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Cardiorespiratory fitness and cardiovascular disease risk factors in children and adolescents

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Stockholm, March 2007

Printed by US-AB
Nanna Svartz väg 4
171 77 Stockholm
ISBN: 978-91-7357-158-6

To my parents and sister

CONTENTS

ABSTRACT

Cardiovascular disease (CVD) events occur most frequently during or after the fifth decade of life, however, there is evidence that the precursors of CVD have their origin already in the first or second decade of life. Low levels of physical activity and cardiorespiratory fitness are major modifiable determinants for CVD development in adults.

The overall aim of the present thesis was to study the associations between physical activity, cardiorespiratory fitness, fatness and CVD risk factors in children and adolescents from the Estonian and Swedish part of the European Youth Heart Study.

Physical activity was measured by accelerometry, and cardiorespiratory fitness was estimated by a maximal ergometer bike test. Total and central body fat were derived from the sum of five skinfold thicknesses and waist circumference, respectively. Additional measured outcomes included fasting insulin, glucose, triglycerides, high density lipoprotein cholesterol, total plasma homocysteine (tHcy), and blood pressure. Genotyping for the methylenetetrahydrofolate reductase (MTHFR) 677C>T polymorphism was done by DNA sequencing.

The main outcome was that cardiorespiratory fitness was negatively associated with a clustering of CVD risk factors in children. Moreover, the results suggested that there is a minimum cardiorespiratory fitness level associated with a low metabolic risk. The results also indicated that the deleterious consequences ascribed to high levels of total and central fatness, such as insulin resistance, were counteracted by having high levels of cardiorespiratory fitness.

On the other hand, the levels of tHcy in children and adolescents were not influenced by the levels of physical activity, fitness, and fatness even after controlling for presence of the MTHFR 677C>T genotype, the main influence on tHcy levels in these individuals. To improve fitness and reduce fatness in children, moderate and vigorous intensity physical activity may have a greater impact than lower physical activity intensity levels.

The data call for the development and testing of preventive strategies, especially for those children with low cardiorespiratory fitness. Longitudinal and interventional studies are needed in order to clarify if changes in physical activity and cardiorespiratory fitness may favourably influence the levels of CVD risk factors already in these ages, and even into adulthood.

LIST OF PUBLICATIONS

I. Ruiz JR, Ortega FB, Rizzo NS, Villa I, Hurtig-Wennlöf A, Oja L, Sjöström M. High cardiorespiratory fitness is associated with low metabolic risk score in children; The European Youth Heart Study. *Pediatr Res*, 2007; 61: 350-355.

II. Ruiz JR, Rizzo NS, Ortega FB, Sjöström M. Association of insulin resistance markers with fatness and fitness in school-aged children. The European Youth Heart Study. *Diabetologia,* (revised version submitted).

III. Ruiz JR, Hurtig-Wennlöf A, Ortega FB, Patterson E, Nilsson TK, Castillo MJ, Sjöström M. Homocysteine levels in children and adolescents are associated with the methylenetetrahydrofolate reductase 677C > T genotype, but not with physical activity, fitness or fatness: The European Youth Heart Study. *Br J Nutr*, 2007; 97: 255-262.

IV. Ruiz JR, Rizzo NS, Hurtig-Wennlöf A, Ortega FB, Wärnberg J, Sjöström M. Relations of total physical activity and intensity to fitness and fatness in children; The European Youth Heart Study. *Am J Clin Nutr*, 2006; 84: 299-303.

LIST OF ABREVIATIONS

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BMI	Body mass index
CI	Confidence interval
CVD	Cardiovascular disease
DNA	Desoxirribonucleic acid
EYHS	European Youth Heart Study
HDLc	High density lipoprotein cholesterol
HOMA	Homeostasis model assessment
MET	Metabolic equivalent
MTHFR	Methylenetetrahydrofolate reductase
SD	Standard deviation
SPSS	Statistical Package for Social Sciences
tHcy	Total plasma homocysteine
TG	Triglycerides
VO2	Oxygen uptake
VO_{2max}	Maximum oxygen uptake
WC	Waist circumference

Cardiovascular disease (CVD) is the leading cause of global mortality (Smith *et al.*, 2004). CVD events occur most frequently during or after the fifth decade of life, however, there is evidence indicating that the precursors of CVD have its origin in childhood (Strong *et al.*, 1992; McGill *et al.*, 2000). Adverse CVD risk factors during childhood have been shown to track into adulthood (Berenson *et al.*, 1998; Raitakari *et al.*, 2003). Results from longitudinal studies have shown that the presence of multiple CVD risk factors among children is associated with more extensive fatty streaks and fibrous plaques in later life (Berenson *et al.*, 1998). The most recognized CVD risk factors are triglycerides, high density lipoprotein cholesterol (HDLc), total cholesterol, insulin resistance, homocysteine (tHcy), inflammatory proteins, blood pressure, total and central body fat, and cardiorespiratory fitness.

A sedentary lifestyle together with a poor diet, along with tobacco, are the leading modifiable behaviours implicated in the development of CVD and death (Mokdad *et al.*, 2004). Increased energy intake combined with reduced energy expenditure results in body fat accumulation. The consequences on health of excess body fat are evident. Adults who were overweight in childhood have higher levels of blood lipids and lipoproteins, blood pressure, and fasting insulin levels, and thus are at increased risk for CVD compared with adults who were thin as children (Freedman *et al.*, 2001; Steinberger *et al.*, 2001; Thompson *et al.*, 2007). Moreover childhood overweight confers a 5-fold or greater increase in risk for being overweight in early adulthood relative to children who were not overweight at the same age (Steinberger *et al.*, 2001; Guo *et al.*, 2002; Thompson *et al.*, 2007).

The protective effect of intentional physical activity on the above mentioned CVD risk factors has been reported in people of all ages (Strong *et al.*, 2005; Pedersen & Saltin, 2006). However, these findings are often confined to questionnaire-based assessment of physical activity, which often lack the necessary accuracy, especially in young people (Kohl *et al.*, 2000).

One factor related to physical activity is cardiorespiratory fitness. Physical activity and cardiorespiratory fitness are closely related in that fitness is partially determined by physical activity patterns over recent weeks or months.

There is increasing evidence indicating that high levels of cardiorespiratory fitness provides strong and independent prognostic information about the overall risk of illness and death, especially related to cardiovascular causes (LaMonte & Blair, 2006).

Cardiorespiratory Fitness

Cardiorespiratory fitness reflects the overall capacity of the cardiovascular and respiratory systems and the ability to carry out prolonged exercise (Taylor *et al.*, 1955). Hence, cardiorespiratory fitness has been considered as a direct measure of the physiological status of the individual.

The gold standard for the measurement of cardiorespiratory fitness is the maximal rate of oxygen uptake (VO_{2max}). VO_{2max} is the rate at which an individual is able to consume oxygen. VO_2 can be measured during indirect calorimetry in a maximal test, or can be estimated through different equations from the performance achieved in maximal or submaximal tests. The level of cardiorespiratory fitness is highly associated with the performance of other health-related fitness parameters in young people and in adults. A cross-sectional study with almost 3000 adolescents showed that the performance of several health-related fitness tests (handgrip strength, bent arm hang, standing-long jump, 4x10m shuttle run test, and seat and reach) was higher in adolescents with high levels of cardiorespiratory fitness compared to those with lower levels of cardiorespiratory fitness (Ortega *et al*., 2005).

Cardiorespiratory fitness, cardiovascular fitness, cardiorespiratory endurance, aerobic fitness, aerobic capacity, aerobic power, maximal aerobic power, aerobic work capacity, physical work capacity, and maximal oxygen uptake (VO_{2max}), refer to the same concept and are used interchangeably in the literature.

Cardiorespiratory fitness is influenced by several factors including age, sex, health status, and genetics. It has been suggested that up to 40% of variation in the level of cardiorespiratory fitness is attributable to genetic factors (Bouchard *et al.*, 1986). However, as stated before, the level of cardiorespiratory fitness is mainly determined by physical activity patterns. In children and adolescents, there is a positive association between objectively measured physical activity and cardiorespiratory fitness (Brage *et al.*, 2004; Gutin *et al.*, 2005; Andersen *et al.*, 2006). Less is known about how physical activity intensity levels influence the levels of cardiorespiratory fitness and other CVD risk factors in children.

Cardiorespiratory fitness and CVD

The association between the level of cardiorespiratory fitness and the risk of all-cause and cause-specific mortality in adults was first studied by Blair and colleagues (Blair *et al.*, 1989). They showed that higher levels of cardiorespiratory fitness appear to delay all-cause mortality primarily due to decreased rates of CVD and cancer.

The same results were corroborated two decades later in men (Myers *et al.*, 2002) and women (Gulati *et al.*, 2003; Mora *et al.*, 2003; Gulati *et al.*, 2005). Recent reports indicate that these findings are also valid in apparently healthy persons, and persons with a disease, such as diabetes mellitus, hypertension, metabolic syndrome and several types of cancer (LaMonte & Blair, 2006).

High cardiorespiratory fitness during adolescence has also been associated with a healthier cardiovascular profile during these years, and also later in life (Castillo-Garzon *et al.*, 2007), and the benefits seems independent of the level of body weight (Figure 1). However, the association between cardiorespiratory fitness and CVD risk factors in children is still uncertain probably because of low research priority that research on this topic has had. Furthermore, most children are asymptomatic for CVD.

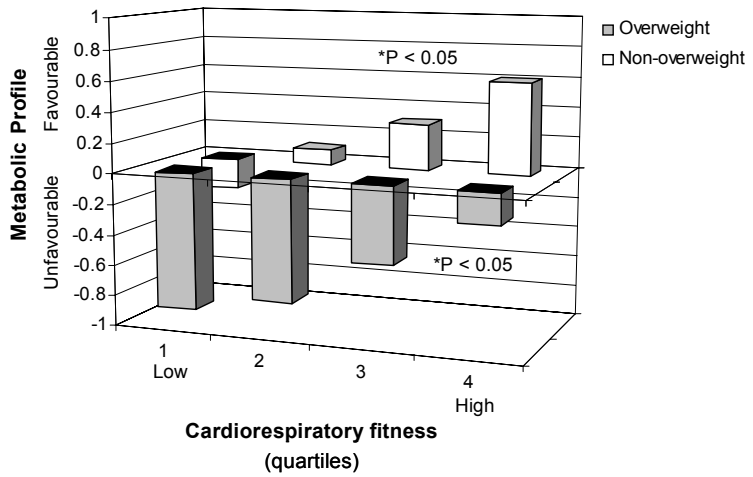

Figure 1. Metabolic profile (standardized values of triglycerides, low density lipoprotein cholesterol, HDLc and glycaemia) and cardiorespiratory fitness in overweight and non-overweigh Spanish adolescents aged 12-18.5 years. *P for trend in both overweight and non-overweight categories (Castillo-Garzon *et al.*, 2007).

It has been suggested that cardiorespiratory fitness be included in the European Health Monitoring System for the adult population (Sjöström *et al.*, 2005), but the question of whether cardiorespiratory fitness should be assessed in European health monitoring systems from the early stages of life remains to be answered. Understanding the association between cardiorespiratory fitness and CVD-related outcomes in children and adolescents would help to establish whether cardiorespiratory fitness could be proposed as a health marker or not at these ages.

The overall aim of the investigations summarized in this thesis was to increase our understanding of the associations between physical activity measured by accelerometry, cardiorespiratory fitness, fatness and CVD risk factors in children and adolescents.

The specific aims of the separate studies were as follows:

- To examine the associations of cardiorespiratory fitness with a clustering of metabolic risk factors in children, and to examine whether there is a cardiorespiratory fitness level associated with a low metabolic risk (**Study I**).
- To examine the associations between markers of insulin resistance and 1) body fat and waist circumference, taking into account cardiorespiratory fitness, and 2) cardiorespiratory fitness at different levels of body fat and waist circumference in children (**Study II**).
- To examine the associations of total plasma homocysteine with physical activity, cardiorespiratory fitness and fatness in children and adolescents (**Study III**).
- To examine the associations of total physical activity and intensity levels with cardiorespiratory fitness and fatness in children (**Study IV**).

The thesis work has been developed with data obtained from the Estonian and Swedish part of the European Youth Heart Study (EYHS). The EYHS is a school-based, cross-sectional study designed to examine the interactions between personal, environmental and lifestyle influences on risk factors for future CVD (Riddoch *et al.*, 2005). Study design, sampling procedure, participation rates and study protocol have been reported elsewhere (Poortvliet *et al.*, 2003; Wennlöf *et al.*, 2003).

Data collection took place during the school year 1998/1999. In Estonia, the city of Tartu and its surrounding rural area was the geographical sampling area. In Sweden, 8 municipalities (Botkyrka, Haninge, Huddinge, Nynäshamn, Salem, Södertälje, Tyresö, and Örebro) were chosen for data collection. The local ethical committees approved the study (University of Tartu no. 49/30-1997, Örebro City Council no. 690/98, and Huddinge University Hospital no. 474/98). Written informed consent was obtained from parents of the children and from both the parents of the adolescents and the adolescents themselves. All children and adolescents gave verbal assent.

Participants

The basic characteristics of the participants and the variables examined in each sub-study are presented in Table 1.

Physical Examination

Body weight was measured to the nearest 0.1 kg (SECA digital balance beam, calibrated according to the manufacturer's manual), and height to the nearest 0.5 cm (Harpenden transportable stadiometer), with the children clad only in their underwear. Body mass index (BMI) was calculated as weight/height squared (kg/m^2).

Skinfold thickness was measured with a Harpenden caliper (Baty International, Burguess Hill, U.K.) at the biceps, triceps, subscapular, suprailiaca and triceps surae areas on the left side of the body according to the criteria described by Lohman *et al.* (Lohman *et al.*, 1991). All measurements were taken twice and in rotation, and the mean value was calculated. If the difference between the two measurements was more than two millimeters a third measurement was taken and the two closest measurements were averaged.

Waist circumference was measured in duplicate with a metal anthropometric tape midway between the lowest rib and the iliac crest at the end of a gentle expiration.

Table 1. Summary of the characteristics of the sub-studies.

Study	Country	Subjects	Age	Variables studied
I. High cardiorespiratory fitness is associated with low metabolic risk score in children	Estonia & Sweden	444 girls 429 boys	9-10 years	Cardiorespiratory fitness, skinfold thickness, TG, HDLc, blood pressure, insulin, glucose
II. Association of insulin resistance markers with fatness and fitness in prepubertal children	Estonia & Sweden	444 girls 429 boys	9-10 years	Cardiorespiratory fitness, BMI, WC, skinfold thickness, insulin, glucose, pubertal status
III. Homocysteine levels in children and adolescents are associated with the MTHFR 677C>T genotype, but not with physical activity, fitness or fatness	Sweden	138 girls 163 boys 194 girls 185 boys	9-10 years 15-16 years	Cardiorespiratory fitness, physical activity, BMI, skinfold thickness, homocysteine, MTHFR 677C>T genotype, intake of folate and vitamin B_{12}, pubertal status
IV. Relations of total physical activity and intensity to fitness and fatness in children	Estonia & Sweden	401 girls 379 boys	9-10 years	Cardiorespiratory fitness, physical activity, BMI, skinfold thickness, pubertal status

TG, triglycerides; HDLc, high density lipoprotein cholesterol; BMI, body mass index; WC, waist circumference, MTHFR, methylenetetrahydrofolate reductase.

The mean between the two measurements was used for further calculations. If the two measurements differed by more than one centimetre, a third measurement was taken, and the two closest measurements were averaged.

BMI and skinfold thickness were used as markers of total adiposity, whereas waist circumference was used as marker of central adiposity.

The individuals were also categorized as normal-weight or overweight (including obesity) following the International Obesity Task Force proposed gender- and age-specific BMI cut-off points (Cole *et al.*, 2000).

Identification of pubertal development was assessed according to Tanner and Whitehouse (Tanner & Whitehouse, 1976). Pubertal stage was recorded by a researcher of the same gender as the child, after brief observation. Breast development in girls, and genital development in boys, were used for pubertal classification.

Physical Activity

Physical activity was measured during four consecutive days (during two week days and at least one weekend day) with an activity monitor (MTI model WAM 7164, Manufacturing Technology Inc., Shalimar, Florida, formerly known as Computer Science and Applications Inc.) worn at the lower back. At least three days of recording, with a minimum of 10 hours registration per day, was set as an inclusion criterion.

Total physical activity was expressed as total counts recorded, divided by total registered time (counts/min). The time engaged in moderate physical activity and vigorous physical activity was calculated and presented as the average time per day during the complete registration. Moderate physical activity (3-6 metabolic equivalents, METs), and vigorous physical activity (>6 METs) intensities were based upon the cut-off limits published elsewhere (Trost *et al.*, 2002). The time spent in at least moderate intensity level (>3 METs) was calculated as the sum of time spent in moderate or vigorous physical activity. Each minute over the specific cut-off was summarized in the corresponding intensity level category.

Validation studies examining the accelerometer used in this study and the construction of summary variables for intensity of movement suggest that it is a valid and reliable measure of children's and adolescent's physical activity (Trost *et al.*, 1998; Puyau *et al.*, 2004). The precision of objective assessment of physical activity in children is superior to subjective methods, however there are some limitations which should be highlighted. The accelerometer must be removed during swimming, contact sports, showering, and bathing. Any activity involving minimal vertical displacement of the body (i.e. cycling) is also underestimated. Four to five days of activity monitoring have been proposed as a suitable duration for accurate and reliable estimates of usual physical activity behavior in children (Trost *et al.*, 2000). Four days of data were available in most of the participants in this study.

There is controversy about the best way to express physical activity. When expressed as energy expended in movement, heavier adolescents seem to engage in relatively large amounts of physical activity because they use more energy to move their bodies for a given amount activity compared to lighter adolescents.

However, when physical activity is expressed as movement, heavier adolescents will appear to engage in less physical activity than lighter peers. The time spent in physical activity of various intensities seems more pertinent for purpose of making exercise recommendations (Ekelund *et al.*, 2002).

Cardiorespiratory Fitness

Cardiorespiratory fitness was determined by a maximum cycle-ergometer test as described elsewhere (Hansen *et al.*, 1989). The subjects cycled at 50-70 revolutions per minute on an electronically braked Monark cycle-ergometer (Monark 829E Ergomedic, Vansbro, Sweden). The test protocol was sex and age-specific, and is presented in detail in Table 2. The test was finished when the subject could no longer maintain the pedalling frequency of at least 30 revolutions per minute, even after vocal encouragement.

Table 2. Test protocol.

Gender	Age (years)	Weight (kg)	Initial work rate (W)	Δ Work rate (W)	Stages (seconds)
Girls & boys	9-10	< 30	20	20	180
Girls & boys	9-10	> 30	25	25	180
Girls	15-16	-	40	40	180
Boys	15-16	-	50	50	180

Kg, kilogram; W, power output; Δ, increase.

A true exhaustive effort was considered to have been achieved if the subject had a heart rate higher than 185 beats per minute, and at the same time the leader observed that the child could no longer keep up. Heart rate was monitored continuously by telemetry (POLAR Vantage NV, Kempele, Finland).

The power output was calculated as: $W_1 + (W_2 \cdot t/180)$, where W_1 is the work rate at the final fully completed stage, W_2 is the work rate increment at the final incomplete stage, and t is time in seconds at the final incomplete stage.

The “Hansen formula” for calculated maximal oxygen uptake (VO_2max) in ml/min was: 12 x calculated power output + 5 x body weight in kg (Hansen *et al.*, 1989). Cardiorespiratory fitness was expressed as VO_{2max} per kilogram of body mass (ml/kg/min) because of the homogeneity in age, pubertal status, height, weight, and obesity grade of the children, and for the purpose of comparing the results with previous publications.

However, cardiorespiratory fitness was also expressed as W/kg, as a more direct score. The test used has been previously validated in children of the same age (Riddoch *et al.*, 2005), and is highly correlated in children with directly measured VO_2max (r = 0.95 and r = 0.90, in girls and boys, respectively) (Hansen *et al.*, 1989).

Blood Variables

With the subject in the supine position, blood samples were taken by venipuncture after an overnight fast, using vacuum tubes (Vacuette, Greiner Lab Technologies Inc). The fasting state was verbally confirmed by the subject before blood sampling.

Blood was centrifuged for 10 minutes at 2000 g, serum was separated within 30-60 minutes, and the samples were stored at -80°C. Serum concentrations of triglycerides were measured using the lipase/glycerol kinase/glycerol phosphate oxidase enzymatic method, high density lipoprotein cholesterol (HDLc) was measured using the homogeneous polyanion/cholesterol esterase/oxidase enzymatic method, and glucose using the hexokinase method. All were analysed on an Olympus AU600 autoanalyser (Olympus Diagnostica GmbH, Hamburg, Germany).

The insulin for the Estonian subjects was analyzed with an enzyme immunoassay (DAKO Diagnostics Ltd., Ely, England). All analyses were performed at Bristol Royal Infirmary, UK, with the exception of insulin for the Swedish subjects, which was performed at Huddinge University Hospital, Sweden (Elecsys, Roche Diagnostics GmbH, Mannheim, Germany). The homeostasis model assessment (HOMA) was calculated as described by Matthews et al. (Matthews *et al.*, 1985): fasting insulin (mU/L) x fasting glucose (mmol/L) / 22.5.

Total plasma homocysteine (tHcy) in acidified citrated plasma (Willems *et al.*, 1998) was assayed using a fluorescence polarization immunoassay on a IMx® unit (Abbott Laboratories, IL, USA). Single-point measurements were performed for all analytes.

Genetics

Total blood DNA was extracted and purified from 200 µL of whole blood anticoagulated with EDTA, using the QIAamp DNA Blood Mini Kit by the spin procedure, according to the instructions of the manufacturer (QIAGEN Inc., Valencia, CA, USA). Genotyping of the 677C>T variant in the methylenetetrahydrofolate reductase (MTHFR) gene was performed using the Pyrosequencing platform (Biotage AB, Uppsala, Sweden, www.biotage.com), as described recently (Borjel *et al.*, 2006).

Blood Pressure

The 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 according to the manufacturer's recommendation after checking the arm circumference.

The subject was in a seated, relaxed position for at least 6 minutes. Recordings were made every second minute for 10 or more minutes with the aim of obtaining a set of systolic recordings not varying by more than 5 mmHg. The mean value of the last three recordings was used as the resting systolic and diastolic blood pressure, in mmHg.

Clustering of Metabolic Risk Factors

The clustering of metabolic risk factors was computed from the following variables: insulin, glucose, HDLc, triglycerides, skinfold thickness, and blood pressure (systolic and diastolic blood pressure). Each of these variables was standardized as follows: standardized value = (value - mean)/ standard deviation. The HDLc standardized value was multiplied by -1 to confer higher risk with increasing value for the purpose of calculating the metabolic risk score. The mean of the standardized values of systolic and diastolic blood pressure was calculated.

The metabolic risk score was calculated as the mean of the six standardized scores separately for boys and girls. Children being below the 75th percentile of the score were defined as having low metabolic risk, and children being at or above the 75th percentile of the score were defined as having high risk.

The same percentile has been used in different health-related variables (e.g. waist circumference, BMI, triglycerides, insulin levels, systolic blood pressure, total cholesterol to HDLC ratio, plasma leptin, etc.) in a number of population- based studies to define subjects at low (<75th) or high (≥75th) risk (Chu *et al.*, 2000; Wyszynski *et al.*, 2005).

Even if none of the children had clinical disease, a high metabolic risk score may not be a desirable condition.

There is no standard paediatric definition of the metabolic syndrome. Several attempts have been made in adolescents aged 12 to 19 years by using criteria analogous to Adult Treatment Panel (ATP) III (Cook *et al.*, 2003; de Ferranti *et al.*, 2004; Shaibi *et al.*, 2005), however, no child involved in the present study had three or more high values in any of the variables included in the ATP III definition. Other approaches have been made elsewhere in order to compute a clustering of metabolic risk factors in healthy children (Brage *et al.*, 2004). We have computed a similar risk score that has been reported previously because of similarities in ages of the studied children, and study methodology (Brage *et al.*, 2004).

Statistics

Basic descriptive statistics were applied for each sex and age group. All variables were checked for normality of distribution before the analysis, and transformations were applied when necessary. Gender and age group differences were assessed by analysis of variance (ANOVA).

Associations between metabolic risk factors and cardiorespiratory fitness quartiles were assessed by ANOVA, as were the associations between clustering of metabolic risk factors and cardiorespiratory fitness quartiles (Paper I).

The cardiorespiratory fitness threshold to discriminate between either a low or high metabolic risk was calculated by receiver operating characteristic (ROC) curve.

The association between markers of insulin resistance, skinfold thickness and waist circumference was assessed by analysis of covariance (ANCOVA), with skinfold thickness or waist circumference as fixed factors, HOMA, insulin or glucose as a dependent variables, and cardiorespiratory fitness, age, pubertal status and study location as covariates (Paper II). The association between markers of insulin resistance and cardiorespiratory fitness was assessed by multiple regression analyses, separately by body fat and waist circumference tertiles.

The effect on tHcy of gender, age, and MTHFR 677C>T were analyzed by ANOVA, and the subgroup means were compared by Tukey's test (Paper III). Multiple regressions were used to study the association between tHcy and physical activity, cardiorespiratory fitness, and body fat, as well as to study the association between physical activity, cardiorespiratory fitness and fatness (Paper IV) after controlling for several confounders.

The analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 14.0 & 15.0 for WINDOWS; SPSS Inc, Chicago) and the level of significance was set to 0.05.

The present thesis shows that cardiorespiratory fitness is negatively associated with a clustering of CVD risk factors in children. Moreover, the results suggest that a cardiorespiratory fitness level that confers a low metabolic risk is identifiable. The results also indicate that the deleterious consequences ascribed to high levels of total and central fatness could be counteracted by having high levels of cardiorespiratory fitness. On the other hand, the levels of tHcy in children and adolescents are not influenced by the levels of physical activity, cardiorespiratory fitness, and body fat even after controlling for presence of the MTHFR 677C>T genotype. To improve fitness and reduce fatness in children, moderate and vigorous intensity physical activity may have a greater impact than lower physical activity intensity levels.

The data call for the development and testing of preventive strategies, especially for those children with low cardiorespiratory fitness, and also reinforce the need to include cardiorespiratory fitness testing in national and European health monitoring systems. Results from longitudinal studies are needed to elucidate the influence of having low cardiorespiratory fitness in childhood on the likelihood of having CVD later in life. Moreover, interventional studies are also necessary in order to clarify if improvements in cardiorespiratory fitness and/or changes in behavioural factors, such as increasing the levels and patterns of physical activity, may favourably influence the levels of CVD risk factors already in these ages.

Cardiorespiratory Fitness and Metabolic Risk in Children (Study I)

High cardiovascular fitness is associated with low metabolic risk score in children; The European Youth Heart Study. In the first study, the associations of cardiorespiratory fitness with a clustering of metabolic risk factors in children were studied, and we examined if there was a cardiorespiratory fitness level associated with a low metabolic risk.

The rationale behind this study was that despite evidence of the association between single CVD risk factors and cardiorespiratory fitness in young adult and adult populations, no consensus exists regarding the minimum cardiorespiratory fitness level associated with a healthy (or more favourable) cardiovascular profile in children.

The results clearly indicated that a lower metabolic risk score is associated with higher levels of cardiorespiratory fitness in both girls and boys (Figure 2).

Moreover, the ROC analysis showed significant discriminatory accuracy of cardiorespiratory fitness to identify either a low or high metabolic risk in both sexes. The cardiorespiratory fitness values at these points were 37.0 and 42.1 mL/kg/min in girls and boys, respectively.

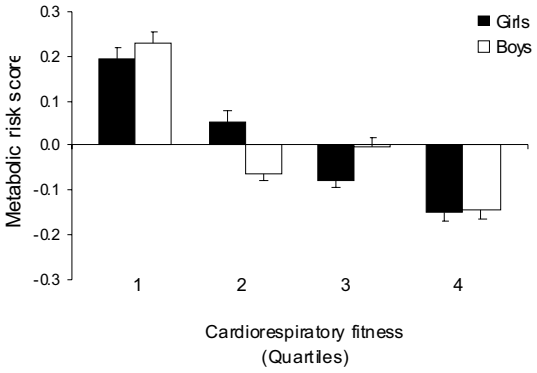

Figure 2. Clustering of cardiovascular disease risk factors (metabolic risk score) and cardiorespiratory fitness in children. Data shown as mean and SD.

The values found in the present study are similar to the cut-off points proposed by the Cooper Institute: ≥ 38 and ≥ 42 ml/kg/min for girls and boys, respectively (The Cooper Institute for Aerobics Research, 1999). These cut-off values were extrapolated from the thresholds established for adults (Blair *et al.,* 1989), while the cut-offs values proposed here have been mathematically calculated within the sample. Of note are the similarities among the cardiorespiratory fitness cut-offs values, despite the differences in the approaches used to calculate them, which support the existence of an optimal cardiorespiratory fitness level already in young persons.

Logistic regression analysis showed that girls with cardiorespiratory fitness levels above 37.0 mL/kg/min were 3.09 times more likely to have a low metabolic risk when compared to those with cardiorespiratory fitness levels below this value. Similarly, boys with cardiorespiratory fitness levels above 42.1 mL/kg/min were 2.42 times more likely to have a low metabolic risk when compared to those with cardiorespiratory fitness levels below this value.

Having optimal values for cardiorespiratory fitness health set from an early age could be useful to identify the target population for primary prevention, as well as for health promotion policies. In this regard, schools may play an important role: firstly by identifying children with low cardiorespiratory fitness, and secondly by promoting positive health behaviours, such as encouraging children to engage in physical activity, as well as decreasing time spent in sedentary activities.

Cardiorespiratory Fitness, Fatness and Insulin Resistance in Children (Study II)

Markers of insulin resistance are associated with fatness and fitness in school-aged children; The European Youth Heart Study. In the second study, the association between markers of insulin resistance and body fat and waist circumference, taking into account cardiorespiratory fitness were studied. The association of markers of insulin resistance with cardiorespiratory fitness within different levels of total and central fatness (i.e. low, middle and high) were also examined.

The aim for this study was to examine if the detrimental consequences attributed to high adiposity may be attenuated by having high levels of cardiorespiratory fitness.

The results showed that HOMA is positively associated with both body fat (as a marker of total adiposity) and waist circumference (as a marker of central adiposity) after adjusting for several confounders including cardiorespiratory fitness (Figure 3).

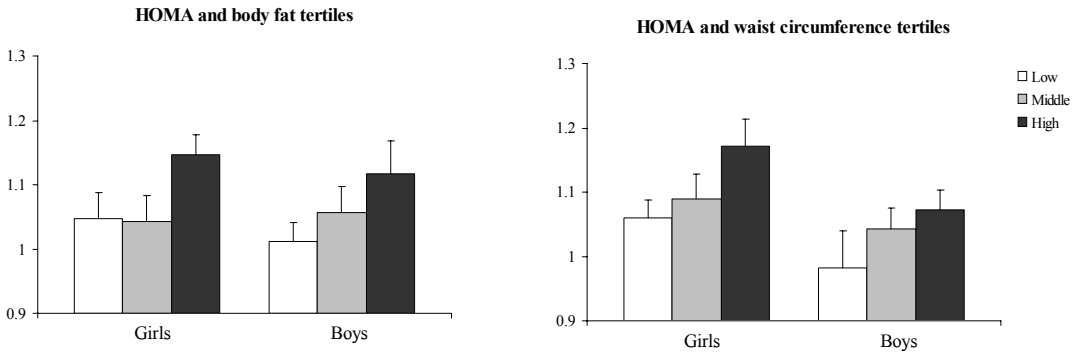

Figure 3. Homeostais model assessment (HOMA) stratified by body fat (expressed as skinfold thickness) and waist circumference tertiles in girls and boys. Values are mean, and errors bars represent 95% confident intervals. Data were analyzed by one-way analysis of covariance after adjusting for cardiorespiratory fitness, age, pubertal status and study location. Low, Middle and High, equals first, second and third tertile, respectively. Body fat and waist circumference, were transformed to the natural logarithmic, and HOMA was raised to the power of 1/3. HOMA was positively associated (all $P < 0.001$) with body fat and waist circumference in both girls and boys.

In addition, cardiorespiratory fitness was related to HOMA in those children with high levels of body fat and waist circumference (Figure 4).

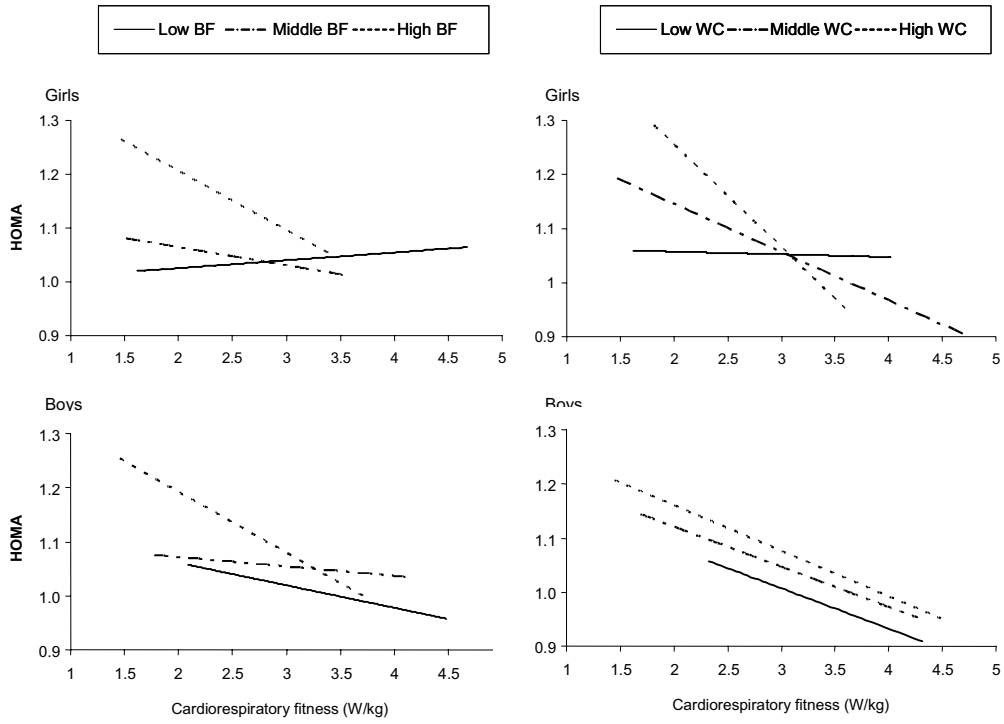

Figure 4. Relationship between homeostasis model assessment (HOMA) and cardiorespiratory fitness by body fat (BF) and waist circumference (WC) tertiles for girls and boys. Low, middle and high equals to first, second and third tertile, respectively. HOMA was transformed data to the power of 1/3 before analysis. Girls: low BF, r = 0.040 (P = 0.703); middle BF, r = -0.062 (P = 0.456); high BF, r = -0.218 (P = 0.002); low WC, r = -0.011 (P = 0.862); middle WC, r = -0.221 (P = 0.012); high WC, r = -0.407 (P < 0.001). Boys: low BF, r = -0.093 (P = 0.197); middle BF, r = -0.042 (P = 0.623); high BF, r = -0.252 (P = 0.016); low WC, r = -0.312 (P = 0.018); middle WC, r = -0.167 (P = 0.032); high WC, r = -0.197 (P = 0.004).

Cardiorespiratory fitness explained a significant proportion on the HOMA variance in those children with high levels of body fat and waist circumference (Table 3).

Several studies support the link between metabolic risk factors and adiposity in children (Caprio *et al.*, 1995; Gutin *et al.*, 2004). Moreover, results from North American adolescents showed that both percentage of body fat (measured with dual-energy X-ray absorciometry) and cardiorespiratory fitness are associated with fasting insulin in adolescents (Gutin *et al.*, 2004).

Table 3. Multiple regression coefficients (β) and coefficient of determination (R^2) examining the association of HOMA with cardiorespiratory fitness separately by body fat (expressed as skinfold thickness) and waist circumference tertiles (T), after controlling for sex, age, pubertal status and study location

Outcome variable	Cardiorespiratory fitness	*β*	*P*	R^2
HOMA	1st T: Low body fat	0.095	0.232	0.087
	2nd T: Middle body fat	-0.009	0.909	0.079
	3rd T: High body fat	-0.241	0.001	0.156
HOMA	1st T: Low waist circumference	-0.010	0.905	0.100
	2nd T: Middle waist circumference	-0.148	0.063	0.086
	3rd T: High waist circumference	-0.250	0.001	0.215

Collectively, the findings indicate the deleterious consequences of having high total and central fatness already in young individuals, and also suggest that these associations could be attenuated by having high levels of cardiorespiratory fitness.

Physical Activity, Fitness, Fatness and tHcy in Children and Adolescents

(Study III)

Homocysteine levels in children and adolescents are associated with the methylenetetrahydrofolate reductase 677C > T genotype, but not with physical activity, fitness or fatness. The third study focused on the associations of tHcy with physical activity, cardiorespiratory fitness and fatness, after controlling for potential confounders including the MTHFR 677C>T genotype, in children and adolescents.

This topic was addressed because tHcy has been suggested to be an independent risk factor for several multi-system diseases (Virtanen *et al.* 2005).

The levels of tHcy seem susceptible to modification by different lifestyle factors, such as physical activity, intake of folate and vitamin B_{12}, obesity and others. Moreover, the levels of tHcy have been negatively associated with cardiorespiratory fitness in women (Kuo *et al.*, 2005).

The results suggest that tHcy levels are not influenced by physical activity, cardiorespiratory fitness, and body fat (expressed as skinfold thickness and BMI) in young individuals (Figure 5).

On the other hand, tHcy levels are significantly higher in the MTHFR 677TT subgroup compared to the MTHFR 677CC and MTHFR 677CT subgroups in both children and adolescents.

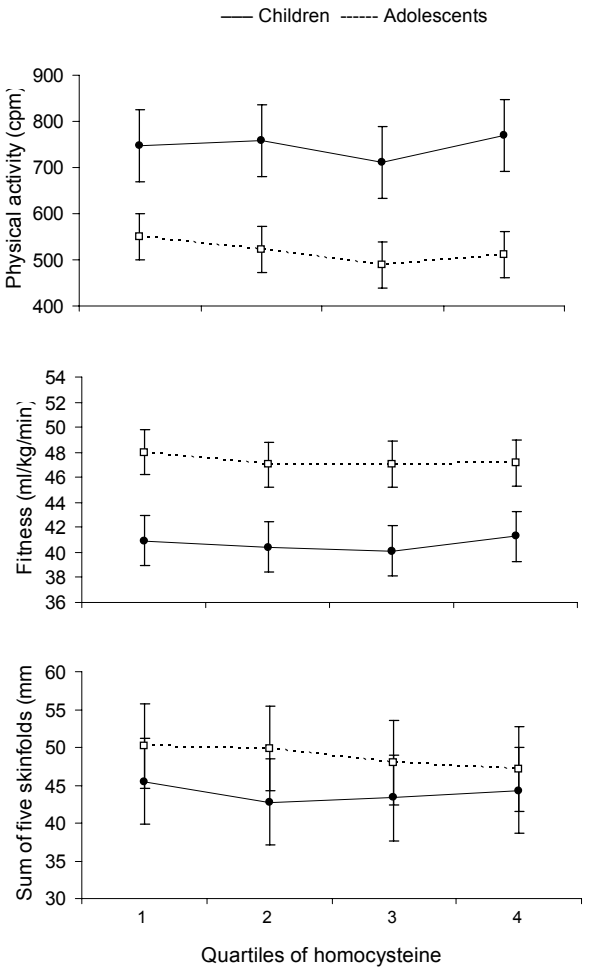

Figure 5. Mean values of total physical activity, cardiorespiratory fitness, and sum of five skinfolds stratified by quartiles of homocysteine for children and adolescents. Errors bars represent 95% CIs.

The association between levels of tHcy and physical activity has been evaluated in a few interventional studies with obese individuals, resulting in a reduction of the tHcy levels after the intervention period (Gallistl *et al.*, 2000; Randeva *et al.*, 2002). We did not find any association between total physical activity and tHcy levels, either when the association of physical activity intensity levels with tHcy levels was examined.

Negative associations between tHcy levels and cardiorespiratory fitness have been reported in women (Kuo *et al.*, 2005), which is not in concordance with the results obtained in the present study. It must be borne in mind that the individuals involved in this study were healthy children and adolescents with no existing cardiovascular pathologies, and it may be that tHcy is not as sensitive to cardiorespiratory fitness as other traditional cardiovascular risk factors are. Similarly, while body fat has been associated with tHcy levels in obese children and adolescents (Gallistl *et al.*, 2000), I did not find an association between body fatness (as expressed as skinfold thickness or as BMI) and tHcy levels, even when the analyses were performed separately for normal-weight or overweight-obese categories.

Physical Activity, Fitness and Fatness in Children (Study IV)

Relations of total physical activity and intensity to fitness and fatness in children; The European Youth Heart Study. In this study, the association of total physical activity and intensity levels with cardiorespiratory fitness and fatness in children was studied.

The aim of this study was to clarify how the amount and intensity of physical activity are associated with cardiorespiratory fitness and fatness in children as young as 9 years.

The results showed that the intensity of physical activity, especially vigorous physical activity, is negatively associated with body fatness (Figure 6), whereas both the amount and intensity of physical activity are positively associated with cardiorespiratory fitness in children.

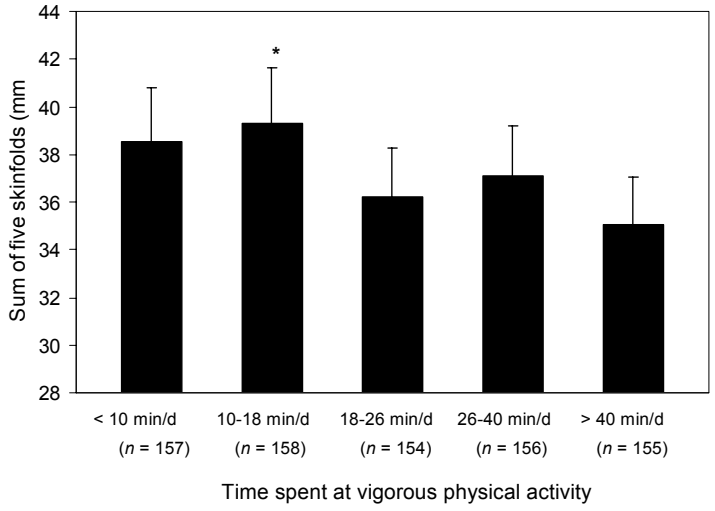

Figure 6. Body fat (expressed as sum of five skinfolds) and time spent at vigorous physical activity. *A significant difference (P < 0.001) was observed between those who accumulated > 40 min of vigorous physical activity per day and those who accumulated 10-18 min/d at this intensity level. Data shown as mean and 95% CIs.

The association between vigorous physical activity and cardiorespiratory fitness and fatness is consistent with other studies (Rowlands *et al.*, 1999; Gutin *et al.*, 2005), which suggest that intensity rather than amount of physical activity may be more important in relation to the prevention of obesity in children. However, it is reasonable to recommend moderate physical activity, especially for obese children and adolescents, until higher intensities can be attained.

Study Strength and Limitations

The cross-sectional nature of the studies in the present thesis does not allow us to infer causality from the results. The cardiorespiratory fitness test requires relatively minor equipment (i.e. cycle-ergometer and a heart rate monitor) and can be easily performed in a clinical setting. However, a maximal test requires a maximal effort to be done, which may not be adequate for populations with clinical pathologies.

The HOMA model is a method for assessing β-cell function and insulin resistance from fasting insulin and glucose concentrations, whereas the gold standard for measuring insulin sensitivity/resistance is the euglycemic-hyperinsulinemic clamp. However, the latter method is too invasive and costly, and may not be suitable for large epidemiological studies. HOMA has been compared with a number of well validated methods used to measured insulin resistance and β-cell function (Wallace *et al.*, 2004).

I do not know if an extrapolation of these results may be made for children with subclinical manifestations of cardiovascular pathology, or for obese children. Nevertheless, with regular reports of increasing childhood metabolic-related diseases prevalence world wide, the results of this study are noteworthy.

The inclusion of children as young as 9 years of age is of importance as they, *a priori*, are not affected by many of the factors that influence and adult's cardiovascular system, such as smoking, alcohol, illness, drugs, etc. The objective measurement of physical activity is another notable strength of this study.

I. Cardiorespiratory fitness is negatively associated with a clustering of metabolic risk factors in children. The results suggest that there is a minimum cardiorespiratory fitness level required in order to have a low metabolic risk.

II. Cardiorespiratory fitness is negatively associated with HOMA in children with high levels of body fat and waist circumference.

III. Physical activity, cardiorespiratory fitness, and body fat are not significantly associated with tHcy levels in children and adolescents.

IV. Vigorous intensity physical activity is negatively associated with body fat in children, whereas both total and at least moderate-vigorous physical activity are positively associated with cardiorespiratory fitness.

The present studies were carried out at the Unit of Preventive Nutrition, Department of Biosciences and Nutrition at NOVUM, Karolinska Institutet, Sweden, in collaboration with the Estonian Centre of Behavioural and Health Sciences, Tallinn, Estonia, and the Department of Physiology, School of Medicine, University of Granada, Spain.

I want to express my sincere gratitude to:

Michael Sjöström, tutor, for giving me the opportunity to work in his group, and in such a good project. Thanks for your tremendous support, constructive criticism, and for believing in me, always. I will not give up either!

Anita Hurtig-Wennlöf, co-tutor, for your wise and generous advices both in professional and personal life. Thanks for giving me the drops of patience that are often lacking in my Spanish temperament.

Manuel Castillo and Angel Gutierrez, co-tutors, for sharing your most critical view of my work, and your deep knowledge of physiology.

Olle Carlson, for helping and pushing me with the statistics. Having your approval makes scientific life much easier…

Francisco Ortega, Nico Rizzo, Julia Wärnberg, Emma Patterson, and **Torbjörn Nilsson**, all co-authors, for your support, good discussions, and always wise advice.

Pekka Oja, for your very useful comments and critical review of manuscripts.

Jan-Åke Gustafsson, for giving the opportunity to become a graduate student at the Department of Bioscience and Nutrition and for your support to the Unit of Preventive Nutrition. Thanks for your words, they are engraved in my memory.

The Swedish EYHS team at PrevNut: Maria Hagströmer, Patrick Bergman, and Eric Poortvliet. Emma, thanks also for the linguistic help! Thanks to all PrevNuters for the nice Friday fika!

The Estonian EYHS team: I am indebted to Prof. **Maarike Harro**, recently deceased, for giving me the opportunity to use the Estonian data, and for your always positive approach. Thanks also to **Toomas Veidebaum** for your helpful collaboration, and my co-authors **Inga Villa,** and **Leila Oja**.

My special thanks are to:

Francisco Ortega for your strong support in every fight! Yes, *arrieros we are*…To **Dirk Meusel** and **Signe Altmäe**, life in Stockholm would not have been the same without you, thanks for the Estonian saunas with German humor on Sunday evenings! To **Juan Jesus Carrero**, for sharing many interesting coffee breaks, and trips and dreams to somewhere.

The studies included in this thesis were supported financially by grants from the Stockholm County Council, the Estonian Science Foundation, and the Ministerio de Educación y Ciencia de España.

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0031-3998/07/6103-0350
PEDIATRIC RESEARCH
Vol. 61, No. 3, 2007
Printed in U.S.A.

High Cardiovascular Fitness Is Associated with Low Metabolic Risk Score in Children: The European Youth Heart Study

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ABSTRACT: The aim of the present study was to examine the associations of cardiovascular fitness (CVF) with a clustering of metabolic risk factors in children, and to examine whether there is a CVF level associated with a low metabolic risk. CVF was estimated by a maximal ergometer bike test on 873 randomly selected children from Sweden and Estonia. Additional measured outcomes included fasting insulin, glucose, triglycerides, HDLC, blood pressure, and the sum of five skinfolds. A metabolic risk score was computed as the mean of the standardized outcomes scores. A risk score <75th percentile was considered to indicate a low metabolic risk. CVF was negatively associated with clustering of metabolic risk factors in children. Receiver operating characteristic curve analysis showed a significant discriminatory accuracy of CVF in identifying the low/high metabolic risk in girls and boys ($p < 0.001$). The CVF level for a low metabolic risk was 37.0 and 42.1 mL/kg/min in girls and boys, respectively. These levels are similar to the health-related threshold values of CVF suggested by worldwide recognized organizations. In conclusion, the results suggest a hypothetical CVF level for having a low metabolic risk, which should be further tested in longitudinal and/or intervention studies. (***Pediatr Res* 61: 350–355, 2007**)

CVF is as a direct marker of physiologic status and reflects the overall capacity of the cardiovascular and respiratory systems, and the ability to carry out prolonged physical exercise (1). High CVF during childhood and adolescence has been associated with a healthier cardiovascular profile during these years (2–5) and also later in life (6). Associations between high CVF and a favorable plasma lipid profile in both overweight and nonoverweight Spanish adolescents have been reported (3). Moreover, CVF has been associated with body fat (4), with features of the metabolic syndrome (2), and with arterial compliance (5) in young population, which supports the concept that CVF may exert a protective effect on the cardiovascular system from an early age. Recently, low CVF has been considered as a risk factor in children and adolescents to the same extent as low HDL cholesterol (HDLC) or insulin resistance (7).

It is biologically plausible that high CVF provides more health protection than low CVF even in healthy children and adolescents, as has been found in adults (8–10). Low CVF seems to be an independent predictor of metabolic syndrome in men and women (9), and low CVF could be another mechanism of overall cardiovascular disease. Moreover, CVF seems to prevent premature mortality regardless of body weight status or the presence of metabolic syndrome in adult men (10). Collectively, cross-sectional, longitudinal, and interventional studies support the notion that CVF is a key correlate of the metabolic syndrome phenotype in the young and adult population.

Despite evidence of the association between CVF and single cardiovascular disease risk factors in young and adult populations, whether health criteria values for CVF can be identified and the implications of these from the public health perspective are still uncertain. In this respect, several health-related threshold values of CVF have been suggested by worldwide recognized organizations (11,12). However, no consensus exists regarding the minimum CVF level associated with a clustering of cardiovascular disease risk factors in children. It has been suggested that clustering of cardiovascular disease risk factors is a better measure of cardiovascular health in young people than single risk factors, and that composite risk score could, to some extent, compensate for day-to-day fluctuations in the single risk factors (7).

For public health strategies and preventive purposes, it is of interest to have evidence-based health criteria values for CVF from an early age. The aim of the present study was to examine the associations of CVF with a clustering of metabolic risk factors in children, and to examine whether there is a CVF level associated with a low metabolic risk.

METHODS

The study involved 1140 children aged 9–10 y, of which 873 (444 girls, 429 boys) from the Estonian and Swedish (539 and 334, respectively) part of

Received August 18, 2006; accepted October 18, 2006.

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The Swedish part of the study was supported by grants from the Stockholm County Council (MS), and the Estonian part was supported by a grant from the Estonian Science Foundation No. 3277 and 5209, and by Estonian Centre of Behavioural and Health Sciences. JRR and FBO were supported by a grant from Ministerio de Educación y Ciencia de España (AP2003-2128, AP2004-2745).

DOI: 10.1203/pdr.0b013e318030d1bd

Abbreviations: AUC, area under the curve; **CVF,** cardiovascular fitness; **ROC,** receiver operating characteristic

the European Youth Heart Study (EYHS) provided a complete data set. The EYHS is a school-based, cross-sectional study designed to examine the interactions between personal, environmental, and lifestyle influences on the risk factor for future cardiovascular diseases. Study design, selection criteria, and sample calculations have been reported elsewhere (13,14). The local ethical committees approved the study (Örebro City Council no. 690/98, Huddinge University Hospital no. 474/98, and University of Tartu no. 49/30-1997). One parent or legal guardian provided written informed consent, and all children gave verbal consent.

Physical examination. Height, weight, and skinfold thickness (biceps, triceps, subscapular, suprailiac, and triceps surae) were measured by standardized procedures. A description of the measurements has been published elsewhere (4). Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). The sum of five skinfold thicknesses was used as an indicator of body fat rather than BMI, because it has been suggested that BMI is not a good measurement of body fat in children (15), and because fatness rather than weight has been shown to be associated with poor health (16). Skinfold thickness has been shown to highly correlate with dual-energy x-ray absorptiometry–measured body fat percentage in children of similar ages (17). Identification of pubertal development was assessed according to Tanner (18). Pubertal stage was assessed by a researcher of the same gender as the child by brief observation. Breast development in girls and genital development in boys were used for pubertal classification.

Blood pressure. The systolic and diastolic blood pressures were measured with an automatic oscillometric method (Dinamap model XL, Critikon, Inc., Tampa, FL). The equipment has been validated in children (19). The subject was in a seated and relaxed position, and recordings were made every second minute for 10 min with the aim of obtaining a set of systolic recordings not varying by more than 5 mm Hg. The mean value of the last three recordings was used as the resting systolic and diastolic blood pressure, in mm Hg.

Blood samples. Serum concentrations of triglycerides, HDLC, glucose, and insulin were measured after an overnight fast. A detailed description of the blood analysis has been reported by Wennlöf *et al.* (20).

Clustering of metabolic risk factors. The clustering of metabolic risk factors was computed from the following variables: insulin, glucose, HDLC, triglycerides, skinfold thickness, and blood pressure (systolic and diastolic). Each of these variables was standardized as follows: standardized value = (value – mean)/SD. The HDLC standardized value was multiplied by –1 to confer higher risk with increasing value for the purpose of calculating the metabolic risk. The standardized values of systolic and diastolic blood pressure were averaged. The metabolic risk score was calculated as the mean of the six standardized scores separately for boys and girls. Children being below the 75th percentile of the score were defined as having low metabolic risk, and children being at or above the 75th percentile of the score were defined as having high risk. Moreover, low (high) metabolic risk was considered when the individual had <1 SD (≥ 1 SD) of this score.

CVF. CVF was determined by a maximum cycle-ergometer test as described elsewhere (21). Briefly, the workload was preprogrammed on a computerized cycle-ergometer (Monark 829E, Ergomedic, Vansbro, Sweden) to increase every third minute until exhaustion. Criteria for exhaustion were a heart rate $\geq$185 beats per minute, failure to maintain a pedaling frequency of at least 30 revolutions per minute, and a subjective judgment by the observer that the child could no longer keep up, even after vocal encouragement. The power output was calculated as being equal to $W_1 + (W_2 \cdot t/180)$, where W_1 is a work rate at fully completed stage, W_2 is the work rate increment at final incomplete stage, and t is time in seconds at final incomplete stage. The "Hansen formula" for calculated VO_{2max} in mL/min was is equal to 12 $\times$ calculated power output + 5 $\times$ body weight in kilograms (21).

Statistical analysis. The data are presented as mean (SD) unless otherwise stated. All variables were checked for normality of distribution before the analysis, and transformations were applied when necessary. Skinfold thickness and triglycerides were logarithmically transformed, and the square root of insulin values was calculated. Untransformed data are presented for ease of interpretation. Gender differences were assessed by ANOVA, inasmuch as there was a significant interaction between gender and CVF. Associations between metabolic risk factors and CVF quartiles were assessed by ANOVA, as were the associations between clustering of metabolic risk factors and CVF quartiles. Differences of metabolic risk factors among CVF quartiles were assessed by Tukey's test. There was not a significant interaction between pubertal development and CVF.

The CVF threshold to discriminate between either a low or high metabolic risk by ROC curve was calculated. ROC curve is a plot of all the sensitivity/specificity pairs resulting from varying the decision threshold (22). Sensitivity (or true-high rate) is the proportion of the sample correctly identified as having a high metabolic risk. Specificity is the proportion of subjects correctly identified as having a low metabolic risk. On the *y* axis, sensitivity is plotted, and, on the *x* axis, is the 1 – specificity (false-high rate). False-high rate is the proportion of subjects having a low metabolic risk that have been incorrectly identified as having a high metabolic risk. The perfect test that correctly classifies all subjects has a true-high rate of 1 and false-high rate of 0. Therefore, the optimal combination of true-high rate and false-high rate is the point closest to the perfect test (upper left corner of the graph). To identify the best threshold, the distance between the perfect test and each sensitivity and 1 – specificity pair was calculated, and, then, the pair closest to 1 was chosen. The AUC and 95% confidence interval (CI) were calculated. The AUC represents the ability of the test to correctly classify children having a low/high metabolic risk. The values of AUC range between 1 (perfect test) to 0.5 (worthless test). Finally, binary logistic regression was used to study the relationship between metabolic risk and CVF. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 13.0 for Windows; SPSS Inc., Chicago, IL), and the level of significance was set at $\alpha = 0.05$.

RESULTS

None of the analyzed variables showed a statistically significant difference between the included subjects and the subjects who did not provided a whole set of data. Valid CVF data were obtained in 85% of the studied subjects. Pubertal development status was obtained from 96% of the children; 97% had blood pressure measurements and 98% had blood measurements. The descriptive characteristics of the study sample are shown in Table 1. One way ANOVA showed that, in girls, skinfold thickness, insulin, and triglycerides decreased across CVF quartiles, whereas, in boys, skinfold thickness and insulin decreased across CVF quartiles (Table 2). The clustering of metabolic risk factors also decreased across CVF quartiles in both girls and boys (Fig. 1). Tukey's test showed that, in girls, skinfold thickness was 22%, 27%, and 36% higher in the first CVF quartile (*i.e.* lowest and/or worst CVF quartile) compared with the second, third, and fourth CVF quartiles (*i.e.* highest and/or best CVF quartiles), respectively. In boys, skinfold thickness was 26%, 31%, and 40% higher in the first CVF quartile compared with the second, third, and fourth CVF quartiles, respectively. Insulin values were 1%, 15%, and 21% higher in the first CVF quartile compared with the second, third, and fourth CVF quartiles in girls, respectively. In boys, insulin values were 23%, 13%, and 20% higher in the first CVF quartile compared with the second, third, and fourth CVF quartiles, respectively. Triglyceride values were 13% higher in the first CVF quartile

Table 1. *Baseline characteristics of 873 children (444 girls, 429 boys)*

	All	Girls	Boys
Age (y)	9.6 (0.4)	9.5 (0.4)	9.6 (0.4)
Height (m)	1.38 (0.6)	1.38 (0.7)	1.38 (0.6)
Weight (kg)	32.1 (5.8)	32.0 (6.2)	32.1 (5.5)
BMI (kg/m^2)	16.7 (2.1)	16.7 (2.3)	16.8 (2.0)
Tanner % (1/2/3/4/5)	69/23/8/0/0	54/44/2/0/0	99/1/0/0/0
Sum of five skinfolds (mm)	40.2 (16.9)	44.7 (18.2)*	35.7 (14.2)
Insulin (pmol/L)	41.4 (23.5)	44.7 (24.6)*	38.0 (21.9)
Glucose (mmol/L)	5.0 (0.4)	4.9 (0.4)*	5.1 (0.3)
HDLC (mmol/L)	1.5 (0.3)	1.4 (0.3)*	1.5 (0.3)
Triglycerides (mmol/L)	0.7 (0.3)	0.8 (0.3)*	0.7 (0.3)
Systolic BP (mm Hg)	102.5 (9.0)	101.9 (8.8)	103.1 (9.2)
Diastolic BP (mm Hg)	60.4 (7.1)	60.6 (7.0)	60.1 (7.3)
Metabolic risk score	0.0 (0.5)	0.03 (0.5)*	−0.03 (0.5)
CVF (mL/kg/min)	40.1 (6.3)	37.1 (5.0)*	43.0 (6.0)

Data shown as mean (SD).
* $p < 0.01$ for differences between sexes.

Table 2. *Associations between metabolic risk factors and CVF quartiles in girls and boys*

	Girls					Boys				
	CVF quartiles (mL/kg/min)					CVF quartiles (mL/kg/min)				
	1st 31.2 (2.3)	2nd 36.2 (0.9)	3rd 39.1 (0.8)	4th 46.5 (8.9)	*p* Value	1st 36.0 (2.7)	2nd 41.9 (0.9)	3rd 45.4 (1.3)	4th 51.9 (4.2)	*p* Value
Sum of five skinfolds (mm)	56.8 (24.4)	44.3 (15.8)	41.3 (12.5)	36.3 (10.3)	<0.001 1 > all; 2 > 4*	47.1 (19.8)	34.7 (9.9)	32.6 (9.3)	28.4 (6.3)	<0.001 All > 4; 1 > 2,3*
Insulin (pmol/L)	49.2 (26.2)	48.8 (31.4)	42.0 (17.6)	38.9 (19.2)	<0.001 1 > all; 2 > 3, 4; 3 > 4*	44.2 (26.9)	33.9 (14.2)	38.5 (19.6)	35.4 (23.9)	<0.001 1 > all; 3, 4 > 2; 3 > 4*
Glucose (mmol/L)	4.9 (0.4)	4.9 (0.4)	4.9 (0.3)	4.8 (0.3)	0.376 —	5.1 (0.4)	5.1 (0.3)	5.1 (0.3)	5.0 (0.3)	0.165 —
HDLC (mmol/L)	1.4 (0.3)	1.4 (0.3)	1.4 (0.3)	1.5 (0.3)	0.067 —	1.5 (0.3)	1.5 (0.3)	1.5 (0.3)	1.5 (0.3)	0.743 —
Triglycerides (mmol/L)	0.8 (0.3)	0.8 (0.3)	0.8 (0.3)	0.7 (0.3)	0.026 1 > 4*	0.7 (0.4)	0.7 (0.3)	0.7 (0.3)	0.6 (0.3)	0.307 —
Systolic BP (mm Hg)	103.3 (9.2)	101.6 (8.6)	101.7 (9.1)	101.1 (8.2)	0.289 —	104.4 (9.9)	103.1 (9.3)	102.4 (8.6)	102.4 (8.9)	0.223 —
Diastolic BP (mmHg)	61.2 (7.0)	60.2 (7.5)	60.8 (6.3)	60.4 (7.1)	0.578 —	60.3 (7.2)	60.0 (7.2)	59.4 (7.3)	60.7 (7.5)	0.613 —

Data shown as mean (SD). *p* Value determined by ANOVA. BP, blood pressure.
* $p < 0.05$ from Tukey's test for differences among quartiles.

than in the fourth CVF quartile in girls (Table 2). The metabolic risk score was 12%, 23%, and 29% higher in the first CVF quartile than in the second, third, and fourth CVF quartiles in girls, respectively, whereas, in boys, it was 23%, 19%, and 30% higher in the first CVF quartile than in the second, third, and fourth CVF quartiles, respectively (Fig. 1). The results did not change when skinfold thickness was adjusted for height.

ROC analysis showed a significant discriminating accuracy of CVF in identifying a low/high metabolic risk in girls (AUC = 0.68, 95% CI: 0.62–0.73; $p < 0.001$), and in boys (AUC = 0.67, 95% CI: 0.61–0.73; $p < 0.001$) (Fig. 2). In girls, the optimal pair of true-high and false-high rates was 0.65 and 0.33, respectively, and 0.65 and 0.39 in boys. The CVF values at these points were 37.0 and 42.1 mL/kg/min in girls and boys, respectively. Logistic regression analysis showed that girls with CVF levels above 37.0 mL/kg/min had an increased odds ratio of having a low metabolic risk when compared with those with CVF levels below this value (odds ratio, 3.09; 95% CI: 1.98–4.82; $p < 0.001$). Boys with CVF levels above 42.1 mL/kg/min had an increased odds ratio of having a low metabolic risk than those with CVF levels below this value (odds ratio, 2.42; 95% CI: 1.56–3.76; $p < 0.001$). The CVF levels and the odds ratios remained the same when the low metabolic risk was considered to be at <1 SD (data not shown). However, the AUC was slightly bigger than the AUC obtained when the cutoff was established at <75th percentile for both genders (data not shown). Because no interaction was found by country, all the analyses were performed with Estonia and Sweden together to have a stronger statistical power. In addition, the results remained similar when the analyses were performed separately for countries.

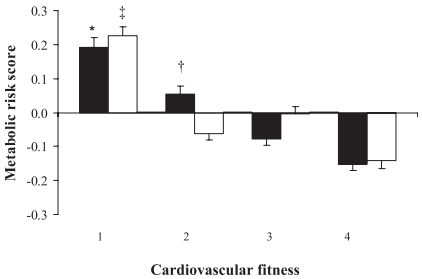

Figure 1. Associations of cardiovascular fitness (quartiles) with clustering of metabolic risk factors (metabolic risk score) in girls (*black bars*) and boys (*white bars*). Data shown as mean and SD. Girls in the first quartile (*) had a higher metabolic risk score than in the second, third, and fourth quartiles ($p = 0.006$, $p = 0.002$, $p < 0.001$, respectively), and girls in the second quartile (†) had a higher metabolic risk score than in the fourth quartile ($p = 0.018$). Boys in the first quartile (‡) had a higher metabolic risk score than in the second, third, and fourth quartiles ($p = 0.007$).

DISCUSSION

The main findings of this study were that CVF is associated with clustering of metabolic risk factors in children. Moreover, the results suggest a hypothetical CVF level for having a low metabolic risk. Having health criterion values for CVF from an early age is of special interest because it can be used to quantify another risk factor for cardiovascular disease in primary prevention and health promotion policies. CVF values could be also adopted by the schools as a "fitness standard" to encourage children to engage in physical activity to achieve and go beyond the required CVF levels. Because the roots of cardiovascular diseases have been found in childhood (23), lifestyle modification during these years may be effective in lowering cardiovascular disease risk in later life.

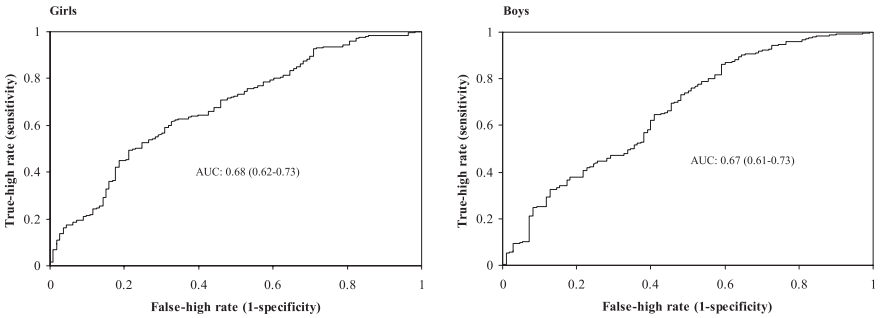

Figure 2. ROC curve summarizing the potential of CVF to identify low/high metabolic risk score in girls and boys. AUC indicates the area under the curve (95% CI).

CVF has a large genetic component (up to 40%) (24). However, it is mainly determined by a person's activity level (4,25–27). Recent findings have shown that variation in CVF was significantly explained by at least moderate to vigorous [3–6 metabolic equivalents (MET)] physical activity (4). Further analysis revealed that children who engaged in at least 26 min/d of vigorous (>6 MET) physical activity had significantly higher CVF than those who accumulate 10–18 min/d of vigorous physical activity. Taken together, these results suggest that those children with a CVF level below that required to have a low metabolic risk may be able to reach the desirable CVF level with adequate aerobic physical activity.

There is no standard pediatric definition of the metabolic syndrome. Several attempts have been made in adolescents aged 12–19 y by using criteria analogous to Adult Treatment Panel (ATP) III (28–30). Other approaches have been made elsewhere to compute a metabolic risk to signify a clustering of metabolic risk factors in healthy Danish children aged 9–10 y (2). The risk score computed here is similar to that which was reported previously (2) because of similarities in ages of the studied subjects and methodology, and because no one child involved in the present study had three or more abnormalities in any of the parameters included in the ATP III definition (data not shown). The risk score computed here was arbitrarily dichotomized defining children having values equal or above 75th percentile of the score as being at high risk. The same percentile has been used in different health-related variables (*e.g.* waist circumference, BMI, triglycerides, insulin levels, systolic blood pressure, total cholesterol to HDLC ratio, plasma leptin, etc.) in a number of population-based studies to define subjects at low (<75th) or high (≥75th) risk (31,32). Even if none of the children had clinical disease, a high metabolic risk may not be a desirable condition.

The test used to measure CVF has been previously validated in children of the same age (20), and it was highly correlated with directly measured VO_{2max} ($r = 0.95$ and $r = 0.90$ in girls and boys, respectively) (21). Maximum tests have been shown to be more accurate than submaximal tests to predict VO_{2max} (33). CVF was expressed as VO_{2max} per kilogram of body mass because of the homogeneity in age, pubertal status, height, weight, and obesity grade of the children, and for the purpose of comparing the results with previous publications.

Previous studies in children and adolescents have shown associations between CVF and cardiovascular risk factors (2–6), whereas others failed to find associations between CVF and features of metabolic syndrome in overweight Latino youths aged 8–14 y with a positive family history of type 2 diabetes (30). Direct associations between increased CVF and clustering of metabolic risk factors in both girls and boys have been shown here, similar to another recent study in Danish children of the same age (2). Associations between increased CVF and a favorable lipid profile and fasting glycemia in both overweight and nonoverweight Spanish adolescents (the AVENA study) showed a similar situation, and the main outcome was that CVF was an indicator of a favorable plasma lipid profile in male adolescents (3).

In the AVENA study, ROC analysis showed a significant discriminatory accuracy of age- and sex-normalized CVF to identify either the presence or absence of a favorable plasma lipid profile in males but not in females (3). The similar ROC analysis performed in the present study showed a significant discriminatory accuracy of CVF to identify either a low or high metabolic risk in both girls and boys (Fig. 2). Girls with CVF levels above 37.0 mL/kg/min were 3.09 times more likely of having a low metabolic risk when compared with those with CVF levels below this value. Similarly, boys with CVF levels above 42.1 mL/kg/min were 2.42 times more likely to have a low metabolic risk when compared with those with CVF levels below this value. Taken together, these results suggest that high CVF is associated with low metabolic risk in children.

The mathematically calculated CVF levels in our study are similar to the health-related CVF threshold suggested by worldwide recognized organizations (11,12). Based on expert judgment, the European Group of Pediatric Work Physiology considered a VO_{2max} of ≥35 mL/kg/min for girls and ≥40 mL/kg/min for boys as a "Health Indicator" (11). The Cooper Institute for Aerobics Research suggested ≥38 and ≥42 mL/kg/min for girls and boys, respectively, as a criterion standard

for the "Healthy Fitness Zone" (12). The cut-off points proposed by the Cooper Institute for adolescents were extrapolated from the thresholds established for adults. The approaches used to calculate the CVF thresholds were different in the previous studies and in our study, as well as the measured outcomes, age, and cultural and social factors of the study subjects. However, the similarities among the results support the existence of a hypothetical health criterion value for CVF in children.

The percentage of the population failing to reach the required level of CVF is of concern and suggests the need to interpret with prudence the meaning of these values. Among girls, 44% did not reach the CVF level, and neither did 40% of the boys. Longitudinal studies are needed to show whether those children having a CVF above the suggested values have a lower incidence of cardiovascular diseases later in life than those having a CVF below the suggested value.

The observations of the present study are limited by the cross-sectional design nature, *i.e.* direction of causality cannot be determined. The CVF fitness test requires relatively minor equipment (*i.e.* cycle-ergometer and a heart rate monitor) and can be easily performed in a clinical setting. However, a maximal test requires a maximal effort be made, which may not be adequate for a population with clinical pathology. The low AUC observed in this study together with the modest odds ratio for having a low metabolic risk in subjects with low/high CVF levels may indicate a low discriminatory ability for identifying children likely to have a high metabolic risk. However, it must be kept in mind that the children involved in this study were apparently healthy children with no previously diagnosed cardiovascular pathologies. We also do not know whether an extrapolation of the association may be made for children with subclinical manifestations of cardiovascular pathology. Nevertheless, with regular reports of increasing childhood metabolic-related diseases prevalence worldwide, the results of this study are noteworthy. The inclusion of a large number of subjects of the same age and the objective measurement of CVF are notable strengths of this study.

In conclusion, the present study shows that CVF is associated with clustering of metabolic risk factors in children. Moreover, the results suggest a hypothetical CVF level for having a low metabolic risk. The CVF levels shown here are similar to the health-related threshold values of CVF suggested by the European Group of Pediatric Work Physiology (11), and by the Cooper Institute for Aerobics Research (12). From a clinical point of view, these results add supporting evidence to the body of knowledge indicating the importance of having a high CVF from an early age. Longitudinal and/or intervention studies are needed to examine the impact of having low CVF in childhood on the likelihood of having cardiovascular diseases later in life.

Acknowledgments. This work is dedicated to the late Maarike Harro, University of Tartu and the National Institutes of Health Development, Tallinn, Estonia. The authors thank Olle Carlsson for statistical assistance and Pekka Oja for highly valuable comments, both guest scientists at our unit.

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II

Diabetologia, revised version submitted

Markers of insulin resistance are associated with fatness and fitness in school-aged children; The European Youth Heart Study

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Words in abstract: 249

Words in text: 3022

ABSTRACT

Aims/hypothesis To examine the association between markers of insulin resistance and 1) body fat and waist circumference, taking into account cardiorespiratory fitness in school-aged children; 2) cardiorespiratory fitness at differing levels of body fat and waist circumference.

Methods Cross-sectional study of 873 children aged 9.6 ± 0.4 years from Estonia and Sweden. Weight, height and waist circumference were measured. Body fat was expressed as the sum of five skinfold thicknesses. Cardiorespiratory fitness was estimated by a maximal ergometer bike test. The studied markers of insulin resistance were fasting insulin and glucose, and homeostasis model assessment (HOMA).

Results HOMA and fasting insulin were positively associated with body fat and waist circumference after adjusting for cardiorespiratory fitness, age, pubertal status and study location. HOMA and fasting insulin were negatively associated with cardiorespiratory fitness in children in the third (highest) tertile of body fat and waist circumference after controlling for sex, age, pubertal status and study location. Fasting glucose was negatively associated with cardiorespiratory fitness in children in the third (highest) tertile of waist circumference, but it was not associated when body fat was taken into account.

Conclusions/interpretation In school-aged children, HOMA and fasting insulin are significantly associated with body fat and waist circumference. In addition, cardiorespiratory fitness explained a significant proportion of the HOMA and fasting insulin variance in those children with high levels of body fat and waist circumference. The findings suggest that the deleterious consequences ascribed to high fatness could be counteracted by having high levels of cardiorespiratory fitness.

Keywords: Body fat, cardiorespiratory fitness, children, diabetes, glucose, HOMA, insulin resistance, obesity, physical activity.

ABBREVIATIONS

EYHS: European Youth Heart Study

HOMA: Homeostasis model assessment

INTRODUCTION

One of the consequences of the pediatric obesity epidemic is thought to be an increased incidence of type 2 diabetes among children worldwide [1]. Longitudinal studies have shown that childhood obesity is closely related to markers of insulin resistance later in life [2]. Both total and central adiposity are also strongly negatively associated with cardiorespiratory fitness in young people [3-5]. Therefore, the detrimental consequences attributed to adiposity may be partially due to the influence of low cardiorespiratory fitness.

Findings from cross-sectional and prospective studies show that markers of insulin resistance and other cardiovascular disease risk factors are already negatively associated with high cardiorespiratory fitness in adolescents [6-8]. However, studies examining the interaction of markers of insulin resistance, fatness and fitness in a large sample of school-aged children are sparse and equivocal [9, 10]. The purpose of the present study was to examine the association between markers of insulin resistance and 1) body fat and waist circumference, taking into account cardiorespiratory fitness, and 2) to examine the association of markers of insulin resistance with cardiorespiratory fitness at differing levels of body fat and waist circumference in school-aged children.

SUBJECTS, MATERIAL AND METHODS

The study involved 1140 children aged 9.6 ± 0.4 years from the Estonian and Swedish parts of the European Youth Heart Study (EYHS). A complete data set was available in 873 children (444 girls, and 429 boys), except for pubertal status, which was available in 672 children (77%). None of the analysed variables differed significantly between the included children (n = 873) and those who did not provide a whole set of data (n = 1140). The EYHS is a school-based, cross-sectional study designed to examine the interactions between personal, environmental and lifestyle influences on the risk factor for future cardiovascular diseases. Study design, selection criteria and sample calculations have been reported elsewhere [11, 12]. Data collection took place during the school year 1998/1999. In Estonia, the city of Tartu and its surrounding rural region was the geographical sampling area. In Sweden, eight municipalities (Botkyrka, Haninge, Huddinge, Nynäshamn, Salem, Södertälje, Tyresö, and Örebro) were chosen for data collection. All schools with more than 20 children in the age group in question were initially included. The schools were stratified with regard to school grade and to the mean income level in their catchments 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. The local ethical committees approved the study (Örebro City Council no. 690/98, Huddinge University Hospital no. 474/98, and University of Tartu no. 49/30-1997). One parent or legal guardian provided written informed consent, and all children gave verbal consent.

Physical examination

Height and weight were measured by standardized procedures. BMI was calculated as weight/height squared (kg/m^2). Skinfold thickness were measured with a Harpenden caliper at the biceps, triceps, subscapular, suprailiaca and triceps surae areas on the left side of the body according to the criteria described elsewhere [13]. All measurements were taken twice and in rotation, and the mean value was calculated. If the difference between the measurements was more than two mm, a third measurement was taken and the two closest measurements were averaged. The sum of the five skinfold thicknesses (hereafter referred as "body fat") rather than BMI was used as an indicator of total body fat, because BMI has been suggested to be less valid measurement of body fatness in children [14], and because BMI does not discriminate between muscle and fat mass. Waist circumference (cm) was measured in duplicate with a metal anthropometric tape midway between the lowest rib and the iliac crest at the end of a gentle expiration. The mean between two measurements was used for further calculations. If the two measurements differed by more than one centimetre, a third measurement was taken, and the two closest measurements were averaged.
Identification of pubertal development was also assessed [15]. Pubertal stage was recorded by a researcher of the same gender as the child, after brief observation. Breast development in girls, and genital development in boys, was used for pubertal classification.

Blood samples

Serum concentrations of glucose and insulin were measured after an overnight fast. The homeostasis model assessment (HOMA) was calculated [16]: fasting insulin (mU/L) x fasting glucose (mmol/L) / 22.5. A detailed description of the blood analysis has been reported elsewhere [17].

Cardiorespiratory fitness

Cardiorespiratory fitness was determined by a maximum cycle-ergometer test [18]. Briefly, the workload was pre-programmed on a computerized cycle-ergometer (Monark 829E Ergomedic, Vansbro, Sweden) to increase every third minute until exhaustion. Criteria for exhaustion were a heart rate ≥ 185 beats per minute, failure to maintain a pedalling frequency of at least 30 revolutions per minute, and a subjective judgment by the observer that the child could no longer keep up, even after vocal encouragement. The power output was calculated as $= W_1 + (W_2 \cdot t/180)$, where W_1 is a work rate at fully completed stage, W_2 is the work rate increment at final incomplete stage, and t is time in second at final incomplete stage. Cardiorespiratory fitness was expressed as the maximal power output per kilogram body mass (W/kg). The test used to measure cardiorespiratory fitness has been previously validated in children of the same age [19].

Statistical Analysis

The data are presented as means ± SD unless otherwise stated. To achieve normality in the residuals, waist circumference, skinfold thickness, insulin, and glucose were transformed to the natural logarithm, and HOMA was raised to the power of 1/3. Gender differences were assessed by one-way analysis of variance (ANOVA), and adjusted for mass significance [20]. The association between markers of insulin resistance, body fat and waist circumference was assessed by one-way analysis of covariance (ANCOVA); with waist circumference or body fat variables as fixed factors; HOMA, insulin or glucose as a dependent variables, and cardiorespiratory fitness, age, pubertal status and study location as covariates. Since there was an interaction effect between sex and both body fat and waist circumference, all analyses were performed separately for boys and girls. Both body fat and waist circumference were recoded into tertiles to be entered into the models. In girls, mean body fat values for the first, second and third tertiles were 26.8, 36.3 and 59.4 mm, respectively.

In boys, mean body fat values for the first, second and third tertiles were 25.9, 35.2 and 57.6 mm, respectively. In girls, mean waist circumference values for the first, second and third tertiles were 53.4, 58.7 and 65.5 cm, respectively. In boys, mean waist circumference values for the first, second and third tertiles were 53.6, 57.2 and 61.7 cm, respectively. For those relationships where waist circumference was involved, an additional adjustment for height or body fat was done.

Following bivariate correlation analysis, multiple regressions were used to study the association between markers of insulin resistance and cardiorespiratory fitness after controlling for sex, age, pubertal status and study location. Regression analysis was performed separately by body fat and waist circumference tertiles (low, middle and high, equals first, second and third tertile, respectively). No interaction effects between sex and cardiorespiratory fitness was found, therefore, all analyses were performed jointly for boys and girls. Semipartial correlation was used as the measure of the relationship between cardiorespiratory fitness and the part of the outcome (HOMA, fasting insulin and glucose) that is not explained by the other predictors in the model (sex, age, pubertal status and study location). In other words, it is a measure of the variance in the outcome that fitness alone share. The analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 14.0 for WINDOWS; SPSS Inc, Chicago) and the level of significance was set to 0.05.

RESULTS

The descriptive characteristics of the study sample are shown in Table 1. Girls had significantly lower waist circumferences, glucose and fitness levels than boys, while boys had lower body fat, HOMA, and insulin than girls. The Estonian children (both girls and boys) had lower body fat and waist circumference than did the Swedish children, whereas the former had higher HOMA and insulin levels.

All the fatness-related parameters were positively inter-correlated in both girls and boys, with correlation coefficients ranging from 0.751 to 0.844. Cardiorespiratory fitness was significantly associated with body fat, BMI, and waist circumference in girls (r = -0.437, -0.407, -0.312, respectively, all $P < 0.001$) and boys (r = -0.562, -0.487, -0.439, respectively, all $P < 0.001$).

The associations of HOMA, fasting insulin and glucose with body fat and waist circumference for girls and boys are shown in Figure 1. HOMA and fasting insulin were positively associated (all $P < 0.001$) with body fat and waist circumference in both girls and boys (Figure 1). Fasting glucose was not significantly associated with body fat or waist circumference (Figure 1). For those relationships where waist circumference was involved, an additional adjustment for height or body fat did not alter the results.

Figure 2 shows simple bivariate relationships between HOMA and cardiorespiratory fitness by body fat and waist circumference tertiles for girls and boys. The results of the regression models with HOMA, fasting insulin and glucose as the outcome variables and cardiorespiratory fitness as the predictor variable, conducted by body fat and waist circumference tertiles, are shown in Table 2. HOMA and fasting insulin were negatively associated with cardiorespiratory fitness in those children with high levels of body fat, and waist circumference (those in the third tertile).

Subsequent analysis examining the association of markers of insulin resistance with cardiorespiratory fitness separately by BMI tertiles was similar to those with body fat. For those relationships where waist circumference was involved, additionally controlling for height or body fat did not alter the results. Similar results were obtained when the regression analyses were performed separately for girls and boys. No significant effect of pubertal status on the outcome was observed in any of the analysis performed (data not shown).

DISCUSSION

The results of the present study showed that HOMA and fasting insulin were positively associated with body fat and waist circumference in school-aged children. In addition, cardiorespiratory fitness explained a significant proportion on the HOMA and fasting insulin variance in those children with high levels of body fat and waist circumference.

The methodology used allowed us to examine the association of markers of insulin resistance with fitness within different levels of total and central fatness (i.e. low, middle and high). The findings suggested that the deleterious consequences ascribed to high fatness may be counteracted by having high levels of cardiorespiratory fitness. Taken together, these findings suggest that interventions designed to prevent hyperinsulinemia and related metabolic disorders should focus not only to reduce fatness but also to improve fitness.

Numerous studies support a link between metabolic risk factors and adiposity in children [6, 21]. Fasting insulin appears to be related more to total adiposity than to central adiposity [6, 22]. However, one study in youths found that total body fat was the predominant factor influencing insulin sensitivity, but that visceral fat may have additional effects on fasting insulin [23]. In our study, both body fat (as a marker of total adiposity) and waist circumference (as a marker of central adiposity) explained a significant proportion of the HOMA and fasting insulin variance after adjusting for potential confounders including cardiorespiratory fitness. Waist circumference has been shown to be a powerful marker of abdominal fat accumulation and visceral adiposity tissue in young people [24, 25]. Waist circumference is widely used as a surrogate of central fat distribution in young and adult people [25]. Direct measurements of visceral adiposity with magnetic resonance imaging or computed tomography may provide further information, however, this can not be proposed for field studies due to their cost and technical difficulties [26].

The results of the association between markers of insulin resistance and cardiorespiratory fitness separately by BMI tertiles were similar to those with body fat. Limits of BMI are well recognized, as well as its feasibility to be used in epidemiological studies. Recently, a cross-sectional study on 407 children aged 7-16 years reported that BMI was a good predictor of the variance of subcutaneous adipose tissue (88.9% of variance) [25]. Therefore, when no better possibilities are available, BMI could be used as a marker of subcutaneous adipose tissue in children.

The association between fasting insulin and cardiorespiratory fitness is consistent with findings reported in North American adolescents [6, 7], whereas other studies failed to find associations between markers of insulin resistance and cardiorespiratory fitness [9, 10]. Gutin et al. [6] reported that percentage of body fat (measured with dual energy X-ray absorptiometry) and cardiorespiratory fitness explained a significant proportion of the fasting insulin variance in boys. In girls, fasting insulin was associated only with percentage of body fat, suggesting that the detrimental impact of high fatness and low fitness was greater in boys than in girls. In our study, cardiorespiratory fitness explained a significant proportion of the HOMA and fasting insulin variance in both girls and boys with high levels of fatness. The North American adolescents were both older (14-18 years) and biologically more mature (Tanner stages 4-5) than the children involved in our study. Rapid and dynamic changes in various metabolic systems, including hormonal regulation, changes in body fat content and body fat distribution, as well as transient changes in insulin resistance are known to occur during growth and puberty [1], which may partially explain the differences between studies.

Results from the Study of Latino Adolescents at Risk for Diabetes revealed that insulin sensitivity or secretion was not independently associated with cardiorespiratory fitness in a relatively small sample of overweight Hispanic children aged 8-13 years with a family history of type 2 diabetes [9]. Moreover, cardiorespiratory fitness was not shown to be different in children with impaired glucose tolerance compared to those with normal glucose tolerance [10], suggesting that fitness may influence insulin dynamics indirectly through fat mass, especially in those groups of children at increased risk of early cardiovascular disease. Differences between study subjects and methodologies make comparisons difficult. Moreover, it must be borne in mind that the children involved in our study were apparently healthy children with no previously diagnosed cardiovascular pathologies.

Recent findings from the National Health and Nutrition Examination Survey [7] showed that insulin sensitivity was significantly associated with physical activity (measured by questionnaires) and cardiorespiratory fitness in boys. In girls, insulin sensitivity was not significantly associated with physical activity or with cardiorespiratory fitness but was significantly associated with BMI. The differences between genders may be due to the fact that females seem to be, in some way, protected from the prejudicial effects of excess fatness by the role of estrogens [27, 28]. Similarly, this mechanism also seems to protect young women from the deleterious consequences of low cardiorespiratory fitness on insulin resistance. This might be true due to the fact that fitness levels are lower in girls than in boys, whereas fatness and fasting insulin levels are higher in the former. This is consistent with other studies on young people [6, 7, 29-32].

Cardiorespiratory fitness is highly influenced by the person's activity level [3, 4, 33, 34]. Objectively measured physical activity has also been negatively associated with body fat activity level [3, 4], and with fasting insulin levels [35].

Similarly, HOMA was positively associated with regular exercise independently of total and central adiposity in adolescents [36]. Collectively, these findings indicate that regular physical activity may play a key role in reducing fat mass and markers of insulin resistance, and improving cardiorespiratory fitness in youths.

In the present study fasting glucose was not associated with fatness and fitness. Similarly, fasting glucose was not associated with total physical activity in school-aged Danish girls and boys [35]. In fact, the clinical value of fasting glucose in the first decade of life has been questioned. Recent findings show that fasting glucose is weakly associated with clustering of cardiovascular disease risk factors in young people [37]. This can be explained by the fact the insulin resistance may increase before children display impaired glucose tolerance. In the very early stages of insulin resistance, the pancreas may compensate this metabolic disturbance by excreting higher quantities of insulin [38].

The limitations of the present study include its cross-sectional design, therefore, the direction of causality can not be determined. HOMA model is a method for assessing β-cell function and insulin resistance from fasting insulin and glucose concentrations, whereas the gold standard for measuring insulin sensitivity/resistance is the euglycemic-hyperinsulinemic clamp. However, the latter method is too invasive and costly, and may not be suitable for large epidemiological studies. HOMA has been compared with a number of well validated methods used to measured insulin resistance and β-cell function [39].

In conclusion, the results showed that HOMA and fasting insulin were associated with body fat and waist circumference in school-aged children. In addition, cardiorespiratory fitness explained a significant proportion on the HOMA and fasting insulin variance in those children with high levels of body fat and waist circumference.

Collectively, the findings suggest that the deleterious consequences ascribed to high fatness could be counteracted by having high levels of cardiorespiratory fitness. The assessment of the effect of an exercise intervention focused on reducing fatness and improving fitness is warranted.

ACKNOWLEDGEMENTS

We are indebted to Prof. Maarike Harro for her enthusiastic and foresight in her scientific work. We thank Prof. Olle Carlsson from the Unit for Preventive Nutrition, Department of Biosciences and Nutrition at NOVUM, Karolinska Institutet, for statistical assistance.

The Swedish part of the study was supported by grants from the Stockholm County Council (MS), and the Estonian part was supported by a grant from the Estonian Science Foundation No. 3277 and 5209, and by Estonian Centre of Behavioural and Health Sciences and ESF grants No. 3277, 5209 and 6788. JRR and FBO were supported by a grant from Ministerio de Educación y Ciencia de España (AP2003-2128, AP2004-2745). TV was supported by grants from the Ministry of Education and Science (0942706s06 and 0192112s02). None of the authors had any conflict of interest.

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Table 1. Descriptive characteristics of the children.

	All (n=873)		Girls (n=444)		Boys (n=429)		*P* for gender
	Mean	SD	Mean	SD	Mean	SD	
Age (y)	9.6	0.4	9.5	0.4	9.6	0.4	0.147
Pubertal status (I/II/III/IV/V)[a]	80/18/2/0/0		70/26/4/0/0		90/10/0/0/0		
Weight (kg)	32.1	5.8	32.0	6.2	32.1	5.5	0.828
Height (m)	1.4	0.1	1.4	0.1	1.4	0.1	0.677
Body mass index (kg/m^2)	16.7	2.1	16.7	2.3	16.8	2.0	0.846
Body fat (mm)[b]	40.2	16.9	44.6	18.2	35.7	14.2	<0.001
Waist circumference (cm)[b]	58.0	5.4	57.1	5.9	58.9	4.8	<0.001
HOMA[c]	1.3	0.8	1.4	0.8	1.2	0.8	<0.001
Insulin (mU/L)[b]	41.4	23.5	44.7	24.5	38.0	21.9	<0.001
Glucose (mmol/L)[b]	5.0	0.4	4.8	0.4	5.1	0.3	<0.001
Cardiorespiratory fitness (W/kg)	2.9	0.5	2.7	0.4	3.2	0.5	<0.001

All values are mean ± SD, or [a]percentages.

Analysis was performed on [b]log-transformed data, and [c]transformed data to the power of 1/3, but nontransformed data are presented in the table.

HOMA: homeostasis model assessment; Body fat is expressed as skinfold thickness.

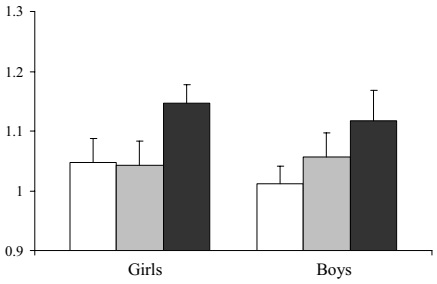
Table 2. Multiple regression coefficients (β), coefficient of determination (R^2), and semipartial correlations (*sr*) examining the association of markers of insulin resistance with cardiorespiratory fitness separately by body fat and waist circumference tertiles.

Outcome variable	Cardiorespiratory fitness	β	*P*	R^2	*sr*
HOMA[b]	Low body fat	0.095	0.232	0.087	0.08
	Middle body fat	-0.009	0.909	0.079	-0.08
	High body fat	-0.241	0.001	0.156	-0.23
Insulin[a]	Low body fat	0.108	0.174	0.088	0.09
	Middle body fat	-0.005	0.948	0.065	-0.01
	High body fat	-0.220	0.002	0.149	-0.21
Glucose[a]	Low body fat	-0.084	0.283	0.126	-0.07
	Middle body fat	-0.010	0.899	0.132	-0.01
	High body fat	-0.126	0.081	0.067	-0.12
Outcome variable	Cardiorespiratory fitness	β	*P*	R^2	*sr*
HOMA[b]	Low waist circumference	-0.010	0.905	0.100	-0.01
	Middle waist circumference	-0.148	0.063	0.086	-0.13
	High waist circumference	-0.250	0.001	0.215	-0.22
Insulin[a]	Low waist circumference	0.009	0.911	0.099	0.01
	Middle waist circumference	-0.139	0.069	0.110	-0.13
	High waist circumference	-0.223	0.002	0.183	-0.20
Glucose[a]	Low waist circumference	-0.056	0.492	0.056	-0.05
	Middle waist circumference	0.002	0.977	0.152	0.01
	High waist circumference	-0.183	0.010	0.104	-0.16

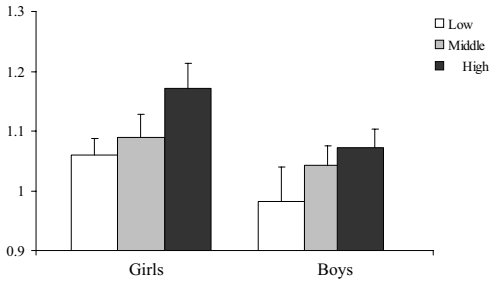
All the analyses were controlled for sex, age, pubertal status and study location. Analysis was performed on [a]log-transformed data, and [b]transformed data to the power of 1/3. Low, middle and high equals to first, second and third tertile, respectively.

Fig. 1. Markers of insulin resistance stratified by body fat (expressed as skinfold thickness) and waist circumference tertiles in girls and boys. Values are mean, and errors bars represent 95% confident intervals. Data were analyzed by one-way analysis of covariance after adjusting for cardiorespiratory fitness, age, pubertal status and study location. Low, Middle and High, equals first, second and third tertile, respectively. HOMA: homeostais model assessment. Body fat, waist circumference, fasting insulin and glucose were transformed to the natural logarithmic, and HOMA was raised to the power of 1/3. HOMA and fasting insulin were positively associated (all $P < 0.001$) with body fat and waist circumference in both girls and boys. Fasting glucose was not significantly associated with body fat or waist circumference.

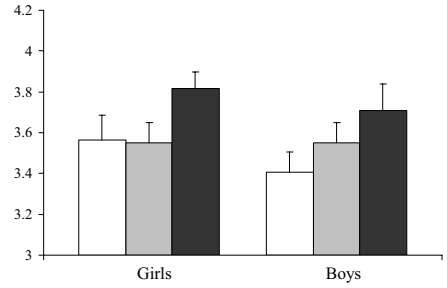
HOMA and body fat tertiles



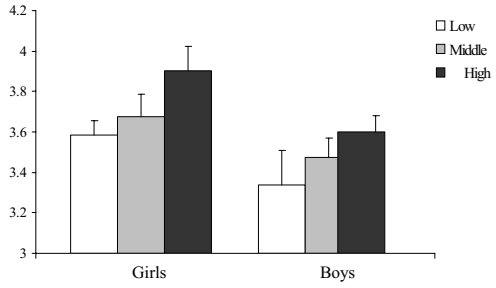
HOMA and waist circumference tertiles



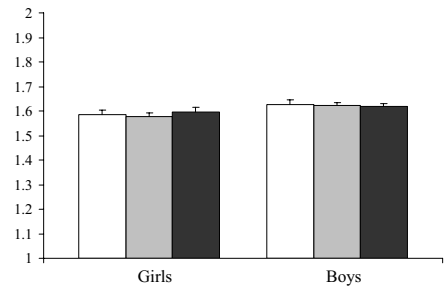
Insulin and body fat tertiles



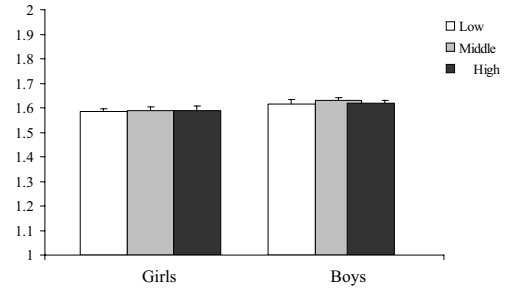
Insulin and waist circumference tertiles



Glucose and body fat tertiles



Glucose and waist cincumference tertiles



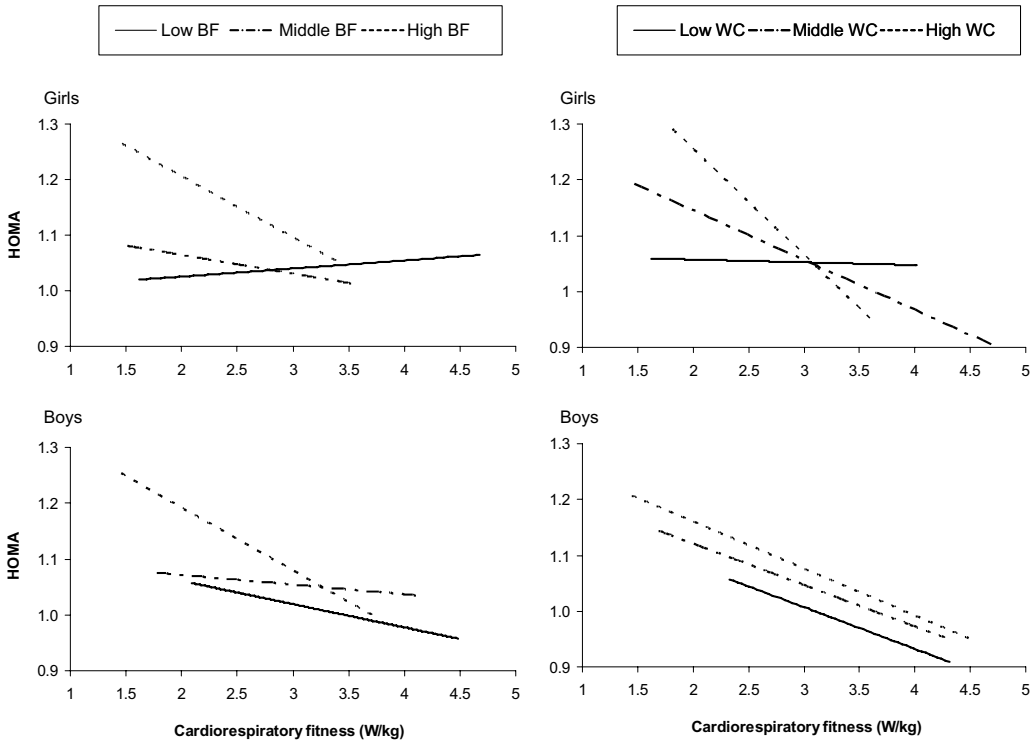


Fig. 2. Relationship between homeostasis model assessment (HOMA) and cardiorespiratory fitness by body fat (BF) and waist circumference (WC) tertiles for girls and boys. Low, middle and high equals to first, second and third tertile, respectively. HOMA was transformed data to the power of 1/3 before analysis. Girls: low BF, $r = 0.040$ ($P = 0.703$); middle BF, $r = -0.062$ ($P = 0.456$); high BF, $r = -0.218$ ($P = 0.002$); low WC, $r = -0.011$ ($P = 0.862$); middle WC, $r = -0.221$ ($P = 0.012$); high WC, $r = -0.407$ ($P < 0.001$). Boys: low BF, $r = -0.093$ ($P = 0.197$); middle BF, $r = -0.042$ ($P = 0.623$); high BF, $r = -0.252$ ($P = 0.016$); low WC, $r = -0.312$ ($P = 0.018$); middle WC, $r = -0.167$ ($P = 0.032$); high WC, $r = -0.197$ ($P = 0.004$).

III

British Journal of Nutrition (2007), **97**, 255–262
DOI: 10.1017/S0007114507280535

Homocysteine levels in children and adolescents are associated with the methylenetetrahydrofolate reductase 677C > T genotype, but not with physical activity, fitness or fatness: The European Youth Heart Study

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(Received 28 April 2006 – Revised 25 July 2006 – Accepted 22 August 2006)

To examine the associations of total plasma homocysteine (tHcy) with physical activity, cardiorespiratory fitness and fatness in children and adolescents, a cross-sectional study of 301 children (9–10 years old) and 379 adolescents (15–16 years old) was conducted. Physical activity was measured by accelerometry. Cardiorespiratory fitness was measured with a maximal ergometer bike test. Body fat was derived from the sum of five skinfold thicknesses. Genotyping for the methylenetetrahydrofolate reductase (MTHFR) 677C > T polymorphism was done by DNA sequencing. Fasting tHcy level was the outcome variable. Multiple regressions were used to determine the degree to which variance in tHcy was explained by physical activity, cardiorespiratory fitness and body fat, after controlling for potential confounders including MTHFR 677C > T genotype. tHcy levels were neither associated with any measure of level and pattern of physical activity nor with data on cardiorespiratory fitness, or body fat, in any age group after controlling for potential confounders including MTHFR 677C > T and even when subgroups 677TT and 677CC + CT were analysed separately. Mean values of tHcy were significantly higher in the TT subgroup compared with CC and CT subgroups in children (TT 7·4 μmol/l, CC 6·3 μmol/l, CT 6·6 μmol/l, $P<0·001$ and $P=0·019$, respectively) and adolescents (TT 16·9 μmol/l, CC 8·3 μmol/l, CT 9·0 μmol/l, both $P<0·001$). The results suggest that physical activity, fitness and body fat are not associated with tHcy levels in children and adolescents, even after controlling for presence of the MTHFR 677C > T genotype, the main influence on tHcy levels in these subjects.

Homocysteine: Physical activity: Cardiorespiratory fitness: Body fat

Elevated total plasma homocysteine (tHcy) levels are associated with increased oxidative stress and endothelial damage (Welch & Loscalzo, 1998). tHcy has been suggested to be an independent risk factor for several multi-system diseases (Virtanen *et al.* 2005), including CHD (Homocysteine Studies Collaboration, 2002; Vrentzos *et al.* 2004), stroke (Ford *et al.* 2002), dementia and Alzheimer's disease (Seshadri *et al.* 2002), risk of hip fracture (McLean *et al.* 2004) and pregnancy complications (Hague, 2003).

Lifestyle factors such as smoking, lack of physical activity, excessive alcohol intake, obesity, high coffee consumption as well as nutritional deficiencies in folate and vitamin B_{12} are associated with elevated levels of tHcy (Jacques *et al.* 2001; Bates *et al.* 2002; Silaste *et al.* 2003; Chrysohoou *et al.* 2004; van Beynum *et al.* 2005). In addition, a major genetic determinant of tHcy levels has been identified, the methylenetetrahydrofolate reductase (MTHFR) polymorphism 677C > T (Frosst *et al.* 1995). Age and gender seem to play a specific role. Levels of tHcy are higher in men than in women and this gender effect seems to be enhanced during and after puberty (De Laet *et al.* 1999; Bates *et al.* 2002; van Beynum *et al.* 2005).

Objectively measured physical activity has been negatively associated with traditional cardiovascular risk factors in children and adolescents (Strong *et al.* 2005). Poor cardiorespiratory fitness is another important health problem (Myers *et al.* 2002; Carnethon *et al.* 2003). Recently, cardiorespiratory fitness has been negatively associated with features of metabolic syndrome in children and adolescents (Mesa *et al.* 2006; Ruiz *et al.* 2006*a*) and it has been negatively associated with tHcy in adult women (Kuo *et al.* 2005). Studies examining the possible interplay between physical activity, cardiorespiratory fitness and fatness with tHcy levels in children and adolescents are lacking. For public health strategies and preventive purposes, it is of interest to understand the relative influence of modifiable factors on tHcy levels on early ages. Therefore, we examined the associations of tHcy with physical activity, cardiorespiratory fitness and fatness, after controlling for

Abbreviations: MTHFR, methylenetetrahydrofolate reductase; tHcy, total plasma homocysteine.

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potential confounders including the MTHFR 677C > T genotype, in children and adolescents.

Methods

Subjects

The subjects were participants in the Swedish part of the European Youth Heart Study, a school-based, cross-sectional study of risk factors for future CVD in a random sample of healthy children (9–10 years old) and of adolescents (15–16 years old) (Poortvliet *et al.* 2003). Data collection took place from September 1998 to May 1999 in thirty-seven schools in central Sweden. The present report is based on the 680 subjects who had both tHcy and MTHFR genotypes measured (301 children, 379 adolescents). Study design, sampling procedure and participation rates have been reported elsewhere (Wennlöf *et al.* 2003). The study was approved by the Research Ethics Committees of Örebro County Council and Huddinge University Hospital. Written informed consent was obtained from the parents of the children and from both the parents of the adolescents and the adolescents themselves.

Before any testing was performed, the parents completed a questionnaire in which part of the questions addressed the child's previous and current health status. Socioeconomic status was also assessed via the questionnaire and defined by the maternal educational status (coded as 0 = below university education and 1 = university education). Using the maternal educational status as socioeconomic status indicator has the advantage of having a high response rate and relatively unbiased responses in comparison with questions regarding income (Kaplan & Keil, 1993).

Physical examination

Height and weight were measured by standardized procedures. BMI was calculated as weight/height^2 (kg/m^2). Skinfold thicknesses were measured with a Harpenden caliper (Baty International, Burgess Hill, UK) at the biceps, triceps, subscapular, suprailiaca and triceps surae areas on the left side of the body according to the criteria described by Lohman *et al*. (1991). These measures have been shown to correlate highly with dual-energy X-ray absorptiometry-measured body fat percentage in children of similar ages (Gutin *et al.* 1996; Rodriguez *et al.* 2005). All measurements were taken twice and in rotation and the mean value was calculated. If the difference between the two measurements was > 2 mm, a third measurement was taken and the two closest measurements were averaged. The sum of the five skinfold thicknesses (hereafter referred to as 'skinfold thickness') was used as an indicator of body fat rather than BMI, because BMI has been suggested to be a less valid measurement of body fatness in children (Rennie *et al.* 2005) and because fatness rather than weight has been shown to be associated with poor health (Allison *et al.* 2002). However, for the purpose of comparing the results with previous publications, the subjects were also categorized as normal-weight or overweight-obese following the International Obesity Task Force proposed gender- and age-adjusted BMI cut-off points (Cole *et al.* 2000).

Identification of pubertal development was assessed according to Tanner & Whitehouse (1976). Pubertal stage was recorded by a researcher of the same gender as the child, after brief observation. Breast development in girls and genital development in boys were used for pubertal classification.

Measurement of physical activity

Physical activity was measured during four consecutive days (two weekdays and at least one weekend day) with an activity monitor (MTI model WAM 7164; Manufacturing Technology Inc., Shalimar, FL, USA) worn at the lower back. At least 3 d of recording, with a minimum of 10 h registration per d, was set as an inclusion criterion. Total physical activity was expressed as total counts recorded, divided by total daily registered time (counts/min). The time engaged in moderate physical activity and vigorous physical activity was calculated and presented as the average time per d during the complete registration. Moderate physical activity (3–6 metabolic equivalents) and vigorous physical activity (>6 metabolic equivalents) intensities were also calculated and were based upon the cut-off limits published elsewhere (Trost *et al.* 2002). The time spent in at least moderate intensity level (>3 metabolic equivalents) was calculated as the sum of time spent in moderate or vigorous physical activity. Each minute over the specific cut-off was summarized in the corresponding intensity level group. Validation studies examining the accelerometer used in the present study and the construction of summary variables for intensity of movement suggest that it is a valid and reliable measure of children's and adolescent's physical activity (Trost *et al.* 1998; Puyau *et al.* 2004). The precision of objective assessment of physical activity in children is superior to subjective methods (Kohl *et al.* 2000); however, there are some limitations that should be highlighted. The accelerometer must be removed during swimming, contact sports, showering and bathing. Any activity involving minimal vertical displacement of the body (i.e. cycling) is also underestimated. A period of 4–5 d of activity monitoring has been proposed as a suitable duration for accurate and reliable estimates of usual physical activity behaviour in children (Trost *et al.* 2000). Data for 4 d were available for most of the participants in the present study.

Measurement of cardiorespiratory fitness

Cardiorespiratory fitness was determined by a maximum cycle-ergometer test, as described elsewhere (Hansen *et al.* 1989). Briefly, the workload was pre-programmed on a computerized cycle ergometer (Monark 829E; Ergomedic, Vansbro, Sweden) to increase every third minute until exhaustion. Heart rate was registered continuously by telemetry (Polar Sport Tester; Polar Electro Oy, Kempele, Finland). The criteria for exhaustion were a heart rate ≥ 185 beats per min, failure to maintain a pedalling frequency of at least 30 rpm and a subjective judgement by the test leader that the child could no longer keep up, even after vocal encouragement. The power output was calculated as $= W_1 + (W_2 \cdot t/180)$, where W_1 is a work rate at fully completed stage, W_2 is the work rate increment at final incomplete stage and t is time in seconds at final incomplete stage. The 'Hansen formula' for calculated VO_{2max} (ml/min) was $= 12 \times$ calculated power output $+ 5 \times$ body weight (kg) (Hansen *et al.* 1989). Cardiorespiratory fitness was expressed as VO_{2max} per kg body mass (ml/kg per min) because of the homogeneity in stature and obesity grade of the subjects, because it is well established and for ease of comparison with the results of

previous studies. The test used has been previously validated in children of the same age (Riddoch *et al.* 2005) and is highly correlated in children with directly measured VO_{2max} (r 0·95 and r 0·90, in girls and boys, respectively) (Hansen *et al.* 1989).

Dietary assessment

Dietary intake was assessed by an interviewer-mediated 24-h recall. For the 9-year-olds, a qualitative food record, completed the day before the interview, acted as a checklist once the 24-h recall was obtained. A food atlas was used to estimate portion sizes. Dietary data were entered into StorMats (version 4.02; Rudans Lättdata, Västerås, Sweden) and analysed using the Swedish National Food Database, version 99.1 (www.s/v.se). Folate and vitamin B_{12} intakes were calculated in μg/d.

Homocysteine assay and methylenetetrahydrofolate reductase genotyping

With the subject in the supine position, blood samples were taken by venipuncture after an overnight fast using vacuum tubes (Vacuette; Greiner Bio-One GmbH, Essen, Germany). The fasting state was verbally confirmed by the subject before blood sampling. Plasma was separated and stored at −80°C until analysis. Homocysteine in acidified citrated plasma (Willems *et al.* 1998) was assayed using a fluorescence polarization immunoassay on an IMx® unit (Abbott Laboratories, Abbott Park, IL, USA). All CV were under 7·5 %. Total blood DNA was extracted and purified from 200 μl whole blood anticoagulated with EDTA, using the QIAamp DNA Blood Mini Kit by the spin procedure, according to the instructions of the manufacturer (QIAGEN Inc., Valencia, CA, USA). Genotyping of the 677C > T variant in the MTHFR gene was performed using the Pyrosequencing platform (Biotage AB, Uppsala, Sweden), as described recently (Börjel *et al.* 2006).

Statistical analysis

The data are presented as means and standard deviations unless otherwise stated. All variables were checked for normality of distribution before analysis. Skinfold thickness, tHcy, folate and vitamin B_{12} were normalized by transformation to the natural logarithm, and for the total physical activity the square root was calculated.

Gender and age differences were assessed by two-way ANOVA. The levels of tHcy according to MTHFR 677C > T and age group were analysed by two-way ANOVA and mean tHcy levels between MTHFR 677C > T subgroups were compared by Tukey's test.

The tHcy mean values did not differ between the CC and CT groups; therefore, the MTHFR 677C > T genotype was analysed as a recessive trait, i.e. dichotomized into TT and CC + CT groups.

Multiple regressions were used to study the relationship between tHcy and physical activity, cardiorespiratory fitness and body fat, after controlling for potential confounders. Model 1 examined the influence of total physical activity on tHcy after controlling for gender, pubertal development, socioeconomic status, folate and vitamin B_{12} intake and MTHFR 677C > T genotype. Model 2 examined the influence of cardiorespiratory fitness on tHcy after controlling for the same potential confounders included in Model 1. Model 3 examined the influence of body fat (expressed as skinfold thickness or as BMI) on tHcy after controlling for the same potential confounders included in Models 1 and 2. Semipartial correlation (sr) was used as a measure of the relationship between tHcy and physical activity, cardiorespiratory fitness and body fat, after controlling for the effect that one or more additional variables (e.g. pubertal development, socioeconomic status, etc.) had on one of those variables. The analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 14·0 for Windows; SPSS Inc, Chicago, IL, USA) and the level of significance was set to $= 0{\cdot}05$.

Results

Data completeness and baseline characteristics

None of the analysed variables showed a statistically significant difference between included subjects (i.e. those who had both tHcy and MTHFR genotype measurements) and excluded subjects (i.e. those who did not have both). Valid physical activity data were obtained in 71 % of the studied subjects and 84 % had valid cardiorespiratory fitness data, as determined by the criteria explained earlier (see p. 00). Pubertal development status was obtained from 88 %, and 91 % had skinfold thickness measured. Socioeconomic status and dietary intake data were available for 95 and 99 % of the subjects, respectively.

The descriptive characteristics of the study sample are shown in Table 1. The levels of tHcy did not differ between boys and girls in either age group, but significant differences were observed between age groups in both girls and boys. In both children and adolescents, girls had significantly lower values of physical activity related-variables and cardiorespiratory fitness than boys (Table 1). In boys, physical activity related-variables, cardiorespiratory fitness and body fat were significantly different between children and adolescents. Mean values of total physical activity, cardiorespiratory fitness and body fat were not significantly different across tHcy quartiles in both children and adolescents (Fig. 1).

Mean values of tHcy were significantly higher in the TT subgroup compared with CC and CT subgroups in children ($P<0{\cdot}001$ and $P=0{\cdot}019$, respectively) and adolescents (both $P<0{\cdot}001$). No statistically significant differences were found between CC and CT subgroups either in children or in adolescents. The TT genotype was present in 8·6 % of children and 8·7 % of adolescents (Table 2).

Relationship between total plasma homocysteine and physical activity, cardiorespiratory fitness and body fat after controlling for different confounders and separation by age

The results of the regression models using tHcy as the outcome variable are shown in Table 3. Variation in tHcy levels was not significantly explained by total physical activity, cardiorespiratory fitness or body fat (expressed as skinfold thickness) in any age group. The results did not change when body fat was expressed as BMI. Subsequent analysis examining tHcy levels among BMI categories did not show any significant influence (data not shown). Further analysis examining the association of physical activity intensity levels (i.e. moderate, vigorous

Table 1. Descriptive characteristics of the subjects§

(Values are means and standard deviations unless otherwise stated)

	Children				Adolescents			
	Girls (*n* 138)		Boys (*n* 163)		Girls (*n* 194)		Boys (*n* 185)	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	9·6	0·4	9·6	0·3	15·5	0·4†	15.6	0·4‡
Tanner (%)								
1	54		99		0		1	
2	44		1		0		1	
3	2		0		4		2	
4	0		0		46		16	
5	0		0		50		80	
Height (cm)	139·1	6·3	139·0	5·8	165·1	6·5†	176·3	7·8*‡
Weight (kg)	33·6	5·9	33·2	5·6	57·0	7·8†	64·0	10·7*‡
BMI (kg/m^2)	17·3	2·1	17·1	2·2	20·9	2·4†	20·5	2·6‡
Overweight (%)	12·5		9·3		7·8		9·7	
Obese (%)	1·5		2·5		1·0		1·1	
Sum of five skinfolds (mm)	49·4	18·9	38·3	16·0*	62·2	18·8†	40·2	17·4*
Homocysteine (μmol/l)	6·6	1·4	6·4	1·2	9·1	4·4†	9·6	4·3‡
Intake of vitamin B_{12} (μg/d)	4·8	2·3	6·0	7·9*	4·4	2·7†	7·2	8·2*‡
Intake of folate (μg/d)	206·8	75·5	204·5	87·5	227·4	104·1†	285·4	109·1*‡
Total physical activity (counts/min)	654·8	166·6	820·8	275·0*	492·9	161·9†	544·6	197·1*‡
Moderate physical activity (min/d)	155·9	35·4	185·7	47·6*	57·2	22·2†	64·2	31·4*‡
Vigorous physical activity (min/d)	23·2	13·8	36·7	24·2*	12·6	11·5†	15·8	12·1*‡
Moderate-vigorous physical activity (min/d)	179·1	43·5	222·4	63·4*	69·8	28·7†	79·9	38·6*‡
Cardiorespiratory fitness (mm/kg per min)	37·7	5·4	43·0	6·9*	41·3	5·1†	51·8	6·4*‡
Maternal education								
University (%)	70		73		60		63	
Below university (%)	30		27		40		37	

Gender and age differences were assessed by two-way ANOVA.

**P* for gender differences.

†*P* for age differences in girls.

‡*P* for age differences in boys.

§For details of subjects and procedures, see p. 256.

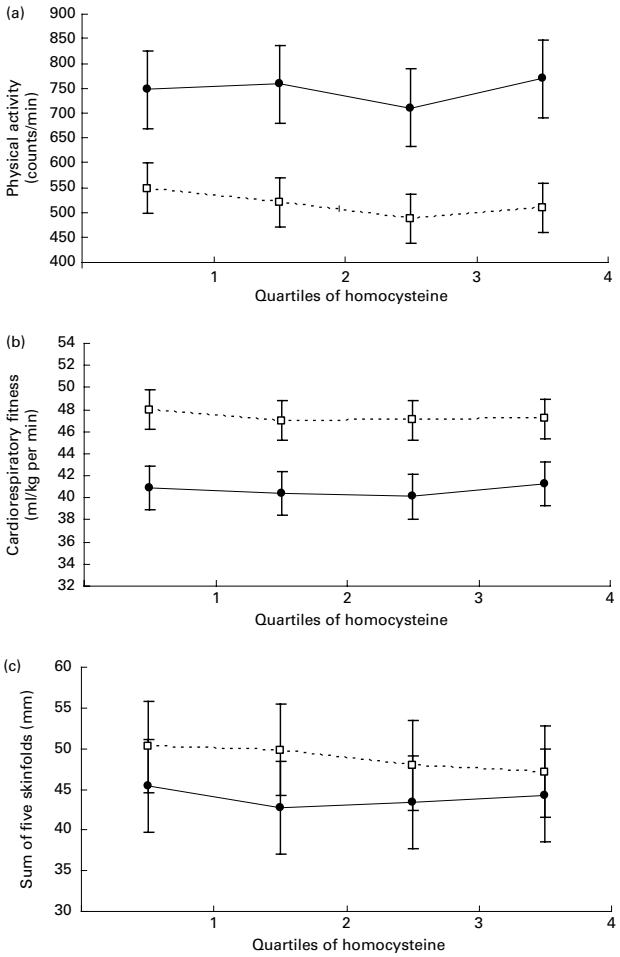

Fig. 1. Mean values of total physical activity (a), cardiorespiratory fitness (b) and sum of five skinfolds stratified by quartiles of homocysteine for children (—●—) and adolescents (—□—). Errors bars represent 95% CI. For details of subjects and procedures, see p. 256.

and moderate-vigorous) with tHcy did not change the results. The results remained the same when the regression models were performed separately for the MTHFR 677TT and MTHFR 677CC + CT subgroups.

Discussion

The results suggest that tHcy levels are not influenced by the studied modifiable factors in young subjects as other well-established cardiovascular risk factors in children and adolescents have been shown to be (Brage *et al.* 2004; Mesa *et al.* 2006; Ruiz *et al.* 2006*a*,*b*). However, tHcy levels were significantly higher in the MTHFR 677TT subgroup compared with the MTHFR 677CC and MTHFR 677CT subgroups in both children and adolescents. Differences in tHcy levels were also observed between age but not gender groups, which is in concordance with previous studies showing that tHcy increases with age (Bates *et al.* 2002). The levels of tHcy seen in the present study were within the normal ranges for these ages (Ueland & Monsen, 2003). Similar tHcy levels have been reported in Belgian (De Laet *et al.* 1999), Dutch (van Beynum *et al.* 2005) and Greek (Papoutsakis *et al.* 2005) children and adolescents. To the best of our knowledge, there are no other data available on the association of tHcy levels with objectively measured

Table 2. Total plasma homocysteine (tHcy) levels according to methylenetetrahydrofolate reductase (MTHFR) 677C > T genotype and age group‡
(Values are means and standard deviations)

	MTHFR 677C > T genotype								
	CC			CT			TT		
	Mean		SD	Mean		SD	Mean	SD	*P* value
Children (*n* 301)									
tHcy (μmol/l)	6·3‘		1·1	6·6		1·4	7·4*	1·6	0·001
n		139			136		26		
%		46·2			45·2		8·6		
Adolescents (*n* 379)									
tHcy (μmol/l)	8·3		1·7	9·0		2·9	16·9†	10·1	< 0·001
n		187			159		33		
%		49·3			42·0		8·7		

* Mean values were significantly different from CC ($P = 0{\cdot}001$) and CT ($P = 0{\cdot}019$) by Tukey's test.
† Mean values were significantly different from CC ($P < 0{\cdot}001$) and CT ($P < 0{\cdot}001$) by Tukey's test.
‡ For details of subjects and procedures, see p. 256.

physical activity, fitness and fatness after controlling for several potential confounders, including MTHFR 677C > T genotype, in children and adolescents.

Physical activity and homocysteine

The association between physical activity and tHcy has been evaluated in few studies. Randeva *et al.* (2002) showed that 6 months of sustained brisk walking for 20–60 min for 3 d/week significantly decreased tHcy levels in young overweight and obese women with polycystic ovary syndrome, a group at increased risk of premature atherosclerosis. Similarly, a weight-reduction programme including physical activities had a positive effect on the tHcy of obese children (Gallistl *et al.* 2001). We did not find any association between total physical activity and tHcy levels, even when the influence of physical activity intensity levels on tHcy levels was examined. When the analyses were performed separately for normal-weight and overweight-obese children and adolescents, no associations were found. Studies examining the influence of objectively measured physical activity and physical activity intensity levels on tHcy in children and adolescents are lacking. Intervention programmes studying the effect of physical activity amount and the influences of different physical activity intensities on tHcy in children and adolescents are needed.

Cardiorespiratory fitness and homocysteine

Cardiorespiratory fitness is a direct marker of physiological status and recent data suggest that fitness is one of the strongest predictors of health outcomes (Myers *et al.* 2002; Carnethon *et al.* 2003). Cardiorespiratory fitness represents the ability of active skeletal muscle to utilize O_2 during exercise. Theoretically, poor cardiorespiratory capacity may be the consequence of pathological changes peripherally affecting the tissues and the associated vasculature or centrally perturbing the heart and coronary arteries. These pathological changes, to some extent, may be attributed to elevated levels of tHcy and the related tissue toxicity in adults. In fact, elevated tHcy levels have been associated with an increased risk of decline in physical function in elderly people (Kado *et al.* 2002). Moreover, high tHcy levels have been negatively associated with estimated cardiorespiratory fitness in adult women (Kuo *et al.* 2005).

High cardiorespiratory fitness during childhood and adolescence has been associated with a healthier metabolic profile during these years (Brage *et al.* 2004; Mesa *et al.* 2006; Ruiz *et al.* 2006*a*). Results from the Alimentación y Valoración del Estado Nutritional en Adolescentes (AVENA) (Food and Assessment of the Nutritional Status of Spanish Adolescents) study showed significant associations between increased cardiorespiratory fitness and a favourable lipid profile and fasting glycaemia in both overweight and non-overweight adolescents aged 13–18 years (Mesa *et al.* 2006). Results from the Swedish and Estonian part of the European Youth Heart Study revealed negative associations between cardiorespiratory fitness and body fat (expressed as skinfold thickness) (Ruiz *et al.* 2006*b*). The same relationship was noted between cardiorespiratory fitness and other features of metabolic syndrome (e.g. insulin resistance, raised TAG, total

Table 3. Standardized multiple regression coefficients (β), SE, semipartial correlations (sr) and standardized coefficient of determination (R^2) examining association of level of plasma total homocysteine with total physical activity, cardiorespiratory fitness and body fat (expressed as skinfold thickness) after controlling for gender, pubertal development, socioeconomic status, folate and vitamin B_{12} intake, and MTHFR 677C > T genotype*

		Children					Adolescents				
Model	Predictor variable	β	SE	*P*	sr	R^2	β	SE	*P*	sr	R^2
1	Physical activity	0·009	0·007	0·90	0·008	0·037	−0·075	0·008	0·30	−0·072	0·017
2	Cardiorespiratory fitness	0·018	0·002	0·81	0·016	0·067	−0·073	0·002	0·32	−0·050	0·042
3	Body fat	−0·010	0·001	0·46	−0·009	0·057	−0·052	0·001	0·88	−0·042	0·039

* For details of subject and procedures, see p. 256.

cholesterol:HDL-cholesterol ratio, etc.) in children aged 9–10 years (Ruiz *et al.* 2006*a*). However, we did not observe any association between cardiorespiratory fitness and tHcy in children and adolescents. It must be borne in mind that the subjects involved in the present study were healthy children and adolescents with no existing cardiovascular pathologies and it may be that tHcy is not as sensitive to cardiorespiratory fitness as are other traditional cardiovascular risk factors.

Body fat and homocysteine

The amount of body fat has been associated with tHcy levels in obese children and adolescents (Gallistl *et al.* 2000). Insulin resistance has been implicated in the relationship, since insulin levels are strongly correlated with body fat (Gallistl *et al.* 2000). We did not find an association between body fatness (as expressed as skinfold thickness or as BMI) and tHcy levels, even when the analyses were performed separately for normal-weight or overweight-obese categories. This may be due to the low number of children and adolescents in the obese BMI category participating in the present study.

The results from the present study should be interpreted with caution due to the limitations of the cross-sectional design that cannot support evidence for causality. Another limitation of the study is the lack of information on other factors that have been shown to influence tHcy levels, such as serum levels of folate and vitamin B_{12} (Jacques *et al.* 2001; Papoutsakis *et al.* 2005; van Beynum *et al.* 2005) and endothelial NO synthase G894T polymorphism (Brown *et al.* 2003). The inclusion of available intake data on folate and vitamin B_{12} was an attempt to overcome this. The inclusion of a large number of subjects and several potential confounders including the MTHFR 677C > T genotype are notable strengths of the present study.

Results from cross-sectional studies have shown strong associations between tHcy and lifestyle-related factors (Jacques *et al.* 2001; Bates *et al.* 2002; Silaste *et al.* 2003; Chrysohoou *et al.* 2004; van Beynum *et al.* 2005). However, findings are different when analysed prospectively (Husemoen *et al.* 2006). The effect of lifestyle intervention based on smoking cessation, increased physical activity and healthy dietary habits on changes in tHcy after 1 year of follow-up was studied in a population-based sample of 915 men and women aged 30–60 years. The results suggested that none of the studied lifestyle changes was associated with changes in tHcy levels, indicating that tHcy may not be reduced by general lifestyle interventions.

One potential explanation for the discrepancy may be that the tHcy-lifestyle associations observed in cross-sectional studies are perhaps not causal. Furthermore, lifestyle-related data are usually obtained by means of questionnaires, which may lead to misreporting. The relationships between tHcy and objectively measured modifiable factors in children and adolescents should be studied prospectively.

In conclusion, the results of the present cross-sectional study suggest that objectively measured physical activity, cardiorespiratory fitness and body fat are not associated with tHcy levels in children and adolescents, even after controlling for presence of the MTHFR 677C > T genotype, the main influence on tHcy levels in these subjects.

Acknowledgements

The study was supported by grants from the Stockholm County Council (M.S.) and Nyckelfonden, Örebro (T.K.N.). J.R.R. and F.B.O. were supported by a grant from Ministerio de Educación y Ciencia de España (AP2003-2128, AP2004-2745).

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IV

Relations of total physical activity and intensity to fitness and fatness in children: the European Youth Heart Study[1–3]

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ABSTRACT

Background: It is unclear how the amount and intensity of physical activity (PA) are associated with cardiovascular fitness (CVF) and body fatness in children.

Objective: We aimed to examine the associations of total PA and intensity levels to CVF and fatness in children.

Design: A cross-sectional study of 780 children aged 9–10 y from Sweden and Estonia was conducted. PA was measured by accelerometry and was expressed as min/d of total PA, moderate PA, and vigorous PA. CVF was measured with a maximal ergometer bike test and was expressed as W/kg. Body fat was derived from the sum of 5 skinfold-thickness measurements. Multiple regression analysis was used to determine the degree to which variance in CVF and body fat was explained by PA, after control for age, sex, and study location.

Results: Lower body fat was significantly associated with higher levels of vigorous PA, but not with moderate or total PA. Those children who engaged in >40 min vigorous PA/d had lower body fat than did those who engaged in 10–18 min vigorous PA/d. Total PA, moderate PA, and vigorous PA were positively associated with CVF. Those children who engaged in >40 min vigorous PA/d had higher CVF than did those who accumulated <18 min vigorous PA/d.

Conclusions: The results suggest that PA of vigorous intensity may have a greater effect on preventing obesity in children than does PA of lower intensity, whereas both total and at least moderate to vigorous PA may improve children's CVF. *Am J Clin Nutr* 2006; 84:299–303.

KEY WORDS Physical activity, cardiovascular fitness, body fat, children, obesity

INTRODUCTION

Obesity among children and adolescents represents an uncontrolled and increasing worldwide epidemic (1, 2). A sedentary lifestyle and the reduction of physical activity (PA) are suggested to be implicated in this trend (3–5). Negative associations between objectively measured PA and fatness in children and adolescents have been shown (6–9).

Low cardiovascular fitness (CVF) is another important health problem (10–13). High CVF during childhood and adolescence has been associated with a healthier cardiovascular profile not only during these years (14–17) but also later in life (18–20). Comparison of habitually active and less active children and adolescents shows better CVF in the former (9, 17, 21).

For preventive purposes, it is of interest to understand the relative importance of the total amount and intensity of PA. New data have shown positive associations between vigorous PA [>6 metabolic equivalents (METs)] and CVF and negative associations between vigorous PA and fatness in adolescents aged 16 y (6). Similar results were found in a small sample of children aged 8–10 y (9). The present study examined the relations of objectively measured total PA and intensity levels to CVF and fatness in a population sample of children aged 9–10 y.

SUBJECTS AND METHODS

Subjects

The present cross-sectional study involved Swedish ($n = 413$) and Estonian ($n = 367$) children aged 9–10 y. The subjects were part of the European Youth Heart Study (EYHS) (22). The pooling of data was considered possible because of the use of common protocols (22). Study design, selection criteria, and sample

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[2] The Swedish part of the study was supported by grants from the Stockholm County Council (to MS) and the Estonian part was supported by a grant from the Estonian Science Foundation (no. 3277 and 5209) and by the Estonian Centre of Behavioural and Health Sciences. JRR and FBO were supported by a grant from Ministerio de Educación y Ciencia de España (AP2003-2128, AP2004-2745).

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Received December 8, 2005.

Accepted for publication March 10, 2006.

calculations are reported elsewhere (22, 23). In Estonia, the city of Tartu and its surrounding rural area was the geographical sampling area. In Sweden, 8 municipalities (Botkyrka, Haninge, Huddinge, Nynäshamn, Salem, Södertälje, Tyresö, and Örebro) were chosen for data collection. The local ethical committees approved the study (Örebro City Council no. 690/98, Huddinge University Hospital no. 474/98). One parent or legal guardian provided written informed consent, and all children gave verbal assent. The study procedures were explained verbally to all children.

Physical examination

Height and weight were measured by the use of standardized procedures. Body mass index (BMI) was calculated as weight/height squared (kg/m^2). Skinfold thicknesses were measured with a Harpenden caliper at the biceps, triceps, subscapular, suprailiac and triceps surae areas on the left side of the body according to the criteria described by Lohman et al (24). These measures have been shown to correlate highly with dual-energy X-ray absorptiometry-measured body fat percentages in children of similar ages (25). All measurements were taken twice and in rotation, and the mean calculated. If the difference between the 2 measurements differed by >2 mm, a third measurement was taken and the 2 closest measurements were averaged. The sum of 5 skinfold thicknesses was used as an indicator of body fat rather than BMI, because BMI has been suggested to not be a good measurement of body fatness in children (7, 8), and because fatness rather than weight has been shown to be associated with poor health (26). Pubertal development was assessed according to Tanner (27). Pubertal stage was assessed by a researcher of the same sex as the child through brief observation. Breast development in girls and genital development in boys was used for pubertal classification.

Cardiovascular fitness

CVF was determined by a maximum cycle-ergometer test as described elsewhere (28). Briefly, the workload was preprogrammed on a computerized cycle ergometer (Monark 829E Ergomedic, Vansbro, Sweden) to increase every third minute until the subject reached exhaustion. Heart rate was registered continuously by telemetry (Polar Sport Tester, Kempele, Finland). The criteria for exhaustion were a heart rate ≥185 beats/min, failure to maintain a pedalling frequency of ≥30 revolutions/min, and a subjective judgment by the observer that the child could no longer keep up, even after vocal encouragement. The power output was calculated as $W_1 + (W_2 \times t/180)$, where W_1 is the work rate at the last fully completed stage, W_2 is the work rate increment at the final incomplete stage, and t is the time in seconds at the final incomplete stage. CVF was expressed as the maximal power output per kilogram body mass (W/kg). The test used to measure CVF was previously validated in children of the same age (29).

Assessment of physical activity

PA was measured over 4 consecutive days (2 weekdays and at least 1 weekend day) with an activity monitor (MTI model WAM 7164; Manufacturing Technology Inc, Shalimar, FL, formerly known as Computer Science and Applications Inc) attached on the right hip. At least 3 days of recording, with a minimum of 10 h registration per day, was set as an inclusion criterion. Total PA (also called *amount of PA*) was expressed as total counts recorded divided by total daily registered time (counts/min). The time engaged in moderate and vigorous PA was calculated and presented as the average time per day during the complete registration. Moderate PA (3–6 METs) and vigorous PA (>6 METs) intensities were based on cutoffs published elsewhere (30). Also, the time spent in at least moderate-intensity PA (>3 METs) was calculated as the sum of time spent in moderate and that spent in vigorous PA (MVPA, min/d). Each minute over the specific cutoff was summarized in the corresponding intensity level group. Validation studies examining the accelerometer used in this study and the construction of summary variables for intensity of movement suggest that this is a valid and reliable measure of children's PA (31, 32).

Controversy exists, however, about the best way to express PA. When expressed as energy expended in movement, heavier adolescents seem to be engaging in relatively large amounts of PA because they use more energy than lighter adolescents do to move their bodies a given amount. However, when PA is expressed as movement (6), heavier adolescents will appear to engage in less PA than their lighter peers. The time spent in activities of various intensities seems more pertinent for the purpose of making exercise recommendations (33).

Statistical analyses

The data are presented as means ± SDs. All variables were checked for normality of distribution before the analysis. The sum of 5 skinfold thicknesses was normalized by transformation to the inverted natural logarithm and by multiplying by minus one. The square root of total PA and of vigorous PA was calculated. Country differences were analyzed by analysis of variance (ANOVA) for boys and girls separately. Sex differences were assessed by ANOVA after adjustments for age and study location. Standard multiple regression was used to determine the degree to which variance in CVF and body fat was explained by PA after control for sex, chronologic age, and study location. The variance explained by the demographic variables was similar when pubertal development rather than age was used in the analyses; thus, age was used in all statistical analyses. The analyses were conducted by using total PA and PA intensities (moderate, vigorous, and MVPA) with either CVF or body fat as the outcome variable. For both outcome variables, a series of models was tested. Model 1 examined the influence of total PA (amount of PA) and its interaction with the covariates. Model 2 examined the influence of moderate PA and its interaction with the covariates. Model 3 examined the influence of MVPA and its interaction with the covariates, and model 4 examined the influence of vigorous PA and its interaction with the covariates. All analyses were adjusted for sex, age, and study location. Statistical significance was set at $P < 0.05$ in all analyses. The analyses were performed with SPSS (version 13.0 for WINDOWS; SPSS Inc, Chicago).

RESULTS

The ANOVA showed that the Estonian boys had a lower sum of 5 skinfold thicknesses and lower total and vigorous PA than did the Swedish boys. The Estonian girls had a lower sum of 5 skinfold thicknesses and lower BMI and vigorous PA than did the Swedish girls. The physical characteristics, CVF, and PA patterns of the 780 children are shown in **Table 1**. The results of the

TABLE 1
Descriptive characteristics of the subjects[1]

	All subjects (n = 780)	Boys (n = 379)	Girls (n = 401)
Age (y)	9.5 ± 0.4	9.6 ± 0.4	9.5 ± 0.4
Weight (kg)	32.6 ± 6.4	32.7 ± 6.0	32.4 ± 6.8
Height (cm)	138.3 ± 6.6	138.6 ± 6.2	138.0 ± 6.9
BMI (kg/m^2)	16.9 ± 2.3	16.9 ± 2.2	16.9 ± 2.4
Body fat (mm)[2]	42.0 ± 19.1	37.3 ± 17.1	46.5 ± 19.8[3]
CVF (W/kg)	3.0 ± 1.4	3.6 ± 1.4	2.4 ± 1.2[3]
Total PA (counts/min)[4]	714.0 ± 274.4	769.9 ± 278.1	661.2 ± 200.9[3]
Moderate PA (min/d)	170.9 ± 56.0	181.5 ± 61.6	161.0 ± 48.0[3]
Vigorous PA (min/d)[4]	26.3 ± 19.6	30.9 ± 22.5	22.0 ± 15.2[3]
MVPA (min/d)	197.3 ± 69.3	212.4 ± 76.8	183.0 ± 58.0[3]

[1] All values are $\bar{x}$ ± SD. CVF, cardiovascular fitness; PA, physical activity; MVPA, moderate to vigorous PA; body fat, sum of 5 skinfold thicknesses.
[2] Inverted natural log-transformed values multiplied by minus one were used in the analysis, but nontransformed values are presented in the table.
[3] Significantly different from boys, $P < 0.05$ (ANOVA after adjustment for age and study location).
[4] Square-root-transformed values were used in the analysis, but nontransformed values are presented in the table.

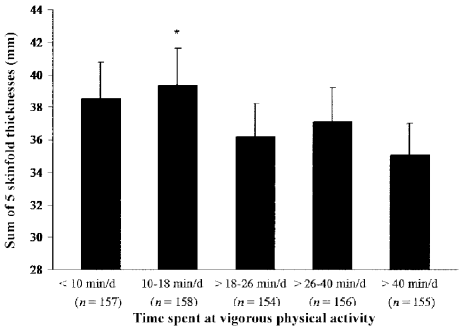

FIGURE 1. Mean sum of 5 skinfold thicknesses (body fat) stratified (in quintiles) by time spent at vigorous physical activity. The sum of 5 skinfold thicknesses was normalized by transforming to the inverted natural logarithm and multiplying by minus one. The square root of vigorous physical activity was calculated. Errors bars represent 95% CIs. Data were analyzed by general linear models with Bonferroni's adjustment for sex, age, and study location. *Significantly different from those who accumulated >40 min vigorous physical activity/d, $P < 0.001$.

ANOVA showed that girls had significantly lower CVF than did the boys and higher body fat. Moreover, girls spent significantly fewer minutes per day in total PA, moderate PA, vigorous PA, and MVPA than did boys.

The statistics of the regression models that used body fat (sum of 5 skinfold thicknesses) or CVF as the outcome variable are shown in **Table 2**. Each model included sex, age, and the study location as covariates. Vigorous PA was the only significant predictor of body fat (model 4); total PA (model 1), moderate PA (model 2), and MVPA (model 3) did not explain significant amounts of variance. The results did not change when BMI was used as an indicator of body fat instead of the sum of 5 skinfold thicknesses. Variation in CVF was significantly explained by total PA (model 1), moderate PA (model 2), MVPA (model 3), and vigorous PA (model 4). Subsequent analyses examining the association between CVF and body fat showed that CVF was also a significant predictor of body fat (expressed as the sum of 5 skinfold thicknesses or BMI).

The children were divided into 5 groups (quintiles) on the basis of their time spent in vigorous PA (ie, >6 METs). General lineal models with Bonferroni's adjustment for sex, age, and study location showed a significant association between activity group and body fatness derived from the sum of 5 skinfold thicknesses ($P < 0.001$). A significant difference ($P < 0.001$) was observed between children who accumulated >40 min (n = 155) of vigorous PA per day and those who accumulated 10–18 min/d (n = 158) at this level of intensity (**Figure 1**). A significant association was also shown between activity group and CVF ($P < 0.001$). Those children who engaged in >40 min/d of vigorous PA (n = 155) had higher CVF ($P = 0.003$) than did those who engaged in <18 min/d (n = 315) at this level of intensity. Also, as shown in **Figure 2**, CVF was higher (P = 0.018) in children who accumulated 26–40 min/d of vigorous PA (n = 156) than in those who accumulated 10–18 min/d at this level of intensity (n = 158).

DISCUSSION

The results of the present study suggest that, after adjustment for demographic factors, the intensity of PA, especially vigorous PA, but not total PA is negatively related to body fatness, whereas both amount and intensity of PA are positively associated with CVF in children. Participation in moderate PA explained a significant proportion of the variance in CVF, whereas moderate PA did not explain a significant proportion of the variance in body fat. Total PA showed a significant positive association with

TABLE 2
Standardized multiple regression coefficients (β), 95% CIs, and standardized coefficient of determination (R^2) for the association of body fat (sum of 5 skinfold thicknesses) and cardiovascular fitness (W/kg) with physical activity (PA) after adjustment for sex, age, and study location

		Body fat[1]				Cardiovascular fitness			
Model	Predictor variable	β	P	95% CI	R^2	β	P	95% CI	R^2
1	Total PA[2]	−0.054	0.115	(−0.00007, 0.00074)	0.13	0.129	<0.001	(0.00737, 0.0219)	0.22
2	Moderate PA	0.018	0.597	(−0.00004, 0.00002)	0.12	0.087	0.008	(0.00022, 0.00143)	0.21
3	Moderate to vigorous PA	−0.011	0.751	(−0.00002, 0.00003)	0.12	0.108	0.001	(0.00034, 0.00132)	0.21
4	Vigorous PA[2]	−0.081	0.02	(0.00019, 0.00222)	0.13	0.124	<0.001	(0.01625, 0.0525)	0.21

[1] Inverted natural log-transformed values multiplied by minus one were used.
[2] The square root of the measures was calculated.

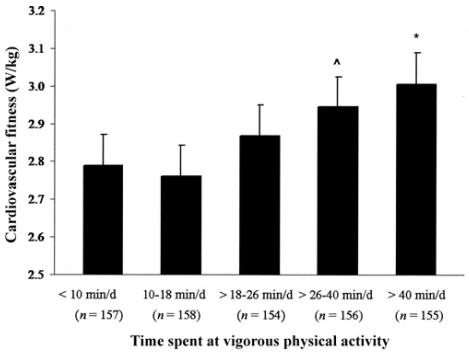

FIGURE 2. Mean cardiovascular fitness stratified (in quintiles) by time spent at vigorous physical activity. The square root of vigorous physical activity was calculated. Errors bars represent 95% CIs. Data were analyzed by general linear models with Bonferroni's adjustment for sex, age, and study location. *Significantly different from those who accumulated <18 min vigorous physical activity/d at this intensity level, $P < 0.001$. ^Significantly different from those who accumulated 10–18 min vigorous physical activity/d, $P = 0.018$.

CVF, whereas total PA did not explain a significant proportion of the variance in body fat. These findings suggest that intensity rather than amount of PA may be more important in relation to the prevention of obesity in children, as has been argued by others (8, 34).

The association between vigorous PA and CVF and fatness is consistent with other findings reported in a recent observational study in North American adolescents (6). Similarly, Rowlands et al (9) found significant correlations between vigorous PA and CVF and body fat, as well as between moderate PA and both CVF and body fat, in a small sample of children aged 8–10 y. In both studies, PA was objectively assessed over 5 or 7 days, respectively, whereas 4 days of data were available for most of the participants in the present study. This slightly shorter monitoring period may have masked the true association between moderate PA and body fat, as well as the association between total PA and body fat. Four to 5 days of activity monitoring has been proposed as a suitable duration for accurately and reliably estimating usual PA behavior in children (35).

The relation between vigorous PA, assessed by accelerometry, and body fat has been observed in several studies of normal-weight American and European children and adolescents (6, 7, 9, 18, 34). The association between PA and body fat seems to be intensity- but not age-dependent. Data from 5 days of accelerometry registration showed that normal-weight North American adolescents spend a mean of only 5 min/d in vigorous PA, and despite this modest time, a significantly positive association with body fat was observed (6). We found similar results in the present study. However, the time engaged in vigorous PA observed in our children was 26.3 ± 19.6 min/d, which is slightly higher than that observed in previous studies. Interventional studies have shown that programs of moderately intense exercise of 30–60 min/d in duration did not influence body fatness in normal-weight children and adolescents (36, 37). Taken together, these results may suggest that relatively large amounts of vigorous PA may be needed to affect adiposity in normal-weight children and adolescents. In fact, it has been suggested that, for nonobese adolescents, the interventions should be high in both intensity and volume (>80 min/d) (38).

The findings in obese children and adolescents are slightly different. In overweight children, beneficial effects in body fat control may be attained with 30-60 min of moderate PA, 3-7 d/wk (39–41). However, obese adolescents who spent the most weekly time engaged in vigorous PA tended to be those who decreased the most in body fat (40, 41). For several reasons, it is reasonable to recommend moderate PA for obese children and adolescents until higher intensities can be attained. Moderate PA is better tolerated than vigorous PA (41), and tiring PA may lead to less PA on the following day (42), although it likely depends on the type of exercise performed (39). Therefore, for obese children and those who have been physically inactive, an incremental approach to the 45–60-min/d goal of moderate PA 5 or more days per week is recommended (43). Increasing activity by ≈10% per week appears to be acceptable and achievable (43), because attempting to achieve too much too rapidly is often counterproductive and may lead to injury.

The cross-sectional nature of this study limits our ability to determine any causality in the results. One limitation of the study is that the accelerometer does not compensate for the relative increase in energy expenditure by increase in body size. To further increase our understanding of PA pattern among normal-weight and obese children, the amount of continuous bouts of PA at different intensity levels should be explored. Moreover, to be able to address the relative intensities of PA, another method has to be used, eg, heart rate monitoring, to collect data on the individual heart rate response as a percentage of maximum heart rate.

We also do not know whether an extrapolation of the association may be made for overweight and obese children. Nevertheless, with regular reports of increasing childhood obesity prevalence worldwide, the results of this study are noteworthy. Although we controlled for several potential confounders, such as age, sex, and study location, other variables such as food intake and genetic aspects may also have an influence. Interventional studies are needed to examine the effect of different PA intensities on CVF and fatness and to establish a threshold of amount and intensity of PA that has a better effect on CVF and body fatness in nonobese and obese children and adolescents. In conclusion, our cross-sectional results suggest that vigorous-intensity PA may have a greater impact in preventing obesity in children than lower PA intensity levels, whereas both total and at least moderate to vigorous PA may improve children's CVF.

We thank Maarike Harro for the opportunity to use the Estonian data and Pekka Oja for highly valuable comments.

JRR and NSR conceived the hypothesis and conducted the statistical analyses for this manuscript. JRR drafted the manuscript. NSR, AH-W, FBO, JW, and MS contributed to the interpretation and discussion of the results. AH-W and MS contributed to the concept and design of the EYHS study. All the authors critically revised the drafted manuscript. None of the authors had any conflict of interest.

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