

Thesis for doctoral degree (Ph.D.)  
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# Cardiorespiratory fitness and cardiovascular disease risk factors in children and adolescents

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**Karolinska  
Institutet**

Stockholm, March 2007



*To my parents and sister*



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Cardiovascular disease (CVD) events occur most frequently during or after the fifth decade of life, however, there is evidence indicating that the precursors of CVD has its origin already in the first or second decade of life. Low levels of physical activity and cardiorespiratory fitness are major modifiable determinants for the development of CVD 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 clustering of CVD risk factors in children. Moreover, the results suggested that there is a cardiorespiratory fitness level for having a low metabolic risk. The results also indicated 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 were not influenced by the levels of physical activity, cardiorespiratory fitness, and body fat 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 in a long perspective of time.





- 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 prepubertal 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 ABBREVIATIONS

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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
VO <sub>2</sub>	Oxygen uptake
VO <sub>2max</sub>	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 ( $\text{VO}_{2\text{max}}$ ).  $\text{VO}_{2\text{max}}$  is the rate at which an individual is able to consume oxygen.  $\text{VO}_2$  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 ( $\text{VO}_{2\text{max}}$ ), refer to the same concept and are used interchangeably in the literature.

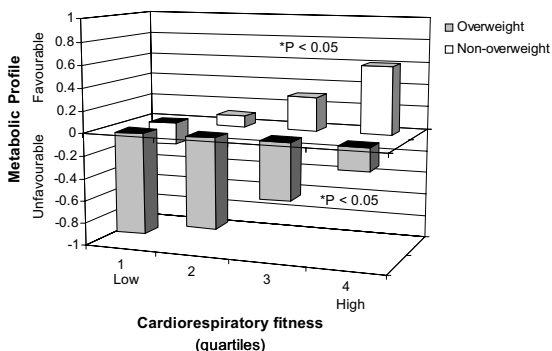
Cardiorespiratory fitness is influenced by several factors including age, sex, health status, and genetics. It has been suggested that up to 40% of variation in the level of cardiorespiratory fitness is attributable to genetic factors (Bouchard *et al.*, 1986). However, as stated before, the level of 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-overweight 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 (Harpender transportable stadiometer), with the children clad only in their underwear. Body mass index (BMI) was calculated as weight/height squared ( $\text{kg/m}^2$ ).

Skinfold thickness was measured with a Harpenden caliper (Baty International, Burgess Hill, U.K.) 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.* (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.

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.

**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 <sub>12</sub> , 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.

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)	$\Delta$ 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;  $\Delta$ , 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 ( $\text{VO}_{2\text{max}}$ ) in ml/min was:  $12 \times \text{calculated power output} + 5 \times \text{body weight in kg}$  (Hansen *et al.*, 1989). Cardiorespiratory fitness was expressed as  $\text{VO}_{2\text{max}}$  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  $\text{VO}_{2\text{max}}$  ( $r = 0.95$  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  $-80^{\circ}\text{C}$ . 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):  $\text{fasting insulin (mU/L)} \times \text{fasting glucose (mmol/L)} / 22.5$ .

Total plasma homocysteine (tHcy) in acidified citrated plasma (Willems *et al.*, 1998) was assayed using a fluorescence polarization immunoassay on a IMx<sup>®</sup> 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](http://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 ( $\geq$  75 th) 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).

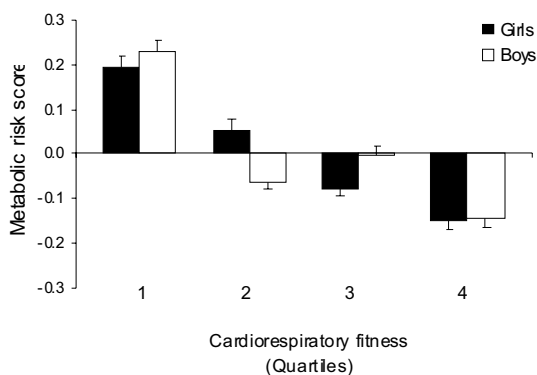
Moreover, the ROC analysis showed significant discriminatory accuracy of cardiorespiratory fitness to identify either a low or high metabolic risk in both sexes. The cardiorespiratory fitness values at these points were 37.0 and 42.1 mL/kg/min in girls and boys, respectively.

The values found in the present study are similar to the cut-off points proposed

by the Cooper Institute:  $\geq 38$  and  $\geq 42$  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.



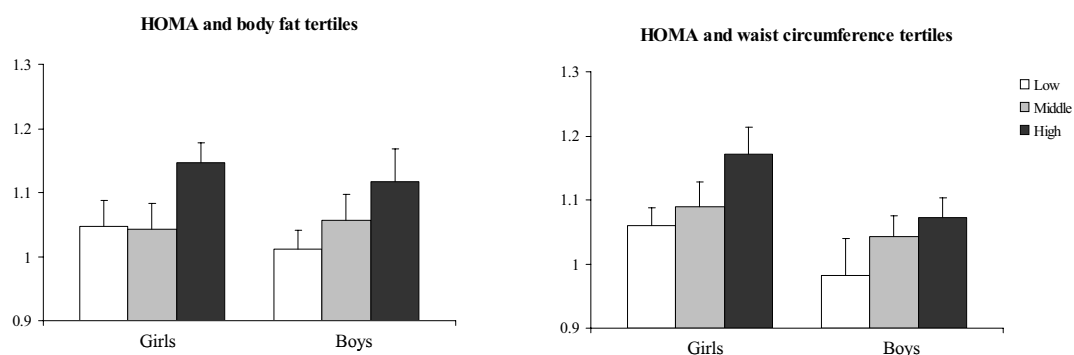
**Figure 2.** Clustering of cardiovascular disease risk factors (metabolic risk score) and cardiorespiratory fitness in children. Data shown as mean and SD.

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

*Markers of insulin resistance are associated with fatness and fitness in prepubertal 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.

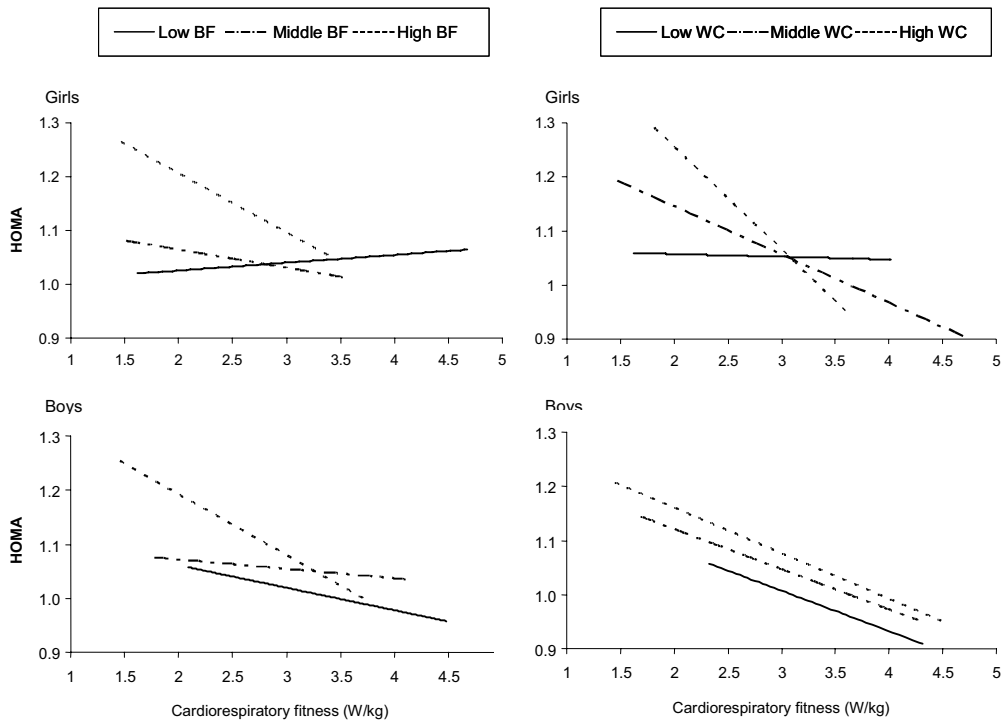
The aim for this study was to examine if the detrimental consequences attributed to high adiposity may be attenuated by having high levels of cardiorespiratory fitness.

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.** Homeostasis model assessment (HOMA) stratified by body fat (expressed as skinfold thickness) and waist circumference tertiles in girls and boys. Values are mean, and error bars represent 95% confidence 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 ( $\beta$ ) 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	$\beta$	$P$	$R^2$
HOMA	1 <sup>st</sup> T: Low body fat	0.095	0.232	0.087
	2 <sup>nd</sup> T: Middle body fat	-0.009	0.909	0.079
	3 <sup>rd</sup> T: High body fat	-0.241	0.001	0.156
HOMA	1 <sup>st</sup> T: Low waist circumference	-0.010	0.905	0.100
	2 <sup>nd</sup> T: Middle waist circumference	-0.148	0.063	0.086
	3 <sup>rd</sup> 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<sub>12</sub>, 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 4).

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.

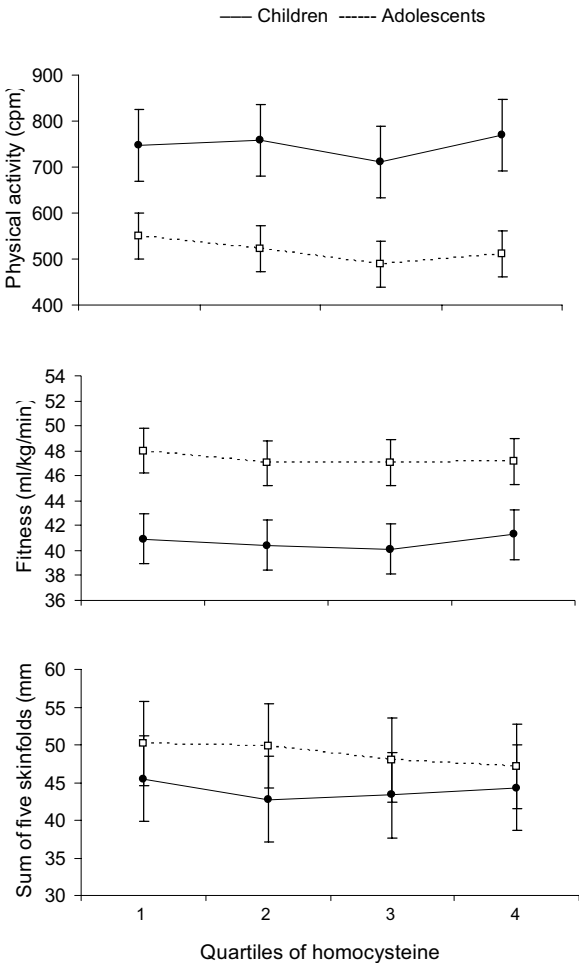


Figure 5. Mean values of total physical activity, cardiorespiratory fitness, and sum of five skinfolds stratified by quartiles of homocysteine for children and adolescents. Errors bars represent 95% CIs.



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.

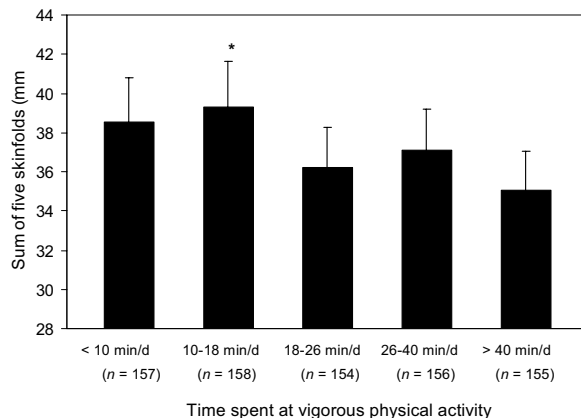
Negative associations between tHcy levels and cardiorespiratory fitness have been reported in women (Kuo *et al.*, 2005), which is not in concordance with the results obtained in the present study. It must be borne in mind that the individuals involved in this study were healthy children and adolescents 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 paper IV, 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 5), whereas both the amount and intensity of physical activity are positively associated with cardiorespiratory fitness in children.



**Figure 5.** 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 do 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  $\beta$ -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 measure insulin resistance and  $\beta$ -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 an 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.

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