strong>DIETARY INTAKE</strong></td>
<td></td>
</tr>
<tr>
<td><strong>PHYSICAL ACTIVITY</strong></td>
<td>7–day–records (III, V)</td>
</tr>
<tr>
<td><strong>TOBACCO USE</strong></td>
<td>Questionnaire adolescent (III, V)</td>
</tr>
<tr>
<td><strong>FAMILY HISTORY OF CARDIOVASCULAR DISEASE</strong></td>
<td>Questionnaire adolescent (VI)</td>
</tr>
<tr>
<td>Early coronary deaths</td>
<td></td>
</tr>
<tr>
<td>CVD morbidity</td>
<td></td>
</tr>
<tr>
<td><strong>SOCIO–ECONOMIC STATUS OF FAMILY</strong></td>
<td>Questionnaire parents (I)</td>
</tr>
<tr>
<td>Parents current or previous occupation</td>
<td></td>
</tr>
<tr>
<td>Parents school attendance</td>
<td>Questionnaire parents (VI)</td>
</tr>
</tbody>
</table>

\(^a\)MBR=Medical Birth Registry, \(^b\)CWC=Child Welfare Clinics records
\(^c\)SHS=School Health Services records
\(^d\)Tanner stage [60]
Participation

The participation in the different study activities is summarised in Table 2. The table shows the total participation in each different study activity.

Table 2. Participation in the study.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Boys n=567 (n (%))</th>
<th>Girls n=465 (n (%))</th>
<th>Total n=1032 (n (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Questionnaire</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adolescents</td>
<td>517 (91)</td>
<td>418 (90)</td>
<td>935 (91)</td>
</tr>
<tr>
<td>parents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>family history CVD</td>
<td>477 (84)</td>
<td>402 (86)</td>
<td>879 (85)</td>
</tr>
<tr>
<td>occupation</td>
<td>479 (84)</td>
<td>400 (86)</td>
<td>879 (85)</td>
</tr>
<tr>
<td>education</td>
<td>410 (72)</td>
<td>341 (73)</td>
<td>751 (73)</td>
</tr>
<tr>
<td><strong>7-day record</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dietary intake</td>
<td>366 (65)</td>
<td>365 (78)</td>
<td>731 (71)</td>
</tr>
<tr>
<td>physical activity</td>
<td>369 (65)</td>
<td>363 (78)</td>
<td>732 (71)</td>
</tr>
<tr>
<td><strong>Clinical examination</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>496 (87)</td>
<td>406 (87)</td>
<td>902 (87)</td>
<td></td>
</tr>
<tr>
<td><strong>Blood samples</strong></td>
<td>477 (84)</td>
<td>402 (86)</td>
<td>879 (85)</td>
</tr>
<tr>
<td><strong>Physical fitness test</strong></td>
<td>442 (78)</td>
<td>327 (70)</td>
<td>769 (75)</td>
</tr>
<tr>
<td><strong>Previous physical growth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>birth weight and height, MBR(^a)</td>
<td>504 (89)</td>
<td>424 (91)</td>
<td>928 (90)</td>
</tr>
<tr>
<td>infancy and childhood, CWC(^b), SHS(^c)</td>
<td>485 (86)</td>
<td>396 (85)</td>
<td>881 (85)</td>
</tr>
<tr>
<td><strong>Infant feeding</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>breast and formula feeding, CWC(^b)</td>
<td>404 (71)</td>
<td>365 (78)</td>
<td>769 (75)</td>
</tr>
</tbody>
</table>

\(^a\) MBR = Medical Birth Registry
\(^b\) CWC = Child Welfare Clinics records
\(^c\) SHS = School Health Services records

Ethics

The study was approved by the research ethics committee of the Medical Faculty, Umeå University. All participants received verbal as well as written information of the purpose and the content of the study and the participation was voluntary.
General discussion

Theoretical model in the study

From the previous review it may be concluded that, although the clinical manifestations of CVD are rare before 40 years of age, atherosclerotic changes in the coronary and cerebral arteries starts much earlier in life. Although we do not fully understand how and why the disease process starts and develops, there is much evidence to say that the disease develops in the interplay between genetic factors, early-life experiences, and life-long environmental exposure, e.g. social factors, smoking, nutrition, and physical activity.

The study variables were included based on current knowledge on CVD risk factors in adults. To clarify the complex interactions and to structure the analysis, a simplified model of the development of CVD was set up, illustrating the theoretical interaction between genetic predisposition, environmental exposure, and the selected outcome variables from birth through childhood and adolescence into adulthood (Figure 5).

DISCUSSION OF METHODOLOGY

Design

The results of the study are based primarily on cross-sectional data in two different age-groups. Such design limits the possibilities to draw causal inferences but may be used to show statistical associations between potentially related study variables. However, some cohort data, i.e. physical growth and infant diet were also included. When comparing the results from the two age-groups, it is important to note that differences between the two age-groups may be due to a development of the study variable with age, or be due to other specific characteristics for the two groups.

Study sample

The sample in this study was large comprising about 45% of the adolescents in these age-groups in the municipality, and the choice of the schools represented both inner city and suburban areas with different kinds of housing. This suggests that the sample may be regarded as representative of adolescents living in the municipality of Umeå, and, most likely, also of Swedish adolescents in general.

Participation

The study was designed, planned and adjusted to be an integrated part of the school activities and, as mentioned before, all study activities were performed at the respective schools. This design is probably a major contributing factor behind the relatively high participation rate in most parts of the study. Except for the dietary recording and the physical fitness test the non-participation rate in the study was similar in boys and girls. The participation rate was higher in the 14-year-olds compared to the 17-year-olds (data not shown).
Figure 5. Theoretical model in the study.
Furthermore, in Sweden almost all adolescents (90%) continue their studies in secondary high school. Students not continuing to secondary high school could theoretically differ in many respects from the other adolescents and therefore an effort was made to enrol also the group of 17-year-old adolescents not attending a secondary high school at the time of the study. However, only a few of these adolescents were willing to participate, mainly because they were engaged in different kinds of work practice activities. Furthermore, parts of the study focus on life-style and overweight and, as expected, adolescents with a higher degree of adiposity, i.e. higher body mass index (BMI) or skinfold measurements, were more difficult to motivate for some activities in the study, i.e. running test, recordings of dietary intake and physical activity. There were, however, no significant differences in the main outcome measurements (s-lipids, s-insulin, s-ferritin, or blood pressure values) between participants and non-participants.

Measurements

In public health research there is a need for methods that are simple and cost-effective, but still valid and reproducible. An additional objective of the Umeå Youth study was therefore to modify and implement health research methods in a normal school setting.

The major parts of the information, i.e. from blood samples, blood pressure measurements, anthropometric measurements, and assessment of pubertal stage, were collected by skilled, specially trained staff by use of standardized techniques. Serum analyses were made at high standard research laboratories using established, validated and well controlled methods, aiming at high validity of data also on the individual level. These measurements were, however, made as single measurements which of course limits their validity, especially for parameters with a high intra-individual variation, e.g. blood pressure. To minimize the variability in s-lipid and s-insulin values all blood samples were taken as fasting morning samples.

Current dietary intake and physical activity was assessed with self-reported 7-day records. The 7-day record has proved to be the most valid self-reporting method for assessing the dietary intake of individuals [61]. There is at present no available validated measurement for assessing physical activity in populations, implying that the 7-day records and the questionnaire regarding physical activity used in this study could not be validated in any simple way. Questionnaires has been suggested as the most suitable measurement of physical activity in population studies of children [62]. The running test was chosen as a simple way of assessing physical fitness, i.e. primarily aerobic capacity, but to some degree also muscular performance. Running a 3 km field-track during physical education classes is a regular activity in Swedish schools, and the test could therefore easily be fitted into the normal schedule. Although the running test is a crude test that may be influenced by different external factors, e.g. motivation of the adolescents, our data suggest that the test is a simple
and useful method to classify the level of physical fitness in adolescents.

**DISCUSSION OF MAIN RESULTS**

The main results of the study are discussed in the context of present knowledge. In a first section the distribution and variation in the selected "outcome variables", or "cardiovascular risk indicators", i.e. s-lipids, s-insulin, s-ferritin, blood pressure, anthropometric measurements, and physical fitness are discussed separately, in relation to age, sex, and available reference values. The co-variation and clustering of the risk indicators are also discussed. In a second section the associations between the risk indicators and genetic and environmental factors, i.e. family history, social environment, previous physical growth and nutrition, current diet, physical activity, and smoking are discussed.

**Cardiovascular risk indicators**

**Serum lipids (I,II,V,VI)**

After fat ingestion the epithelial cells of the small intestine transform the absorbed lipids to chylomicrons which are spherical particles consisting of surface proteins (apolipoproteins), surface lipids (phospholipids, cholesterol), and core lipids (cholesterol esters, triglycerides). The chylomicrons transport lipids (about 150 g/day in an adult) from the intestine to different tissues in the body, successively loosing their TG content through the effect of lipoprotein lipase (delipidation), leaving different rest-particles or remnants. These remnants are normally rapidly removed from plasma by the liver. Moreover, very low-density lipoprotein (VLDL) particles are formed in the liver and they also transport TG to tissues. Delipidation of VLDL results in remnants of cholesterol rich intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) particles [63].

The link between CVD and high serum cholesterol is well established [19]. Dyslipidemia with increased level of cholesterol in the low density lipoprotein (LDL) fraction and with decreased concentration in the high density lipoprotein (HDL) fraction combined with increased concentrations of triglycerides (TG) are regarded as the strongest indicators of an increased risk for coronary heart disease in adults. Oxidization of LDL appears to play a key role in the initiation of the atherosclerotic vascular lesions [64]. Uptake of oxidized LDL by the scavenger LDL-receptor is considered to be the dominating explanation behind the accumulation of lipids into macrophages in the arterial wall, later followed by a proliferation of intimal smooth muscle and endothelial cells and the subsequent formation of atherosclerotic plaques [19,65]. Elevated levels of another specific lipoprotein, lipoprotein (a) (Lpa), have been shown to be independently associated to CVD in adults. Lp(a) is strongly genetically determined and modifies coagulation and fibrinolysis [66]. Several studies indicate that there may be an even stronger association between future CVD and specific patterns of apolipoproteins and also
with genetic polymorphism in specific apolipoproteins [67]. Apolipoprotein B (Apo B) is the dominating protein in triglyceride–rich lipoproteins, chylomicrons and VLDL. Apolipoprotein A–I (Apo A–I) is the dominating protein in HDL and is the activator of the lecithin–cholesterol acetyltransferase (LCAT), which is a key enzyme in the reversed transport of cholesterol from the peripheral tissues. Lowering of total and LDL–cholesterol (LDL–C) has been shown to reduce the mortality rate in CHD in men, although only in those with high values [68].

Present study

Mean total TC values in children and adolescents in different countries parallel the occurrence of adult CVD in these countries [29]. The trends of s–lipid levels in adolescents seem to go in different directions in different parts of the world. Finland show decreasing mean TC values while Japan [72,73], Spain [74] and Italy [75] show increasing values resulting in small mean differences between European countries, including Sweden. These values are contrasted by the much lower values found in adolescents from a country in the developing world (Tanzania) [76]. Our results confirm that s–lipid values in adolescence are related to age and sex. There were only few adolescents who had high TC, LDL–C, or TG values or low HDL–C values. If the internationally accepted cut–off values for high LDL–C (>3.4 mmol/L) [30] is used on this study population 6% of the 14-year-old boys and 9% or the 14-year-old girls had a LDL–C value above this cut–off value compared to 11% and 12% of the 17–year-old boys and girls, respectively. Mean HDL–C was lower and mean TG was higher in the 17–year-old boys compared to the 14–year-old boys, while there were no such differences in girls. The study also confirm that oral contraceptives change serum lipids in girls in an atherogenic direction with higher TC, LDL–C, and Apo B values.

Serum insulin (II,V,VI)

Insulin is essential for glucose and lipid metabolism and takes part in the regulation of transport of glucose and free fatty acids into the cells. In adults
an increasing cellular resistance to insulin, which in turn affects glucose and lipid metabolism, may develop followed by a compensatory hyperinsulinemia [77–80]. Measuring s-insulin concentration is an accepted indirect way of estimating insulin resistance [81].

Children and adolescents
In children and adolescents the importance of s-insulin has mainly been of concern because of its relationship to insulin-dependent diabetes. Serum insulin levels increase during puberty and the increase is probably caused by a combination of higher secretion, reflecting a higher demand of insulin during the pubertal growth spurt, and an increased cellular resistance to insulin [82–83]. There are to date no available reference values for insulin for healthy children and adolescents.

Present study
Serum insulin values were somewhat higher in the younger age-group with the highest level in mid puberty for both boys and girls. The differences between boys and girls, also demonstrated in other studies [84–86], probably reflects differences in maturation between boys and girls.

Serum ferritin (III,V,VI)
A normal adult individual has around four grams of elemental iron distributed into iron in the haemoglobin of the red blood cells (70% of total body iron), iron in the oxygen-binding protein found in muscles (myoglobin, 4%), and iron in enzymes such as cytochromes, catalases, and peroxidases (less than 1%) and storage iron (25%) in the liver, bone marrow and spleen. Iron is transported in small amounts in the blood, bound to the iron transport protein transferrin [87,90]. Body iron status, deficiency or overload, is commonly estimated by measuring s-iron, s-transferrin, and s-iron binding capacity, (transport iron) and s-ferritin (storage iron). Because s-ferritin has been shown to reflect the total amount of storage iron in the body, it is at present generally recognized as the best tool for estimating iron status of an individual or the prevalence of iron deficiency in a population [88–90]. Serum ferritin is known to vary considerably with age and sex. Mean s-ferritin show a successive decrease from a high level during the first year of life in both boys and girls and then stays on the same level for both sexes until puberty. While mean s-ferritin in females seems to remain on the same level during childhood, adolescence and pre-menopausal adult life, it increases after puberty in males. After menopause serum ferritin in females increases to the same level as for males [91]. It should be noted, however, that s-ferritin is only an indirect measure of iron status. Assessment of transferrin receptors is a promising new measure of iron status at tissue level [92–94].

Iron deficiency anaemia is still a major health problem in developing countries [95], and was so also in Sweden some generations ago [1]. In contrast to this, an increasing concern regarding the potential hazards of iron excess has emerged. Iron in high concentrations is
a toxic substance with potentially negative health consequences partly by supporting the formations of free radicals which in turn increase pathological processes such as the oxidization of LDL-C [96–97]. There are no data to support that high iron stores are beneficial, contrary, it has been claimed that iron stores constitute a sign of iron excess and therefore should be kept low [98]. High iron stores, estimated as s–ferritin, have been found to be associated with an excess risk of myocardial infarction in men [99] and also cancer [100,101], but the evidence is still inconclusive [102,103]. Differences in iron stores between adult men and women have been suggested to contribute to the sex–differences seen in CVD morbidity and mortality [104]. Individuals with "the iron loading gene", (genetic haemochromatosis), causing a pathologically increased absorption of iron, are especially vulnerable to an excess intake of iron [105,106].

Children and adolescents

Children, adolescents, and pregnant women are regarded as vulnerable groups for iron deficiency due to their increased demand of iron. In Sweden iron deficiency anaemia is no longer a public health problem, but it has been suggested that low iron stores without anaemia should be regarded as a common health problem in adolescents [107]. The proposed hazards of iron deficiency without anaemia are impaired physical endurance capacity and mental functions, e.g. behaviour and cognitive functions [108–110]. There is, however, no convincing evidence of these suggested negative consequences of iron deficiency without anaemia [111,112].

Present study

Our study confirms that iron stores, estimated as s–ferritin, increase during puberty in boys, but not in girls. Low serum ferritin values (<12 µg/L) were found in 5% of the boys and 15% of the girls. However, apart from low s–ferritin there were no other signs of iron deficiency when using other criteria (s–iron, TIBC, S–Tfr). Of the 17–year-old boys, 7% compared to 1% of the 17–year-old girls had serum ferritin values >100 µg/L. Our findings indicate that the high iron stores of post–pubertal males, compared to post–pubertal females, may reflect sex–related differences in iron metabolism other than menstruation losses and we suggest that age and sex specific reference values for s–ferritin should be decided and used.

Blood pressure (II,V,VI)

The risk for CVD, especially stroke, increases with increasing diastolic blood pressure (BP) in both men and women. There are major differences between reported BP levels between countries but, in contrast to s–cholesterol, the association between blood pressure and CVD is stronger within populations than between populations [113].

Children and adolescents

In contrast to adults, international comparisons of BP values between different populations show similar
mean values during childhood and adolescents for both boys and girls, indicating that environmental circumstances of different populations, and even genetic differences, may have limited bearing on the development of BP in early ages. Both systolic and diastolic BP increases with age and during puberty BP values successively become higher in boys compared to girls. Blood pressure values in children and adolescents, especially systolic BP are, in contrast to adults, closely related to height, and similar to adults also to obesity. Blood pressure shows a large intra-individual variability and repeated measurements are needed to identify those who have truly elevated BP. Studies on tracking of high BP from childhood to adulthood show inconsistent results [48,114,115]. There is some evidence available that high BP in adolescents and young adults is related to anatomic cardiovascular-renal changes, increased left ventricular measurements, altered haemodynamic functions, and increased stiffness of the carotid arteries and aorta [115].

Present study
We found a low prevalence of hypertensive BP values (3% of the boys and 1% of the girls) confirming that hypertension in childhood and adolescence is rare [116].

Anthropometric measurements (II,V,VI)

Obesity, especially central fat obesity, is related to an increased risk of CVD. A high-energy diet in adults, exceeding the energy demands of the individual, may lead to overweight or obesity, and in turn, increase the risk of hypertension, insulin resistance, and non-insulin dependent diabetes [3]. Obese individuals obviously eat more than they need but there are still inconclusive data on possible differences in energy intake or energy expenditure between lean and obese individuals [117]. There is, however, much evidence available that obesity to a large extent is genetically determined [118,119].

Children and adolescents

The association between childhood and adult obesity is weak but increases with the age of the child [120,121]. Obesity in adolescence is related to morbidity and mortality in CVD in adult life [122]. Several suggestions have been made on how to measure obesity in children, e.g. standard deviation scores of weight, weight/height ratios or indices, and skinfold measurements and ratios [123]. All these measurements are also depending on sex, age, and physical development of the child or the adolescent.

Present study
In agreement with others [124,125] we found that measurements of central fat distribution such as the waist/hip circumference ratio or the subscapular/triceps skinfold ratios show no advantages to body BMI or waist circumference alone, as indicators of unfavourable metabolic or physiologic values in adolescents. These findings suggests that BMI, but also waist circumference, seem to be adequate measurements of obesity in adolescents [126]. Body mass index and waist
circumference are both easy to obtain which make these measurements suitable for epidemiological studies. It is however important to emphasize that these measurements must be evaluated against age- and sex specific reference figures or curves.

Physical fitness (V, VI)

A high level of physical fitness has in epidemiological studies been associated with lower prevalence of CVD risk factors and a lower incidence of CHD [127–131].

Physical fitness, or physical capacity, is a function of two major integrated components, aerobic capacity and muscular strength. It may be difficult to separate the effect on CVD of physical capacity, from that of physical activity or leanness. People who are physically fit tend to be more physically active, and also leaner, and it may be argued that they are physically fit, and lean, because they are physically active. Alternatively physically fit individuals may be a selection of people who are genetically fit, and lean, and therefore select to be physically active. Hence, in this study we have defined physical fitness as a constitutional or "outcome", variable while physical activity is defined as an activity or "exposure" variable (Figure 5).

Children and adolescents

The differences in fitness in adolescence seem to be primarily influenced by genetically determined constitutional factors and body composition and only to a smaller part by differences in level of physical activity and exercise [132]. The wide variation in physical development and maturation may also contribute to the large variation in physical fitness in adolescence [133].

Present study

As expected, in both age-groups, boys performed better on the running test than girls and the 17-year-old boys performed better than the 14-year-old boys. Adolescents who were more physically fit had a more favourable serum lipid profile, and among 17-year-old boys also lower s-insulin values compared to those who were less physically fit. The multivariable analysis revealed that these positive associations between physical fitness and s-lipids and s-insulin were mainly explained by differences in BMI, and not by differences in level of physical activity.

Clustering of cardiovascular risk indicators (II, V, VI)

Risk factors for CVD and related diseases tend to cluster. Dyslipidaemia in combination with obesity, insulin resistance, and hypertension is sometimes referred to as the insulin resistance syndrome or syndrome X. Although the pathogenesis of this syndrome is multifactorial genetic factors in combination with a sedentary life-style, including a high-energy-diet rich in saturated fat leading to obesity, are regarded as major determinants [77–80].
Children and adolescents

Some previous studies have indicated that hyperinsulinemia, hyperlipoproteinaemia, obesity and hypertension cluster already in childhood and adolescence [134-136], and also that these factors track into young adulthood [137].

Present study

In our study only a minor proportion of the adolescents had TG, LDL-C, or BP values that were above, and HDL-C values that were below the internationally accepted reference values [30]. Consequently, even fewer had values for several parameters beyond these cut-off limits. However, our results showed that cardiovascular risk indicators, i.e. high s-insulin, TG, LDL-C, and BP values and low HDL-C values clustered in adolescents with high BMI values. Furthermore, adolescents with high physical fitness reported a higher level of physical activity and also a dietary intake containing less fat. As expected, adolescents with the highest physical fitness were leaner compared to those with the lowest physical fitness. They also showed a more favourable s-lipid profile. Moreover, in girls, but not in boys, clustering of risk indicators was more pronounced in adolescents from families of low socio-economic status (SES) compared to adolescents from families of high/medium SES. Girls showed more clustering of "risk habits" compared to boys, i.e. daily smoking, high intake of dietary fat, and low level of physical activity. Clustering of "risk habits" was, however, not associated to SES of the family. The findings indicate that cardiovascular risk indicators do cluster in adolescents and that clustering is more pronounced in girls from families of low SES.

Genetic and environmental associations to cardiovascular risk indicators

Family history of cardiovascular diseases (I)

We know that CVD aggregate in some families but, with few exceptions, we do not know enough of how this heritage is mediated. It is important to recognize that family members do not only share the same genes, but also, to various extent, the same environment, e.g. dietary habits, level of aerobic exercise, and use of tobacco and alcohol. The interest of genetic influence on CVD has mainly focused on s-lipids but other risk factors for CVD, e.g. obesity and hypertension, also aggregate within families [117-119,138,139]. The genetic influence on s-lipids can be mediated through a single major gene (monogenic disorders), e.g. familial hypercholesterolaemia (FH) with high TC and LDL-C and familial combined hyperlipidaemia (FCH) with high TC; LDL-C, and TG values. These rare disorders are related to a very high incidence of CVD in affected adults. The majority of individuals with dyslipidaemia, including children and adolescents, have polygenic dyslipidaemia, i.e. disorders primarily resulting from the expression of a number of genes
combined with environmental contributors such as diet [21].

Children and adolescents
The familial aggregation of dyslipidaemias has been studied by comparing s-lipid values of parents with diagnosed CVD with the s-lipid levels of their children [140,141]. Alternatively, the prevalence of CVD in parents of children with high and low s-lipid values have been compared [142–146]. These studies show that children to parents with diagnosed CVD have higher lipoprotein and apolipoprotein values compared to children without such a history.

Present study
Our study showed that boys with a family history of early coronary deaths and girls with a family history of stroke had an atherogenic s-lipid profile with higher levels of TC, LDL-C, and Apo B values. We do not know if the sex difference reflects any true difference in the hereditary pattern or if the finding is coincidental. Children with a family history of CVD have been shown also to have higher Lp(a) values [147,148], a finding that could not be confirmed in this study. One explanation could be differences in classification of family history.

Social environment (VI)
Social class differences with higher CVD mortality in underprivileged groups of the population have been demonstrated from most industrialised countries [149,150], including Sweden [151]. The decline in CVD mortality seen in the last decades has not occurred in the lower social classes which has resulted in widening social inequalities [152–154]. The social difference in trends in smoking in different social classes is regarded as a major explanation to the social differences in CVD mortality [155]. Other social factors, e.g. social network and social support seem to have an additional independent effect [156].

Children and adolescents
The mortality and health of young children seem to follow the pattern of their parents with increasing mortality and health problems with decreasing socio-economic status of the family [157], while this pattern is not as obvious in adolescence. In Sweden, differences in physical health between families of different SES are now very small [158]. There is very limited information available on the associations between SES of the family and health status, or health habits, of Swedish adolescents in general, and CVD risk indicators in particular.

Present study
Our findings showed that in both boys and girls, low SES and educational level of the parents were related to more smoking, higher BMI, higher dietary fat intake, and in girls also to lower physical fitness. There were, however, neither differences in body height, nor in BP, s-lipid, s-insulin, or s-ferritin values. Obesity is to a varying degree a social handicap [159] and one explanation to the association between low SES and obesity could therefore be a downward social mobility in obese
individuals resulting in a higher prevalence of obesity in low SE groups. Our results also showed that the quality of the diet in the family seem to be depending upon the educational level of the mother and not the educational level of the father, or the SES status of the family. These findings show that the SES differences in dietary intake in Swedish adolescents demonstrated in the beginning of the 1980's [160] still persist. Girls reported more smoking than boys and daily smoking was associated to low SES of the family and smoking in siblings, but predominantly associated to smoking in peers. Tobacco use was considerably higher among adolescents attending vocational programs at secondary high school as compared to theoretical programs. Daily smokers had a more unfavourable s-lipid profile compared to non-smokers. As mentioned before, clustering of cardiovascular risk indicators was more pronounced in girls from families of low SES.

Previous physical growth and nutrition – "programming" (I, II)

Prenatal growth retardation has been reported to be statistically associated with increased CVD mortality, dyslipidaemia, high blood pressure, insulin resistance, and non-insulin dependent diabetes in adulthood [38–40]. Forsdahl reported regional associations between CVD mortality in adults in different municipalities in Norway and infant mortality a generation earlier in the same regions [161]. Barker in retrospective studies from UK demonstrated an association between low birth weight and later CVD deaths in the same individuals and hypothesized that this association was a major explanation behind the regional differences in CVD mortality [39,40]. Based on animal experiments and the retrospective studies of CVD mortality he proposed a biological explanation for the associations between low birth weight and later CVD, suggesting that foetal malnutrition may result in a permanent underdevelopment of essential organs, e.g. the liver and the pancreas, or alternatively, that early metabolic or nutritional "programming" could result in difficulties to cope with a nutritional overload later in life [39,40,162]. Furthermore, low birth weight has also been associated with higher s-cholesterol and fibrinogen levels and low weight at one year of age with high CVD morbidity in adult life [40].

Some previous studies show that a long period of breast feeding is associated with higher s-cholesterol at follow up while other studies, the present study included, show the opposite. It should be noted, however, that studies showing that breast feeding increases s-cholesterol generally have a short follow up period and in most of the studies only TC have been analysed [163]. Breast fed infants have been shown to have higher HDL-C values than formula fed infants when followed up at 3–6 months of age [164]. Only few studies have investigated the effect of breast feeding on adult s-lipid levels and these studies show the same result as our study, i.e. that subjects with short duration of breast feeding, or no breast feeding at all, have higher TC values [41,43,165].
Children and adolescents
Children who are small at birth have been shown to have raised BP and signs of impaired glucose tolerance [166-168]. To our knowledge there are no previous studies evaluating the associations between early physical growth or infant feeding on the one hand and risk indicators for CVD in adolescents on the other.

Present study
We found no significant correlation between s-lipid values and body weight or length at birth but adolescents with high LDL-C values (upper quartile) had had lower attained heights during infancy and childhood indicating an association in an atherogenic direction between poor physical growth during infancy and childhood and s-lipid level in adolescence. The association was, however, weak and the findings should be interpreted with caution. However, in our study we found no association between s-lipids and present SES or educational level of the parents. This fact may indicate that the differences in physical growth during infancy and childhood between adolescents with LDL-C values above the 75th percentile compared to those below, was not explained by differences in SES. Furthermore, similar to the findings for s-lipids, we could not find any significant correlations between weight or length at birth and current s-insulin values. We found, however, that adolescents with high s-insulin values had had higher attained heights and weights later during infancy and childhood compared to those with lower s-insulin values. These results are in contrast with the hypothesis of an association between poor growth during early life and hyperinsulinemia in adulthood [38, 169]. There was also no association between current BP values and weight or length at birth.

Furthermore, we found that adolescents with a short duration of breast feeding (<6 months) or early introduction of infant formula, had a more unfavourable s-lipid profile with higher mean values of TC and Apo B. This finding, combined with other reports [41,43,163-165], could indicate that there may be different short time and long time effects of breast- or formula feeding with respect to s-lipid level. However, the differences were small and must be interpreted with caution. The effects of breast feeding on s-lipids may be difficult to separate from the effect of other current factors influencing s-lipid levels in adolescence. In our study duration of breast feeding was, however, not associated to confounders such as family history of CVD, current weight, current fat intake, or physical activity.

Diet

Serum lipids (IV, V)
Although there is substantial agreement that diet, predominantly the amount and type of fat intake, affects s-lipids and ultimately the occurrence of CVD, it has been difficult to demonstrate a direct association between dietary fat intake and s-lipid levels [23,170 171].
General discussion

Children and adolescents

Ecological studies comparing different countries have shown clear correlation between fat intake in the population and the level of TC and LDL-C in groups of adolescents [172,173]. The importance of the composition of the diet during childhood for the onset and development of atherosclerosis remains still to be proven [23,24,55,]. A reduction of fat intake, or a modification of fat composition with respect to atherogenic fatty acids, have, in intervention studies, been shown to lower s-lipids in children [174,175]. The associations between dietary fat intake and s-lipids seems to be stronger in infants and younger children than in older children [176-178].

Present study

Our study demonstrates that compared to previous dietary studies [179,160] there have been considerable changes in dietary intake in Swedish adolescents. We found significantly lower mean intake of fat and higher intake of carbohydrates. Interestingly, the increase in carbohydrate intake was explained by an increase in complex carbohydrates but not in sucrose. Mean fat intake was 33 E% (percentage of total energy intake) which was close to the level of 30 E% recommended by several authorities [3,23,31,182]. Mean relative intake of saturated fat had, however, not changed and was still rather high (15 E%). Furthermore, except for a relatively low intake of vitamin D and selenium, compared to present dietary recommendations, intake of vitamins and minerals was adequate [181,182]. It is noteworthy, that there were no major differences in dietary habits or nutrient density of the food between 14- and 17-year-old adolescents or between boys and girls.

When controlling for possible confounders, we could not in our study find any significant association between a high dietary fat intake and s-lipid levels except for higher HDL-C in boys and lower TG in girls, i.e. a non-atherogenic association. There are several possible explanations for the difficulties in showing an association between individual fat intake and s-lipid level; e.g. poor dietary data, genetically determined differences in responsiveness to fat intake [183,184], or a threshold effect, i.e. that the effect of fat intake on s-lipids is more pronounced in the low range of fat intake and therefore difficult to show when the population level of fat intake is comparatively high and homogenous [185]. It should also be considered that atherosclerosis develops over decades and the variation in food consumed would of course be greater if the exposure time and hence the cumulative exposure of different diets were considered. Alternatively, there may be other dietary factors, e.g. antioxidants, that may prove to be more important than fat intake, or at least equally important, for the development, or rather the protection against CVD [186].

Serum ferritin (III, V)

The improved iron status in Sweden, and in many other industrialised countries, is probably caused by improved diet in combination with lower prevalence of diseases causing increased iron losses. In populations
with iron deficiency anaemia there is often a high prevalence of iron consuming diseases, predominantly intestinal parasites and malaria, with increased iron losses and a concurrent low intake of dietary iron. It may therefore be difficult to separate the effects of too large iron losses from that of too low iron intake. Furthermore, there is a wide biological potential in the regulation of body iron status. Iron absorption, if not impeded by intestinal disease, can be considerably increased when iron stores are low [187] and it is noteworthy that women have a higher absorption than men, thus balancing a lower iron intake and iron losses through menstruation [188]. It has been difficult to show any correlation between dietary intake of iron and s-iron status within the same population [189 190]. From these observations it may be tempting to conclude that the most important explanation behind the improved iron status seen in many populations is a lower prevalence of iron consuming diseases and not insufficient iron intake. Consequently, the relative importance of iron intake may have been misjudged resulting in too high recommendations of iron intake with a potential risk of dietary iron overload in vulnerable groups.

Present study
We evaluated s-iron status of adolescents in relation to age, sex, and dietary iron intake. We were able to demonstrate that the mean iron intake was high in boys and adequate in girls. Level of iron stores, estimated by s-ferritin, was not associated to total dietary intake, but a low intake of haeme iron in girls, increased the risk of having low s-ferritin. These findings indicate that the differences in iron stores between boys and girls is not explained by a too low iron intake in girls.

Anthropometric measurements (IV, V)
A high-energy diet, especially in combination with a low level of physical activity, could result in obesity. However, most studies show no or an inverse correlation between reported energy intake and body weight [117]. The common explanation for this has been underreporting in obese individuals although metabolic differences between lean and obese individuals can not be excluded [191].
General discussion

Children and adolescents

Studies in children indicate that obese children report a relatively lower energy intake than lean children [192,193]. It has been demonstrated that obese adolescents tend to underreport their dietary intake more than adolescents of normal weight, when their 7-day self-reported records were compared with estimated energy expenditure using the double labelled water method [194].

Present study

The mean energy and fat intake in our study population was substantially lower compared to earlier Swedish studies using comparable methodology [160,179], a trend also reported from other countries [195,196]. One possible explanation could be a gradual reduction in physical activity over the past few decades [197]. Both boys and girls in this study reported relatively low energy intake compared to current reference figures [180]. Furthermore, a large number of adolescents had an energy intake lower than their estimated basic metabolic rate indicating that the low energy intake found may be influenced by underreporting. The mean ratio of energy intake/estimated energy expenditure was low, especially in girls and the ratio decreased with increasing level of BMI. Adjustment for differences in physical activity did not change the pattern of lower energy intake in obese subjects.

Physical activity

Serum lipids and insulin (V)

The positive effect of physical activity on the atherosclerotic process is not fully understood but an increase in HDL-C, a decrease in VLDL-C and TG, and a lowering of BP are thought to contribute [128]. In adults a high level of physical activity is also associated with a lower incidence of non-insulin-dependent diabetes [198].

Children and adolescents

Studies evaluating the effect of physical activity on cardiovascular risk indicators in children and adolescents indicate that high physical activity have a favourable effect on s-lipids and s-insulin values, especially in obese children [199–201].

Present study

In our study we could not demonstrate any independent effect of physical activity on s-lipid or s-insulin values.

Serum ferritin (III, V)

It has been hypothesized that one benefit from physical exercise on CVD and cancer could be a reduction in body iron [202]. Physical activity is known to reduce concentrations of haemoglobin and s-ferritin. This has been regarded mainly as consequence of increased iron losses leading to iron deficiency, the so-called "sports anaemia" [203]. However, alternatively, the lower haemoglobin concentration and iron stores found in physically fit individuals could indicate a physiological adaptation, including a
redistribution of body iron. That "sports anaemia" is not caused by iron deficiency is suggested by the findings that iron supplements given to athletes with supposed iron deficiency have, in most studies, not been effective, unless the subjects were also anaemic [111].

**Children and adolescents**

Physical exercise seem to have a lowering effect on s-ferritin concentrations also in adolescents [204].

**Present study**

We found that the 17-year-old boys with high physical fitness had significantly lower s-ferritin values compared to boys with the lowest fitness. It is noteworthy that the physically fit boys had lower s-ferritin values despite a higher total and haeme iron intake. In the multivariable analysis it appeared that the inverse association between physical fitness and s-ferritin values in boys was mainly explained by differences in BMI. Interestingly, there were no associations between s-ferritin and neither BMI nor iron intake in girls. Our results are in accordance with a recent study in adult men showing higher s-ferritin values in men with low physical activity and high BMI [205].

Apparently, low iron stores were associated with high physical capacity and not, as could be expected, with low capacity. Therefore, one could argue that the lowering effect on iron stores of physical activity and fitness seen in the older boys may in fact be beneficial. If so, this further suggests that the higher s-ferritin levels found in adult men, compared to adult women, may not be regarded as "normal", especially not for women. On the contrary, the higher s-ferritin might, apart from any biological sex difference, partly result from a sedentary life-style with low physical activity and high energy intake, resulting in increased BMI, low physical fitness, and high iron stores. Our findings indicate that s-ferritin is related to BMI in a similar way as other established cardiovascular risk indicators, i.e. s-lipids and s-insulin.

**Anthropometric measurements (V)**

The major part of daily energy expenditure is explained by basic metabolic energy expenditures, or basic metabolic rate (BMR) (50–70% of daily energy expenditure). Voluntary physical activity accounts for less that 25%. The increase in obesity in society parallels the increase in sedentary life-styles [117]. There are large differences between individuals with respect to spontaneous physical activity (fidgeting). Some evidence suggest that there may be genetically determined differences in BMR and in the metabolism of fat, the "obesity gene" [191].

**Children and adolescents**

Even if epidemiological evidence suggest that obesity is to a large degree genetically determined [118,119,138], it is also generally accepted that characteristics of family life, e.g. diet, physical activity and social support are closely linked to the development and maintenance of obesity in children [206,207].
Present study

Our results show that boys spent more time with strenuous physical activity than girls. An interesting difference between boys and girls was found in the association between physical activity and energy intake. It appeared that girls, contrary to boys, did not report any increase in relative energy intake (energy/kg body weight) with increasing level of physical activity. This finding could indicate that girls tend to use physical activity as a measure of weight-control but, on the other hand, the results could also indicate other sex-related differences between boys and girls in their response to increased physical exercise.

Smoking

Serum lipids (VI)

Cigarette smoking is one of the major risk factors for CVD. In adults smoking is associated to higher s-levels of triglycerides, total VLDL-C, and LDL-C, and to lower levels of HDL-C. In addition to altering s-lipid levels, cigarette smoking increases carboxyhaemoglobin levels, clotting factor concentrations, and blood viscosity [208]. In vitro cigarette smoke exposure produces oxidized LDL, facilitating the accumulation of cholesterol esters in macrophages.

Children and adolescents

Cigarette smoking has been shown to be strongly associated with lesions in the aorta in young men [209] and smoking in adolescents have been associated to higher serum levels of triglycerides, VLDL-C and LDL-C, but also lower levels of HDL-C [210]. Maternal cigarette smoking is the major source of passive smoking and adverse alterations in systemic oxygen transport and lipoprotein profiles have been found in pre-adolescent children exposed to long term passive smoking [211]. Smoking among adolescents in Sweden, as in many other countries, has declined in the last decades, particularly in boys, but still on average 25% of 15-year-old adolescents in Sweden report that they smoke regularly. The positive trend has, however levelled off, and there are even reports of increasing prevalence of smoking [212].

Present study

An important finding in our study, confirming reports from others [210], was that in both boys and girls, daily smoking was associated to an unfavourable s-lipid profile. Girls who were daily smokers had higher mean TC, LDL-C, TG, and Apo B values compared to non-smoking girls and the same pattern was seen in boys, although the differences were not significant for TC, and LDL-C, probably explained by the few cases of daily smokers in boys. Smoking boys, but not smoking girls, also had a significantly lower mean HDL-C value compared to non-smokers. The differences in s-lipid values between smokers and non-smokers were independent of BMI, dietary intake, and physical activity. The associations between s-lipid values and daily smoking among girls still remained after adjustment for use of oral contraceptives except for TG.
Prevention

Preventive strategy

"There are no healthy people – just people who have not been sufficiently examined"

Henrik Tikkanen

The prevention strategy against CVD in adults has been divided into a "high risk strategy", i.e. focusing individuals with "risk values", or a "population strategy", i.e. focusing the entire population [2].

The population intervention approach towards CVD in adults is generally regarded as "safe", and non-controversial, although criticism and questions have been raised [213–216].

The different strategies of prevention of future CVD in children and adolescents have recently been evaluated in the National Cholesterol Education Program committee in the United States [31], the European Society for the Prevention of Cardiovascular Disease [30], and European Society of Pediatric Gastroenterology and Nutrition [23]. They all emphasize a population approach comprising measures directed towards teaching the whole childhood and adolescent population a "healthy life-style" with low-fat diet, high physical activity, and non-smoking. In addition to the population approach, they also suggest a selected screening of s-cholesterol of children and adolescents with a family history of premature CVD (<55 years) in parents or grandparents or children with at least one parent with a high s-cholesterol.

Changing lifestyle in adolescents

It has been demonstrated that dietary-, exercise-, and smoking habits track during adolescence and also that these behaviours may be modified [217], but there is no information available to what degree life-style habits continue to track into adulthood. One exception being smoking, where earlier onset has been shown to be predictive of heavier smoking in later life [218]. Our study shows that smoking habits in adolescents are predominantly related to smoking in peers implying that preventive efforts should focus on peer groups of smokers.

In children, the potential hazards of interventions, especially efforts to change diet, have been emphasized [23,24,219]. This concern is predominantly focusing on younger children but it is also important to recognize unforeseen "side effects" of preventive efforts aiming at changes of eating behaviour and physical activity in adolescents. Some adolescents, mostly girls, are very much focused on their body weight [220] and they are often responsive to advice about healthy life-styles. Such girls already have low intake of energy and fat, some of them as part of an anorectic behaviour, and a further reduction of energy intake in this group could therefore influence their growth and health in a negative way [221,222].
Health education at school

Health education has been an integrated part of education in the Swedish schools since the 18th century and is today a compulsory, although not a clearly defined, part of the school curriculum. In the beginning of the 1970's, improved health education in schools was emphasized by several international and national authorities [29,223,224]. However, the effect of present health education methods are poorly evaluated and there is reason to believe that the education is not properly designed for those most in need. There is an urge for further development of methods emphasizing decision-making and setting of goals [225]. A high-quality health education requires both a proper content and proper teaching methods. As the School Health Services in Sweden is also a responsibility of the school authorities, there are good opportunities for collaboration between teachers, nurses and doctors [226].
**Concluding remarks and suggestions for future research**

Although there are some information on tracking of CVD risk factors from childhood into young adulthood, the ultimate proof that CVD starts in childhood requires longitudinal studies following children with identified risk indicators from childhood into adulthood. It is, however, unlikely that such studies, large enough, controlling for all confounders over time, will ever be performed.

In adolescence there are considerable changes in lifestyle, anthropometry, physiology, and biochemistry, and it is therefore difficult to assess single measurements during this age-period. Adult reference values are, for obvious reasons, not applicable in adolescence, implying that age- and sex-specific reference values are needed.

There are reasons to believe that the predictive power of "CVD risk values" in adolescence for adult manifest CVD is low and this conclusion imply that a general screening of CVD risk factors can not be recommended. A selected "high risk screening" of adolescents with a family history of early deaths from CVD in parents of grand-parents (<55 years), is, however, motivated. Instead, preventive measurements should be directed towards primary prevention including recommendations on non-smoking, high physical activity, and a balanced diet low in atherogenic fatty acids.

Some of the differences in CVD mortality between populations may be explained by differences in genetic predisposition or susceptibility and the interaction with different environmental factors, e.g. social environment, smoking, diet, and physical activity. Further studies are needed to explore genetically determined differences in physiological and biochemical response.

Possible side-effects of general health campaigns, e.g. "sports anorexia" and eating disorders in adolescents, must be recognized, studied and considered. It is also important to be aware of a moralistic view on life-style, e.g. "blaming the victim" (Figure 6). Remember: "No nocere"!

Health education at school should focus more on how to improve self-esteem in the adolescents and should try to involve entire peer-groups with unfavourable health habits, especially smoking.

Finally, we believe that this study has contributed new information on cardiovascular risk indicators in adolescents and has also given valuable experiences on school-based health research which may have important implications for future similar research projects.
Concluding remarks and suggestions for future research

Figure 6. From the propaganda for dairy products in Sweden in 1937: "A healthier mankind is the goal, let us all become A-people! Milk, butter and cheese create A-people".

"The focus on the childhood origins of adult disease has been criticised because influences in early life shape the lives people lead and the social environment in which they live and work. It may be the conditions of adult life that are related to ill-health, and the effect of childhood conditions may therefore be indirect. It is clearly not easy to separate the indirect effects on health of early and later life experiences. Nevertheless, it is important to attempt to distinguish between these two sets of influences since their relative importance is crucial to determining the appropriate locus for interventions which may both improve overall adult health and reduce socio-economic differentials. This is a vital area for further research" [227].
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