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PHYSICAL ACTIVITY, CARDIOVASCULAR FITNESS AND ABDOMINAL ADIPOSITY IN CHILDREN AND ADOLESCENTS

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To my family,
"por los que están, y por los que se marcharon y siguen estando"

ABSTRACT

Cardiovascular fitness (CVF) and adiposity, especially abdominal adiposity, are well-known factors associated with general health status in childhood and adolescents. Physical activity (PA) may play a crucial role in CVF enhancement and the prevention of abdominal adiposity accumulation. The apparently obvious association of PA with CVF and adiposity still requires further research, since current knowledge is mostly based on data from self-reported measures but such measures are of limited use in pediatric populations.

The current thesis aimed to examine the associations of objectively measured PA with CVF and abdominal adiposity in more than one thousand children (9-10 years) and adolescents (15-16 years) from the Swedish part of the European Youth Heart Study.

Total PA levels and time spent in PA of different intensities (i.e. moderate and vigorous PA) were measured by accelerometry. Cardiovascular fitness was measured by a maximal cycling test. Body mass index was used to define overweight/obesity, and waist circumference to define an excess of central adiposity. Percentage body fat was estimated from skinfold thickness. Stages of sexual maturation were identified according to Tanner.

The main findings and conclusions were: I) When examining CVF in adolescents, sexual maturation status and percentage body fat, as well as the way in which CVF is expressed, should be taken into account. II) Those adolescents who spend 60 minutes or more in moderate to vigorous PA daily seem to be more likely to have a healthier CVF level, independently of their sexual maturation and adiposity status. III) Low levels of total PA and, in particular, of vigorous PA, are associated with an increased risk for overweight and excess of central adiposity, independently of other important determinant factors, such as television viewing and birth weight. The data also indicate that the adverse association of television viewing with abdominal adiposity could be attenuated if sufficient vigorous PA is accumulated. IV) Cardiovascular fitness is inversely associated with abdominal adiposity and seems to modify the associations between PA and abdominal adiposity. In children and adolescents with low CVF, time spent in vigorous PA seems to be the key component linked to abdominal adiposity.

The findings reported in this thesis will contribute to a better understanding of the associations of PA with CVF and abdominal adiposity, a prerequisite for more efficient health promotion. Future public health recommendations should consider the evidence-based importance of high intensity PA.

Keywords: Physical activity, accelerometry, fitness, aerobic capacity, adiposity, waist circumference, children, adolescents.

LIST OF PUBLICATIONS

I. Ortega FB, Ruiz JR, Mesa JL, Gutiérrez A, Sjöström M. Cardiovascular fitness in adolescents: the influence of sexual maturation status. The AVENA and EYHS studies. Am J Hum Biol, 2007;19:801-808.

II. Ortega FB, Ruiz JR, Hurtig-Wennlöf A, Sjöström M. Physically active adolescents are more likely to have a cardiovascular fitness level adequate for cardiovascular health. Rev Esp Cardiol, (in press).

III. Ortega FB, Ruiz JR, Sjöström M. Physical activity, overweight and central adiposity in Swedish children and adolescents: the European Youth Heart Study. Int J Behav Nutr Phys Act, 2007;4:61.

IV. Ortega FB, Ruiz JR, Hurtig-Wennlöf A, Vicente-Rodríguez G, Rizzo NS, Castillo MJ, Sjöström M. Cardiovascular fitness modifies the associations between physical activity and abdominal adiposity in children and adolescents. The European Youth Heart Study. (submitted).

CONTENTS

LIST OF ABBREVIATIONS

AVENA	Alimentación y Valoración del Estado Nutricional de los Adolescentes / Feeding and assessment of nutritional status of Spanish adolescents
ANCOVA	Analysis of the covariance
ANOVA	Analysis of the variance
BMI	Body mass index
CVD	Cardiovascular disease
CVF	Cardiovascular fitness
DXA	Dual-energy X-ray absorptiometry
EYHS	European Youth Heart Study
FFM	Fat-free mass
GPS	Global positioning system
METs	Metabolic equivalents
MVPA	Moderate to vigorous physical activity
PA	Physical activity
TV	Television
VO_{2max}	Maximal oxygen consumption
%BF	Percentage body fat

1. INTRODUCTION

The transition from childhood to adulthood during the second decade of life is complex. It is a period of rapid growth and development. The adolescent experiences profound biological, emotional, intellectual and social changes, and the patterns of behavior they adopt may have long-term consequences for their health and quality of life.

The influence of such health determinants over long periods of time is clearly exemplified by the case of cardiovascular disease (CVD). Cardiovascular disease events occur in adulthood, but the disease may have its origin already in childhood or adolescence,[1-3] perhaps even earlier,[4, 5] and is influenced by the health behavior of the individual while young .

Childhood obesity is another public health concern.[6] Health promotion and disease prevention in the beginning of the 21st century may therefore focus on young people and their modifiable lifestyle factors such as dietary habits and physical activity (PA) habits, in an attempt to control the overweight and obesity problem. A better understanding of these lifestyle factors and the associations between them and adiposity may improve the possibilities for successful public health work.

In this thesis, the associations of PA with two important risk factors for CVD - cardiovascular fitness (CVF) and adiposity - will be thoroughly examined in a sample of Swedish youths aged 9 (hereafter referred to as "children") and 15 years ("adolescents").

1.1. Physical activity

Physical activity is defined as any bodily movement produced by skeletal muscles that results in energy expenditure.[7] This broad term means that PA includes almost everything that a person does. *Inactivity* is, by contrast, the time spent in behaviors that do not markedly increase energy expenditure. Another related but not interchangeable term is *physical exercise*, defined as a subset of PA that is planned, structured and systematic.[7] To describe and analyze PA, at least three key attributes should be taken into account:

frequency, duration and intensity. Special attention will be paid in this thesis to the intensity of PA.

Physical activity under free-living conditions is difficult to assess, especially in young people, due to its complex nature.[8, 9] More than 30 different methods of assessing PA are described in the literature. The strengths and limitations of these methods, as well as their feasibility for use in pediatric population-based studies, have been thoroughly reviewed.[10-13] In short, methods for assessing PA in young people can be grouped into three categories: reference techniques, objective techniques and subjective techniques.

1) The most commonly used reference techniques are direct observation, assessment of total energy expenditure using doubly labeled water and indirect calorimetry. *Direct observation* is, according to Sirard and Pate,[13] the most practical and appropriate criterion measure of PA and patterns of activity. These authors have critically reviewed seven direct observation techniques and all attained satisfactory inter-observer agreement (84-99%) among simultaneous observations of the same child. Although direct observation provides valuable information about PA levels and patterns, it is extremely complicated to follow a child for a full day.[14, 15] It is feasible to assess a small number of individuals, but not large numbers. The *doubly labeled water* method is recognized as the reference method or "gold standard" for the assessment of total energy expenditure.[11] This technique assesses energy expenditure by estimating carbon dioxide production using an isotope dilution over a minimum of 3 days.[16] This method provides a powerful tool for the accurate measurement of daily energy expenditure, but it has several limitations.[11] The isotopes are expensive and difficult to obtain, and no information about duration, frequency or intensity of the activity is obtained. *Indirect calorimetry* during rest and exercise is used extensively and considered an accurate and valid measure of short term energy expenditure. Although smaller, lighter and portable gas analyzers have been developed in recent years, this method cannot be used for long recording periods (the battery works for only two to three hours) and is obviously very intrusive.

2) Objective techniques are increasingly used, and include equipment such as heart rate monitors, pedometers and accelerometers. The information obtained by *heart rate monitoring* is mainly based on the assumption of a linear relationship between heart rate and work rate. However, heart rate is influenced not only by PA but also by other factors

such as caffeine or medications, anxiety, emotional stress, fatigue, body position, the active muscle group, training status, state of hydration, ambient temperature and humidity.[11, 13] *Pedometers* are relatively inexpensive and simple motion sensors that record the number of steps taken over a period of time.[13] On the other hand, pedometers are not able to measure the intensity or the pattern of the activities. They do not properly record activity during cycling (except when using ankle-mounted pedometers) or increases in energy expenditure due to carrying objects. They are also unable to distinguish between walking and running, or between moving downhill and uphill.[11] *Accelerometers* are more sophisticated motion sensors than pedometers and they record movement through piezo-electric transducers and microprocessors that convert recorded accelerations to a quantifiable digital signal referred to as a "count".[11] This method provides an accurate measure of PA and valuable information about the intensity of PA. However, some limitations, such as its insensitivity to cycling, walking/running uphill *vs.* downhill or other activities as swimming must be recognized.[10] Strengths and limitations of accelerometry are further discussed in the methodological considerations section (5.2.2).

Combinations of objective methods, such as heart rate monitoring plus accelerometry or GPS (Global Positioning System) plus accelerometry, are currently being developed and implemented, and will provide more sophisticated measures of PA. The technological developments are advancing rapidly. However, the intellectual and scientific developments required to make these methodologies useful (e.g. the use of appropriate software for cleaning data and appropriate interpretation of the output), are still in their infancy.

3) Many subjective techniques, such as self-reported questionnaires, interviewer-administered questionnaires, mail surveys, proxy-reports by parents or teachers, and diaries are currently available. Self-report questionnaires are the most widely used in epidemiological research, due to their ease and low costs of implementation. However, important drawbacks have been recognized when using them in pediatric populations.[13] The sporadic nature of children's PA[17] makes these activities difficult to recall, quantify and categorize. Also, the lower cognitive functioning of children compared with adults reduces their ability to accurately recall intensity, frequency and especially duration of the activities.[18, 19]

The reference methods give a high accurate measure of PA, but are too expensive and not feasible enough to be used in large-scale studies. The subjective methods have shown a low accuracy for the assessment of PA in youths, especially in younger children (< 12 y).[20] Among the objective methods, accelerometry has been shown to be feasible and provides accurate information about duration, frequency and intensity of habitual PA under free-living conditions. Consequently, accelerometry was the method chosen for the assessment of PA in the studies included in this thesis.

The associations between PA at young ages and its short/long term consequences on health have been recently reviewed.[21-25] A systematic review of the associations of PA and health in adolescents concluded that PA promotion must start early in life. Although both the quantity and the nature of 'optimal' PA remains unknown and needs further research, the lifelong benefits of high levels of PA early in life on adult health are unequivocal.[26] The present work will provide further insights into how the amount of PA, as well as the type of intensity, is associated with healthier CVF levels and adiposity status.

1.2. Cardiovascular fitness

Physical fitness is a set of attributes related to a person's ability to perform physical activities that require aerobic fitness, endurance, strength or flexibility and is determined by a combination of regular activity and genetically inherited ability.[7] *Cardiovascular fitness* is the physical fitness component that has been studied the most. It reflects the overall capacity of the cardiovascular and respiratory systems and the ability to carry out prolonged exercise.[27] Many terms have been used to define this physical fitness component: cardiovascular fitness, cardiorespiratory fitness, cardiorespiratory endurance, aerobic fitness, aerobic capacity, aerobic power, maximal aerobic power, aerobic work capacity, and physical work capacity. All refer to the same concept and are used interchangeably in the literature.[28] Another important related concept is maximal oxygen consumption, or VO_{2max}. The VO_{2max} attained during a graded maximal exercise is an objective measure of CVF level.[27] The VO_{2max} can be expressed in absolute terms (oxygen uptake/time) or normalized by body weight or fat free mass (FFM). Further methodological issues on the assessment of CVF will be commented on in the discussion sections.

Cardiovascular fitness levels are highly associated with other fitness parameters in young people and in adults. A cross-sectional study of almost 3000 adolescents showed that those adolescents with a high CVF level also had higher muscular strength (in both lower and upper limbs), speed/agility and flexibility.[29] This finding suggests that CVF, if assessed at population level, may be a good indicator of the overall physical fitness level of young people.

In adults, CVF is a powerful predictor of CVD and all-cause morbidity and mortality.[30-33] This seems to be valid in apparently healthy individuals, and also in people with diseases, such as diabetes mellitus, hypertension, metabolic syndrome and several types of cancer.[34] In recent years, an increasing amount of research on physical fitness and health in childhood and adolescence has been published. This information, recently reviewed by Ortega et al.,[35] suggests that:

1) There is strong evidence indicating that CVF levels are associated with total and abdominal adiposity, when adiposity is assessed either by anthropometric indices or by reference methods, such as dual-energy X-ray absorptiometry (DXA), computed tomography or magnetic resonance imaging.[36-45] Whether or not this association is independent of objectively measured PA needs further study.

2) Cardiovascular fitness has been shown to be associated with both traditional and emerging CVD risk factors, such as low- and high-density lipoprotein cholesterol, triglycerides, blood pressure, fasting glucose, fasting insulin, low grade inflammatory markers and homocysteine. In addition, while fatness tends to exacerbate these risk factors, the available information suggests this could be counteracted by having high levels of CVF.[28, 46-66]

3) Together with muscular fitness, CVF enhancements are recommended in pediatric cancer patients/survivors in order to compensate for the chemotherapy-induced neuropathy and muscle atrophies, to attenuate fatigue and improve their quality of life.[67-72]

4) Although the literature on the association between physical fitness and mental health in young people is still scarce, the available information suggests that improvements in CVF have short-term and long-term positive effects on depression, anxiety, mood status and self-esteem in young people, and high CVF also seems to be associated with better academic performance.[73, 74]

The relationships between physical fitness and health outcomes, indicating the main fitness components involved in these associations, are illustrated in Figure 1.

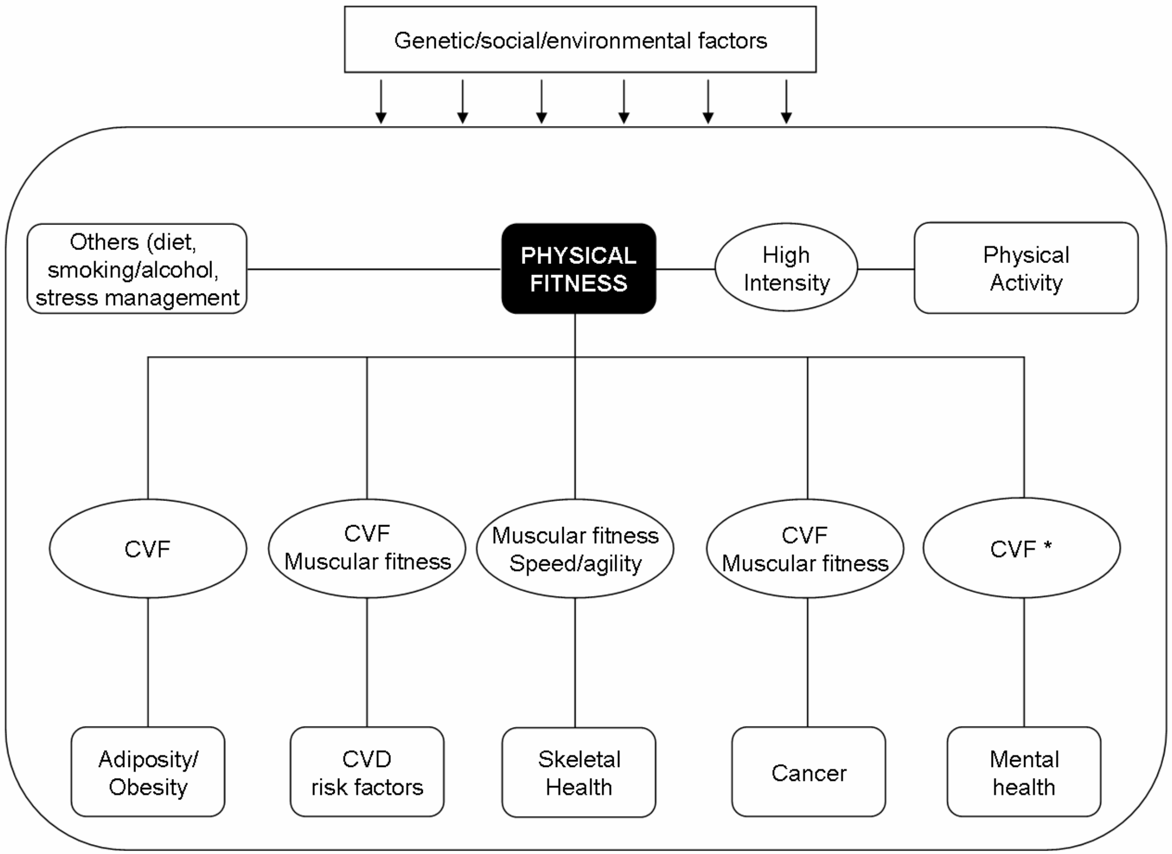

Figure 1. Associations between physical fitness and several health outcomes, showing the main physical fitness components involved in those associations (Figure taken from Ortega et al. [35]). CVF, cardiovascular fitness; CVD, cardiovascular disease. * No information has been found about the other fitness components.

1.3. Adiposity and overweight/obesity

Overweight and obesity are defined as an abnormal or excessive fat accumulation that presents a risk to health (World Health Organization, available at: http://www.who.int/topics/obesity/en/). Childhood obesity is nowadays recognized as a worldwide epidemic and is associated with a variety of adverse consequences both in childhood and later in life.[75] It is not only the total amount of fat that is associated with poor health status, but also the distribution of that fat in the body.[76] Central or abdominal body fatness is associated with CVD risk factors including dyslipidemia, insulin resistance and hypertension in adults, as well as coronary heart disease morbidity and mortality.[77-79] **A**bdominal fatness, instead of total fatness, is nowadays used as a key component in the definition of the metabolic syndrome, both in adults[80-82] and in young people.[83] Most disturbances related to obesity have shown their onset during childhood.[84] Therefore, attention should already be paid to obesity related factors in children.

1.3.1. Total adiposity

Data from the International Obesity Task Force (IOTF)[6] report that ten per cent of the world's school-aged children are estimated to be carrying excess body fat, and thus an increased risk for developing chronic disease in adulthood. Of these overweight children, a quarter are obese, and many of these are likely to have multiple risk factors for type 2 diabetes, heart disease and a variety of other co-morbidities before or during early adulthood. Pulmonary complications, such as restrictive lung disease and the hypoventilation syndrome, have also shown to be related with obesity.[85] The importance of overweight/obesity for health is such that overweight in adolescents is associated with mortality from all causes and disease-specific mortality among men, as well as with morbidity from coronary heart disease and atherosclerosis among men and women.[86] In fact, Must et al., reported that overweight in adolescence was a more powerful predictor of these risks than overweight in adulthood.[86]

The prevalence of overweight is dramatically higher in economically developed regions, but is rising significantly in most parts of the world.[6] Although the prevalence of obesity in Sweden is still low from an international perspective, i.e. is in the lowest tertile,[87] the increase among children and adolescents during recent decades is alarming.[88-92] Useful strategies must be designed and implemented to fight the rise of pediatric obesity. An increase in the available knowledge on adiposity and its relation to lifestyle factors such as PA will be crucial for the achievement of this goal.

1.3.2. Abdominal adiposity

As mentioned above, it is not only the amount of overall fatness, but also the distribution in the body that has important health implications.[76] The study of fat distribution among children and adolescents is complex because there are marked changes in circumferences and skinfold thicknesses during growth and development.[93, 94] The two main central fat depots are located in the abdominal and truncal area.

Waist circumference is a surrogate measure of abdominal adiposity.[95, 96] Within a given body mass index (BMI) category, children and adolescents with larger waist circumferences are more likely to have an unfavorable cardiovascular profile, such as high levels of triglycerides, glucose and blood pressure, as well as an increased risk of having the metabolic syndrome.[97] Factors related to abdominal adiposity should be identified for more efficient health promotion.

1.4. Rationale for this research

Given the well-known relevance of CVF level and obesity, especially abdominal obesity, to general health status, CVF enhancement and abdominal adiposity management at early stages of life will be major goals for health promotion. Physical activity may play a crucial role in the achievement of these goals. For public health recommendation purposes, a better understanding of the associations of PA with CVF and abdominal adiposity is required. The apparently obvious association of PA with CVF and adiposity still requires further research, mainly due to the complexity of assessing PA. Self-report methods have been used most frequently in previous research. However, their use in young people has shown important limitations. In this thesis, the associations of objectively measured PA with CVF and abdominal adiposity were examined in a sample of Swedish children and adolescents.

2. AIMS

The overall purpose of this thesis was to increase the understanding of the associations among PA, CVF and abdominal adiposity. The outcome of the work is presented in four papers (I to IV, Figure 2), each based on a specific aim:

I. To examine the influence of sexual maturation status and body composition, as well as the way in which VO_{2max} is expressed (ml/min/kg, ml/min/kg^{FFM} or l/min), when comparing CVF levels in adolescent populations; and to describe the associations between CVF and sexual maturation status in adolescence.
II. To examine whether meeting the current PA recommendations, i.e. 60 minutes or more of moderate to vigorous PA (MVPA), is associated with a healthier CVF level in adolescents, independently of their adiposity status.
III. To study the independent associations of PA and other factors predisposing to overweight, with total and central adiposity in children and adolescents.
IV. To examine the associations between different objectively measured PA variables and abdominal adiposity in children and adolescents, and to test whether CVF levels modify these associations. Whether the association of CVF with abdominal adiposity is independent of objectively measured PA was also studied.

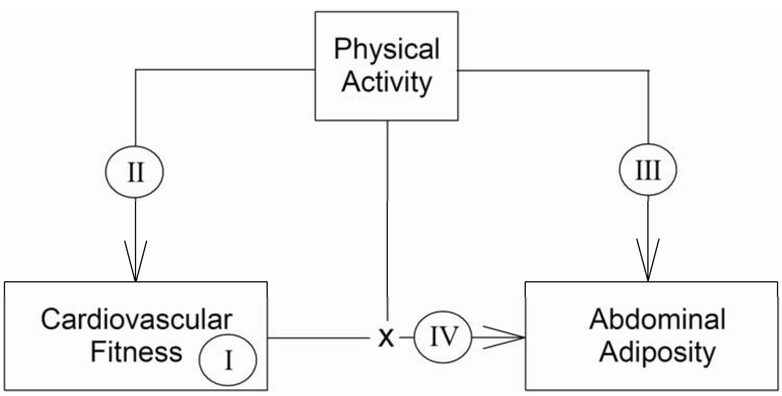

Figure 2. Inter-relationships among the papers/aims of this thesis
I. Study of factors that influence CVF assessment, which will be taken into account in the Papers II, III and IV.
II. Associations between PA and CVF.
III. Associations between PA and adiposity.
IV. Interactions between PA and CVF s in relation to abdominal adiposity.

3. MATERIAL AND METHODS

The work presented in this thesis is based on data from the Swedish part of the European Youth Heart Study (EYHS).[98, 99] The EYHS is an international cross-sectional study aiming to establish the nature, strength, and interactions between personal, environmental, and lifestyle influences on CVD risk factors in European children and adolescents. A minimum of 1000 boys and girls aged 9-10 y and 15-16 y were recruited from five European countries: Denmark, Estonia, Norway, Portugal and Sweden.

In addition, data from a Spanish cross-sectional study, the AVENA study (Alimentación y Valoración del Estado Nutricional de los Adolescentes / Feeding and assessment of nutritional status of Spanish adolescents),[100] were used in Paper I. The AVENA study was conducted in adolescents aged 13-18.5 y. Sampling was multi-staged and stratified by town of origin (Granada, Madrid, Murcia, Santander and Zaragoza), socioeconomic status, sex and age. The final sample size was 2859 (1357 boys and 1502 girls).

This section will thoroughly describe the material and methods used in the EYHS and a brief description for the AVENA study is provided when applicable.

3.1. Ethics

The EYHS (Papers I to IV)

The local ethical committees in Örebro and Stockholm approved the study protocols (Örebro City council case no. 690/98 and Huddinge University Hospital case no. 474/98), and the study was carried out in accordance with the 1975 Helsinki Declaration (as revised in Hong-Kong in 1989) and national guidelines (Medicinska forskningsrådet 1996).

The boards of all collaborating schools approved the use of school facilities during school hours. Conducting the study during school hours and at the school guaranteed that the participants were insured through the comprehensive school insurance system. The children and their families received written information about the purpose and the content of the study. Written consent was obtained from one of the parents or a legal guardian for the 9 y group, and from one of the parents or a legal guardian and from the adolescent in the 15 y group.

The AVENA study (Paper I)

The study protocol was performed according to the Spanish rules and following the ethical standards established in the 1975 Declaration of Helsinki (as revised in Hong-Kong in 1989 and in Edinburgh in 2000), and approved by the Review Committee for Research Involving Human Subjects of the Hospital Universitario Marqués de Valdecilla (Santander, Spain). A comprehensive verbal description of the nature and purpose of the study was given to the adolescents, their parents and their teachers. Written consent to participate was obtained from both parents and adolescents.

3.2. Sample and study design

The EYHS (Papers I to IV)

Data collection took place from September 1998 to May 1999 in 37 schools from eight municipalities (Botkyrka, Haninge, Huddinge, Nynäshamn, Salem, Södertälje, Tyresö, and Örebro) in Sweden. The study design, sampling procedures, selection criteria, participation rates and study protocol have been reported elsewhere.[101]

The total number of children and adolescents differ among the four papers, depending on the main variables involved in the different research questions. The maximum number of individuals with complete and valid data for the main outcomes studied in each paper (i.e. waist circumference, BMI, etc.), were included in the analyses. In paper I, a total of 472 adolescents aged 14 to 16 y, the age range common to both the EYHS and the AVENA study, had complete and valid data on CVF and were selected for the analysis. In paper II, the same number of adolescents was included in the analysis, since they had valid data for CVF and PA. As almost 100% of the children sample met the current PA recommendation (see results section in Paper III), the research question could not be answered using child data and so only the adolescent sample was included. In paper III, a total of 557 children and 517 adolescents, with valid data for waist circumference and BMI, were included in the study. In paper IV, 557 children and 518 adolescents had valid data for waist circumference and were included in the analysis.

The AVENA study (Paper I)

Paper I included a sub-sample of adolescents from the AVENA study. In order to be comparable with the Swedish adolescent sample, adolescents aged 14 to 16 y were selected for the analysis. Data collection for the AVENA study took place from 2000 to 2002. After excluding those individuals with missing CVF data, the final sample size for this study was 1867.

3.3. Measurements

3.3.1. Physical activity

The EYHS (Papers II, III and IV)

Total PA levels and time spent in PA of different intensity were objectively measured by accelerometry. The activity was measured over four consecutive days (at least two weekdays and at least one weekend day) with an activity monitor (MTI model WAM 7164, Manufacturing Technology Inc., Fort Walton Beach, FL, formerly known as Computer Science and Applications Inc.) worn at the right hip. At least three days of recording, with a minimum of 10 hours registration per day was set as a criterion for inclusion. Recorded periods of more than 10 minutes of consecutive zero counts were not considered in the analysis.

Accelerometers provide a chronological measure of frequency, intensity and duration of movement, allowing data to be analyzed over user-defined intervals (epochs). The epoch was set at 1 minute, the most commonly used duration in field-based studies at the time of data collection,[102, 103] allowing data to be collected for an adequate length of time.

Total PA was expressed as the total counts recorded, divided by the 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 (min/day) as follows:

1) Physical activity energy expenditure, as expressed in METs (metabolic equivalents) was estimated using the age-specific equations proposed by Freedson et al:[104-106]

$$METs = 2.757 + (0.0015 \cdot counts/min) - (0.08957 \cdot age\ (y)) - (0.000038 \cdot counts/min \cdot age\ (y))$$

2) Time spent in moderate PA and vigorous PA was based upon cut-off limits published elsewhere,[106, 107] as follows: time spent in activities from 3 to 6 METs was considered as moderate PA (min/day) and time spent in activities of more than 6 METs was considered as vigorous PA (min/day). The time spent in activities of at least 3 METs was also calculated and considered as MVPA (i.e. the sum of time spent in moderate and vigorous activities; min/day).

Finally, the proportion of participants who met the current PA recommendations of 60 minutes or more of MVPA daily[108] was also calculated and used in the analyses in Paper II and Paper III.

The AVENA study

No data on PA was included in the paper involving AVENA data (Paper I).

3.3.2. Cardiovascular fitness

The EYHS (Paper I, II and IV)

Cardiovascular fitness was determined by a maximal cycle-ergometer test.[109] The work rate was pre-programmed on a computerized electronically braked cycle ergometer (Monark 829E Ergomedic, Vansbro, Sweden), to increase every third minute until exhaustion, as follows:

1) In children (boys and girls) with a body weight of 30 kg or more, the initial work rate was set at 25 Watts increasing by 25 Watts every third minute, whereas in those children with a body weight lower than 30 kg, the initial work rate was set at 20 Watts increasing by 20 Watts every third minute.

2) In adolescent boys, the initial work rate was set at 50 Watts, increasing by 50 Watts every third minute, while in adolescent girls the initial work rate was set at 40 Watts, increasing by 40 Watts every third minute.

All the individuals could choose the pedal frequency that was most comfortable for them (usually between 60-80 revolution/min), but a pedal frequency lower than 30 revolution/min was not allowed. Heart rate was registered continuously by telemetry (Polar Sport Tester, Kempele, Finland). The criteria for exhaustion were a heart rate ≥185 beats/min and a subjective judgment by the test leader that the individual could no longer keep up, even after vocal encouragement. The mean maximal heart rate achieved by the participants was 94% of their age-based predicted maximal heart rate. The power output (Watts=W) was calculated as = $W_1 + (W_2 \cdot t/180)$, 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 time in seconds at final incomplete stage. The "Hansen formula" for calculating VO_{2max} in ml/min was = 12 · calculated power output + 5 · body weight in kg.[109] The test and equations used in this thesis have been previously validated in young people.[99, 109]

In Papers I and IV, CVF normalized by FFM (ml/min/kg^{FFM}) was used in the main analyses. The reasons for this decision are given later in the discussion section. In paper

II, CVF normalized by body weight (ml/min/kg) was used since the reference values from the Cooper Institute are expressed in that way. [110, 111] According to the criterion referenced standards for CVF proposed by the Cooper Institute, the threshold for the "Healthy Fitness Zone" for adolescent boys corresponds to a VO_{2max} of 42 ml/min/kg, and to a VO_{2max} of 35 ml/min/kg for adolescent girls.[110, 111] In this paper, the adolescents were classed as having a high-CVF level (those who met the minimum criteria for the "Healthy Fitness Zone") or low-CVF level (those who did not).

The AVENA study (Paper I)

Cardiovascular fitness in the AVENA study was assessed by the 20 m shuttle run test.[112] Running pace was determined by audio signals emitted from a pre-recorded cassette tape; the initial velocity was 8.5 km/h, which was increased by 0.5 km/h every minute (i.e., every stage). Subjects were instructed to run in a straight line, to pivot upon completing a shuttle, and to pace themselves in accordance with the audio signals. The test was finished when the subject failed to reach the end lines concurrent with the audio signals on two consecutive occasions. Scores were recorded as the number of stages completed. The equations of Leger et al.[113] were used to estimate the VO_{2max}. To calculate the VO_{2max} from the result of the 20 m shuttle run test score it is sufficient to introduce the age (A) and the final speed (S=8+0.5 · last stage completed) into the following formula (r=0.7; for children-adolescents aged 8-19 y):[113]

$$VO_{2max}\ (ml/min/kg) = 31.025+3.238 \cdot S-3.248 \cdot A+0.1536 \cdot S \cdot A.$$

The reliability and validity of this test for estimating the VO_{2max} in children and adolescents has been widely documented.[113-115]

3.3.3. Physical examination

The EYHS (Paper I to IV)

The protocol description and explanation for physical examination in EYHS has been previously published.[98, 99] In short, the height and weight of the children were measured by standardized procedures. Body mass index was calculated as weight divided by height squared (kg/m^2). The age- and sex-specific BMI cut-off values proposed by the IOTF[116] were used to categorize the subjects as non-overweight, overweight and obese. For most of the analyses, this variable was dichotomized as non-overweight and overweight, which includes obesity.

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 of two measurements was used for further calculations. If the two measurements differed by more than one centimeter, a third measurement was taken, and the two closest measurements were averaged. In Paper III and Paper IV, the age- and sex-specific waist circumference cut-off values for predicting risk factor clustering proposed by the Bogalusa Heart Study were used to class the individuals as having a high or low metabolic risk (referred to as high/low-risk waist circumference in the results section).[117]

Skinfold thicknesses were measured with a Harpenden caliper (Baty International, Burguess Hill, U.K.) on the left side of the body according to the procedure described by Lohman et al.[118] 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 mean of the two closest measurements was used. Percentage body fat (%BF) was calculated from skinfold thicknesses (triceps and subscapular) using Slaughter's equations.[119] Based on existing data,[120] being above the sex- and age-specific 85th centile of %BF was used in paper III and IV as a cut-off value for defining individuals with high levels of total adiposity (hereafter called high total fatness). Fat-free mass was derived by subtracting fat mass from total body weight.

Pubertal stage was assessed by a trained researcher of the same gender as the child, after brief observation, according to Tanner and Whitehouse.[121] Breast development in girls and genital development in boys was used for classification.

The AVENA study (Paper I)

The protocol for physical examination carried out in the AVENA study, for the variables involved in Paper I, was identical to those in the EYHS; therefore the description of the procedure showed above is applicable to the AVENA study, except for maturation. The assessment of maturation in young people is discussed in the methodological considerations section (5.2.3). The harmonization and standardization of anthropometric measurements, as well as a description of body composition assessment in the AVENA study has been published elsewhere.[100, 122]

3.3.4. Other factors assessed

The EYHS (Papers III and IV)

Television (TV) viewing: The time spent viewing TV was self-reported by the subjects, who were asked “How many hours of TV do you usually watch?” The answer was classified as watching either $\leq$2 h/day or >2 h/day.[123]

Neonatal data: Parents reported their child’s birth weight and subjects were categorized as having birth weights of <2500g, between 2500 and 4000g or >4000g.[124]

Socioeconomic status: The socioeconomic status of the child was defined by the mother’s educational level being below university level or university level.[125, 126]

Parental overweight: Parents self-reported their height and weight, and BMI was calculated. In Paper III, parental overweight status according to international cut-offs for adults was determined ($\geq$25 kg/m^2 = overweight, and $\geq$30 kg/m^2 = obese). The validity of BMI based on self-reported weight and height in adults has been documented elsewhere.[127]

The AVENA study (Paper I)

No data on the above variables were included in the paper involving AVENA data.

3.4. Statistical analysis: overall approach

Descriptive characteristics of the subjects are presented as means and standard deviations, unless otherwise stated. The differences between sex and age groups on continuous variables were assessed by two-way analysis of variance (two-way *ANOVA*, with sex and age as fixed factors). After square root transformation of the vigorous PA and total PA variables, all the residuals showed a satisfactory pattern. Nominal variables were analyzed by *Chi-squared tests*.

Analysis of the covariance (*ANCOVA*) was used (Paper I) to examine differences in CVF between Swedish and Spanish adolescents and differences in CVF among groups differing by sexual maturation status, after adjustment for relevant confounders.

Associations between continuous variables were analyzed by *linear regression* models (Paper IV). In those cases in which the main outcomes were dichotomous variables (e.g. overweight/non-overweight), *binary logistic regression* analysis was performed (Paper II and Paper III).

Interaction factors (i.e. sex × main exposure and age × main exposure) were considered to check whether age and sex modified the associations between exposures and outcomes. In those cases in which significant interactions were found, the analyses were performed separately by sex and/or age groups, otherwise all subjects were analyzed together.

All analyses were performed using SPSS and the significance level was set at 5 %.

4. RESULTS

4.1. Factors that influence cardiovascular fitness assessment (Paper I)

Paper I examined the influence of sexual maturation status and body composition, as well as the way in which VO_{2max} is expressed (ml/min/kg, ml/min/kg^{FFM} or l/min), when comparing CVF levels between adolescent populations. In order to answer this scientific question, a sample of Spanish adolescents from the AVENA study and a sample of Swedish adolescents from EYHS were compared with regard to their CVF levels. The results showed that:

1) Spanish adolescents, both boys and girls, had a higher CVF than their Swedish counterparts, when the VO_{2max} was expressed in relation to weight (ml/min/kg) or FFM (ml/min/kg^{FFM}), but not when expressed as the absolute value (l/min) (Figure 3).

2) Using CVF as VO_{2max} expressed in relation to FFM (ml/min/kg^{FFM}), higher CVF levels were observed in the Spanish adolescents. A smaller, but still significant, difference between countries was found after adjusting for sexual maturation status. After adjustment for both sexual maturation status and %BF, the difference disappeared in boys, while it remained significant in girls (Figure 4).

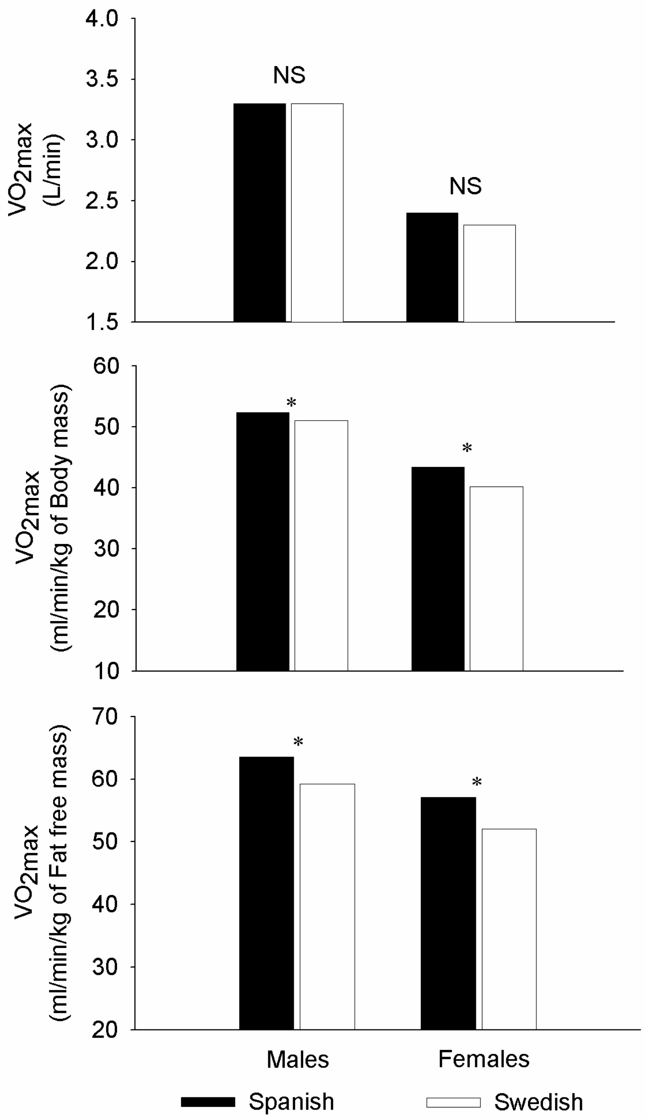

Figure 3. The choice of expression of maximal oxygen consumption (VO_{2max}) may affect the results and interpretation when comparing groups of adolescents (Figure taken from Ortega et al., 2007 [128]).
* Significant differences (all $P<0.05$). NS, non-significant.

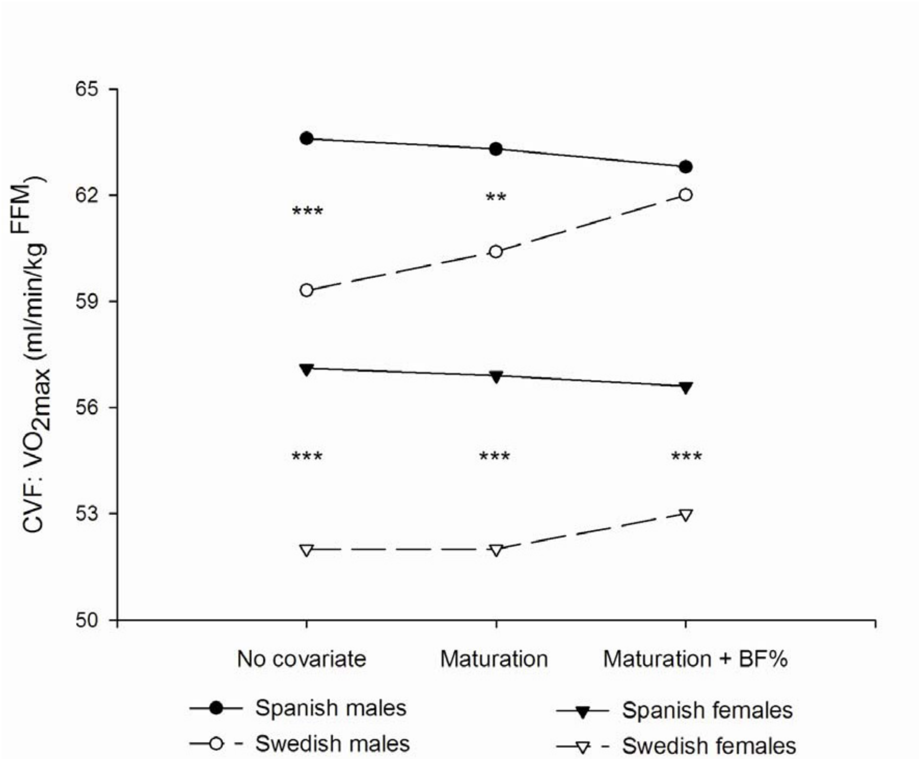

Figure 4. Cardiovascular fitness (CVF), expressed as maximal oxygen consumption normalized by FFM (VO_{2max}, ml/min/kg^{FFM}), in Spanish and Swedish adolescents, in three successive models: unadjusted model (no covariate), adjustment for sexual maturation status (maturation), and adjustment for both sexual maturation status and percentage body fat (maturation + %BF).
** P≤0.01 and *** P≤0.001 Spanish *vs.* Swedish adolescents. Otherwise, non-significant.

Paper I also studied the associations between CVF and sexual maturation status in adolescence. The results revealed that CVF was negatively associated with sexual maturation status in boys (P≤0.001), but not in girls. After adjustment for %BF, CVF was negatively associated to sexual maturation status only in girls (Figure 5 Paper I). No interaction between the studies (countries) and the relationship of CVF with sexual maturation status was found.

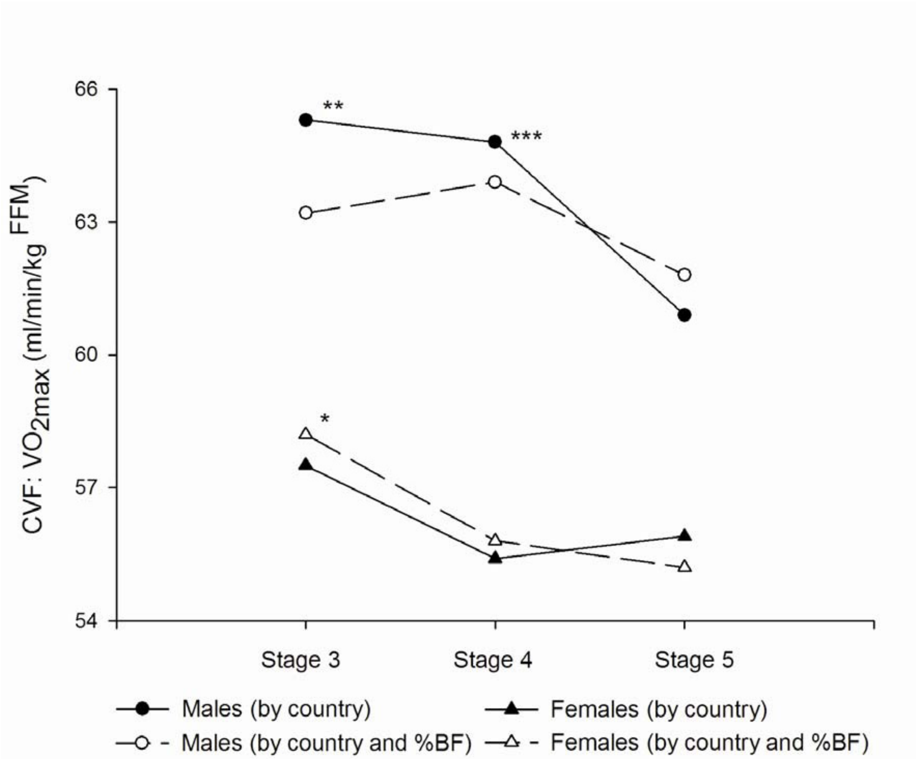

Figure 5. Mean cardiovascular fitness (CVF), expressed as maximal oxygen consumption normalized by FFM (VO_{2max}, ml/min/kg^{FFM}) according to sexual maturation status (Tanner stages 3, 4 and 5), in two successive models: adjusting for country, and adjusting for both country and percentage body fat (%BF).

Pairwise comparisons: * P≤0.05 stage 3 *vs.* 5; ** P≤0.01 stage 3 *vs.* 5; *** P≤0.001 stage 4 *vs.* 5; Otherwise non-significant.

4.2. Associations between physical activity and cardiovascular fitness (Paper II)

Paper II examined whether meeting the current PA recommendations (≥ 60 min/day of MVPA, hereafter "high-PA level") was associated with a healthier CVF level ("high-CVF level") in adolescents. Figure 6 shows the percentage of adolescents who had a low-CVF level by sex and PA level. The highest probability (0.274) of having a low-CVF level was observed in low-PA level girls, while the lowest one (0.032) was observed in high-PA boys.

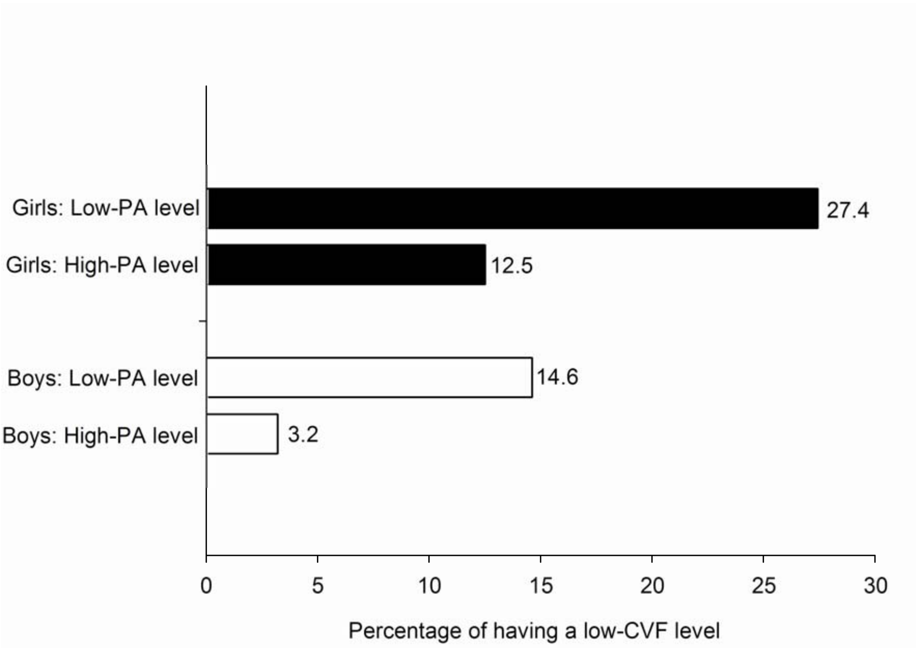

Figure 6. Percentage of individuals with a low cardiovascular fitness (CVF) level by sex and physical activity (PA) level.

Binary logistic regression showed that adolescent girls meeting the current PA recommendations had three-fold higher odds of having a high CVF level than girls that did not meet the recommendations, after controlling for sexual maturation status and body fat (Figure 7). Likewise, the odds of having a high CVF level was 8 times higher among adolescent boys who met the PA recommendations than boys who did not meet the recommendations (Figure 7).

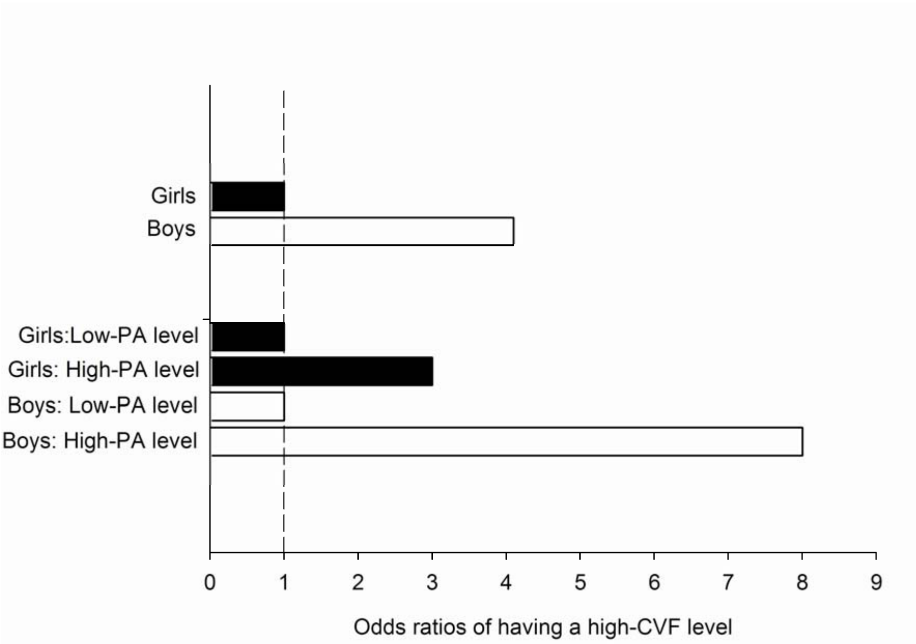

Figure 7. Odds of having a high cardiovascular fitness (CVF) level by sex and physical activity (PA) level in adolescents, after controlling for sexual maturation and adiposity. The dotted line is the reference line.

4.3. Associations between physical activity and adiposity (Paper III)

Paper III examined the associations of PA and other factors predisposing to overweight, with overweight and central adiposity in children and adolescents. It was shown that children and adolescents who had a low level (first tertile) of vigorous PA were more likely to be overweight (including obesity) and to have a high-risk waist circumference than those with a high level (third tertile) of vigorous PA (Figure 8). Similarly, those individuals who had a low or middle level (second tertile) of total PA were more likely to be overweight than those who had a high level of total PA (Figure 8). When high total fatness (>85th sex- and age-specific percentile for %BF) instead of overweight was entered into the models as a dependent variable, only vigorous PA was significantly associated with high total fatness.

Birth weight and TV viewing were also associated with higher odds of having a high-risk waist circumference (Figure 9), yet these associations were attenuated once either total or vigorous PA was included in the model. Those subjects who had two overweight parents were more likely to be overweight and to have a high-risk waist circumference, compared to those whose parents were not overweight (Figure 9), independently of PA variables.

In all the analyses, when sexual maturation status was entered into the model instead of age group, the results were not substantially affected.

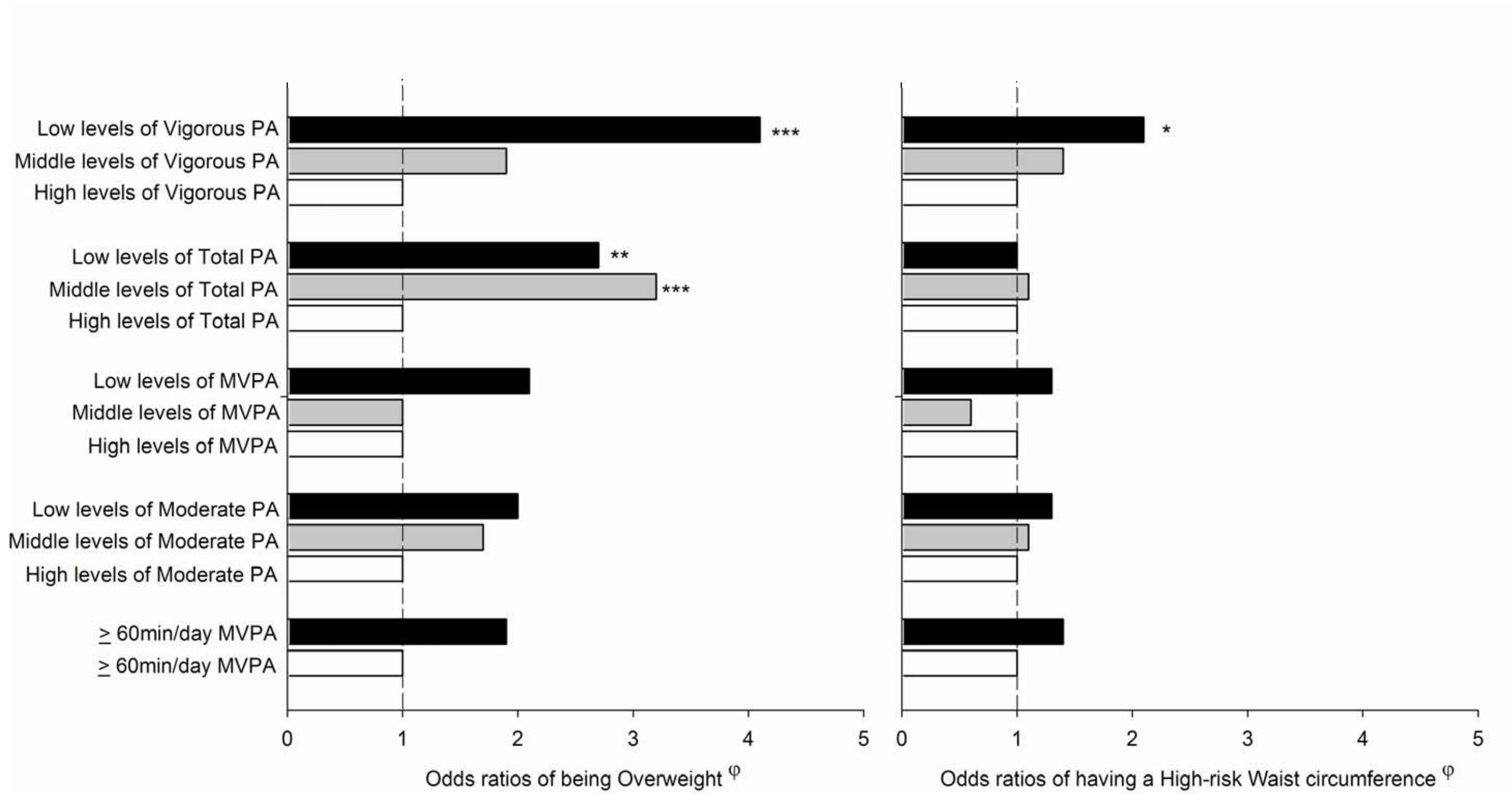

Figure 8. Overweight and high-risk waist circumference according to physical activity (PA) variables.
φ Logistic regression analysis was performed controlling for sex and age. The dotted line is the reference line. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. Low, middle and high PA categories represent the 1^{st}, 2^{nd} and 3^{rd} tertiles (age- and sex-specifically calculated), respectively. MVPA, moderate-vigorous PA. Since the number of children not meeting PA recommendations were very small (≤1%), this analysis was performed in the adolescent group.

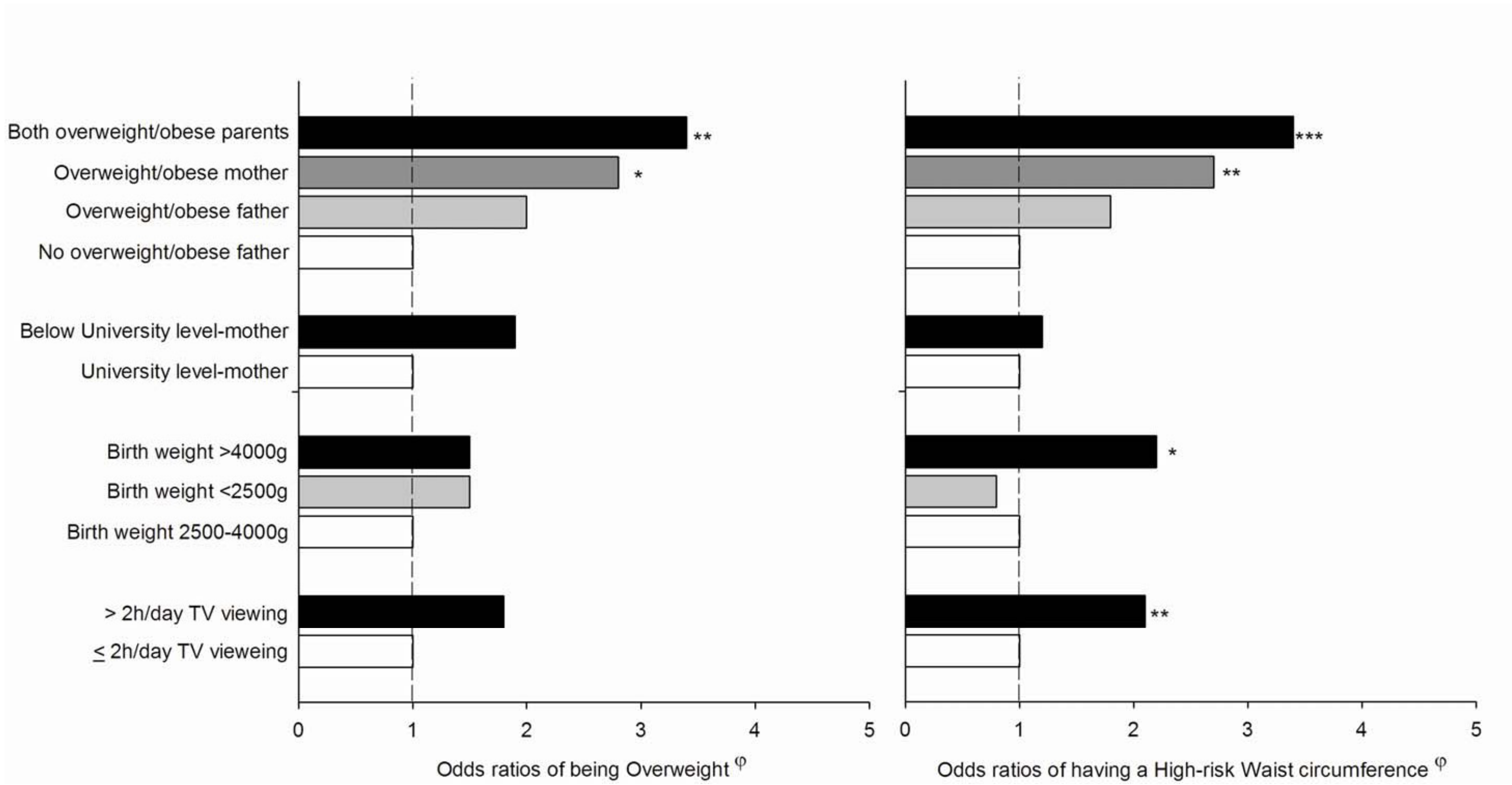

Figure 9. Overweight and high-risk waist circumference according to several factors potentially related to overweight.
φ Logistic regression analysis was performed controlling for sex and age. The dotted line is the reference line. * P≤0.05; ** P≤0.01; *** P≤0.001. TV, television.

4.4. Interactions between physical activity and cardiovascular fitness in relation to abdominal adiposity (Paper IV)

Paper IV examined the associations between PA and abdominal adiposity, as measured by waist circumference, in children and adolescents, and also whether the level of CVF modified these associations. Linear regression did not show any association between the PA variables and waist circumference, after controlling for sex, age and height. Since interactions between CVF and some PA variables (i.e. vigorous and total PA) were found, the analyses were repeated stratifying by CVF level (low/high CVF level, according to sex- and age-specific medians). In individuals with a low CVF level, vigorous PA was inversely associated with waist circumference, while total PA showed a borderline inverse association (Figure 10). Unexpectedly, in the group with a high CVF level, the PA variables were positively associated with waist circumference, including vigorous and total PA variables (Figure 10). In both groups, the results did not substantially change when the analyses were further controlled for maternal educational level, maternal BMI, birth weight or TV viewing.

Paper IV also studied the associations between CVF and waist circumference. The results showed that CVF was inversely associated with waist circumference. This association remained significant after controlling for all the PA variables studied and the above mentioned confounders.

The prevalence of being overweight (IOTF cut-off values), of having an excess of total fatness (>85th percentile of body fat) and of having a high-risk waist circumference (the Bogalusa study cut-off values) were significantly lower in the high CVF group than in the low CVF group.

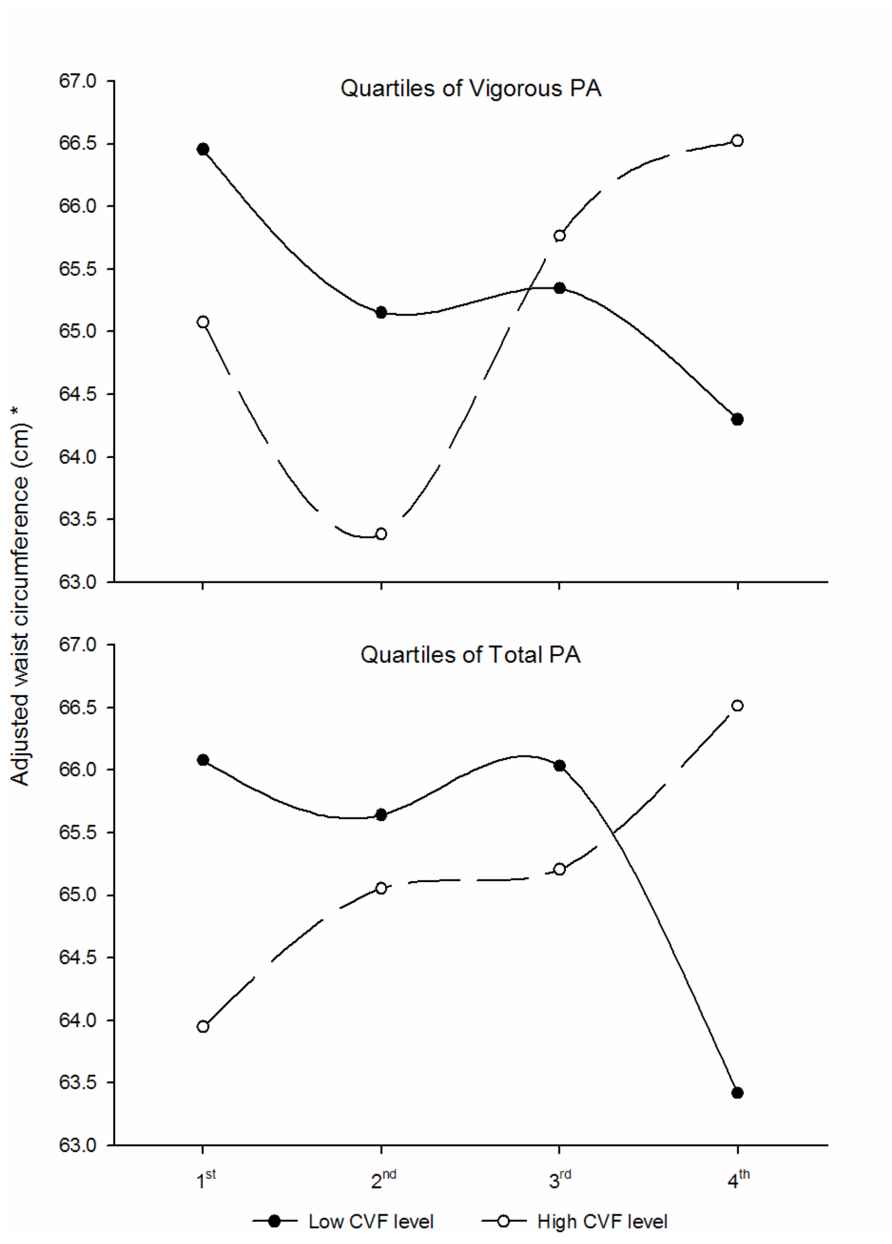

Figure 10. Associations of vigorous physical activity (PA) and total PA, with waist circumference in children and adolescents stratified by cardiovascular fitness (CVF) level. High/low CVF was defined according to sex- and age-specific medians, and the PA quartiles were sex- and age-specifically calculated. All the data were analyzed by linear regression in their continuous form, although data were stratified by quartiles of PA only for illustrative purposes.

* Waist circumference was adjusted for sex, age and height.

5. DISCUSSION

5.1. Discussion of main findings

5.1.1. Factors that influence cardiovascular fitness assessment (Paper I)

The results indicate that the way in which VO_{2max} is expressed (i.e. in absolute terms or in relative terms such as divided by weight or FFM) when comparing CVF of adolescent populations may substantially affect how the results are interpreted. The question of which is the best way of expressing VO_{2max} in adolescents needs to be discussed since it can have important implications.

Data from DXA indicate that FFM is a major determinant of VO_{2max}, even more important than other body composition factors such as weight or %BF in young people.[129] This is most likely due to the fact that a higher stroke volume and, thus, cardiac output is positively associated with FFM. Since cardiac output is the main determinant of the VO_{2max} ,[130] it is reasonable to suggest that association between FFM and VO_{2max} can be due to their common link with cardiac output. Dividing VO_{2max} in absolute terms by FFM seems to be an appropriate procedure when comparing CVF in groups who differ in body size.[129, 131-133] This consideration was taken into account in the present thesis and when possible, VO_{2max} normalized by FFM (ml/min/kg^{FFM}) was used in the analyses (Papers I and IV).

The results also suggest that apparent differences in CVF between adolescents populations (e.g. Spanish and Swedish, or when stratifying a study sample in tertiles) may be actually due to differences in %BF. Hence, body composition, particularly %BF, seems to be a relevant modifying factor, even when VO_{2max} is expressed relative to FFM. This finding is in agreement with previous observations in Portuguese children and adolescents.[134]

The data also showed an inverse association between CVF and sexual maturation status. In this case, the associations were also modified by %BF, supporting the idea that both sexual maturation status and %BF are relevant factors for the study of CVF in young people, and should therefore be accounted for in any analysis involving CVF.

5.1.2. Associations between physical activity and cardiovascular fitness (Paper II)

The data presented here suggest that those adolescents who spend 60 minutes or more in MVPA daily are more likely to have a healthier CVF level (according to the "Healthy fitness zone" proposed by the Cooper Institute[110, 111]), independently of their sexual maturation and adiposity status. Objectively measured PA data agree that PA is positively associated with CVF in young people.[36, 43, 135-137] The main contribution of this study to the previous literature is that it examines, using objective methods, the extent to which meeting the current PA recommendations is associated with a CVF level considered healthy, and not just whether higher levels of PA are linearly associated with higher levels of CVF.

Another interesting finding was that the association between meeting the PA recommendation and CVF level was stronger in boys than in girls. Since the analyses were controlled for sexual maturation status and total adiposity, the sex-differences shown do not seem to be explained by maturation or adiposity.

The available information from large scale epidemiological studies using objective methods for assessing PA,[36, 43, 135, 137] and findings from randomized controlled trials,[138-146] suggest that high intensity PA is positively associated with physical fitness. Also, physical exercise programs properly designed and controlled have proven to improve physical fitness in children and adolescents, independently of age, maturation development and sex.

It can be hypothesized that the sex differences observed in the strength of the association between meeting PA recommendations and having a healthier CVF level could be due to differences in the relative time spent in vigorous PA by boys and girls. It is well accepted that boys are more physically active than girls at all ages,[11] however whether or not the sex differences in the time spent in vigorous PA are greater than in the time spent in moderate PA, as measured by objective methods, has not previously been examined. In relative terms, adolescent boys spent on average 28% more time in vigorous PA, while only 12% more time in moderate PA (unpublished results). In other words, sex differences regarding time spent in PA are twice as high for vigorous PA than for moderate PA. Also, from the total time spent in MVPA, the proportion of time spent in vigorous PA were significantly higher in boys than in girls (19% *vs.* 15%, respectively, P=0.005) (unpublished results). Since vigorous PA is the key PA component associated with CVF, the fact that boys devote a higher proportion of their PA time to vigorous PA could explain the observation that the association between meeting PA recommendations (based on time spent in MVPA) and CVF level was stronger in boys than in girls. This findings need to be confirmed by future studies.

5.1.3. Associations between physical activity and adiposity (Paper III)

Individuals with a low level of vigorous PA seem more likely to be overweight, and more likely to have a high-risk waist circumference, compared to those with a high level of vigorous PA. This finding is in accordance with those data reported by Gutin et al. and Ruiz et al.[36, 135] They observed that a high level of vigorous PA, rather than moderate PA, was associated with a lower total adiposity in children and adolescents. In addition, our data suggest that being physically active, especially at high intensities, is associated with a lower "risk" of being overweight, independently of other important determinant factors such as TV viewing, birth weight or parental overweight status. By contrast, no significant associations were found between moderate PA, MVPA or meeting PA recommendations and adiposity.

Because the use of BMI for defining overweight/obesity in young people has been criticized, we additionally studied the associations discussed above using the high total fatness variable (derived from skinfold thicknesses) instead of the international BMI categories. Among the PA variables, only vigorous PA was associated with high total fatness. Data from a large UK project - the Avon Longitudinal Study of Parents and

Children - in whom PA was measured by accelerometry and fatness by DXA, also suggest that PA of high intensity may be more important than total PA in relation to adiposity.[147] Our findings, together with those reported by others, suggest that high intensity PA may play a key role in the prevention of total and central childhood obesity. However, more data from longitudinal and randomized control trials are needed to support these results.

The data from this study also suggest that sedentary behavior is associated with a twofold higher odds of having a high-risk waist circumference. Television viewing may increase the "risk" through both a reduction in energy expenditure or increased food intake.[148, 149] Although there are potential benefits of viewing TV, such as the promotion of positive aspects of social behavior (e.g., sharing, manners, cooperation), many negative health effects can result.[123] In addition, longitudinal studies investigating the role of TV viewing on the development of obesity in youths suggest that decreased sedentary behavior is protective against relative weight and fatness gains during childhood and adolescence.[150, 151] In the present study, when total PA or time spent in vigorous PA was taken into account, no association was found between TV viewing and high-risk waist circumference. This result suggests that the negative effect of spending more than 2 hours per day viewing TV on central fatness could be attenuated by appropriate levels of vigorous PA.

Finally, Paper III showed that parental overweight seems to be an important determinant for overweight and high-risk waist circumference in children and adolescents. The individuals who had two overweight/obese parents had a threefold higher odds of being overweight and having a high-risk waist circumference, compared to those whose parents were non-overweight. Similar findings have been previously reported in British children.[152] In addition, a longitudinal study reported that in either obese or non-obese children, parental obesity more than doubles the risk of a child being obese in adulthood, particularly when both parents were obese.[153] Obesity in one or both parents probably influences the "risk" of obesity in their offspring because of shared genes and/or environmental factors within families. Our data also showed that this association was independent of total PA, time spent in vigorous PA, TV viewing and birth weight. To the best of our knowledge, no study has previously examined the associations between parental overweight and waist circumference in children and adolescents.

5.1.4. Interactions between physical activity and cardiovascular fitness in relation to abdominal adiposity (Paper IV)

Young people with both low fitness and high fatness levels have an increased risk for metabolic disease[46, 56, 61, 154-156] and require special attention. In this study, the prevalence of being overweight, of having an excess of total fat and of having a high-risk waist circumference, was greater in the low CVF group than in the high CVF group. The group classed as low CVF had both low fitness levels and high fatness levels. In this specific group of individuals, high levels of vigorous PA were associated with a lower abdominal adiposity, independently of sex, age and height.

This association was not affected by other confounders, such as maternal educational level, birth weight or TV viewing. Maternal adiposity is a more important risk factor for high levels of abdominal fatness than paternal adiposity at childhood and adolescence.[157] Consequently, the analyses were additionally controlled for maternal BMI to test whether this factor could influence the results, but similar trends were observed. The fact that vigorous PA might benefit the abdominal adiposity status in those children and adolescents at a higher metabolic risk may have implications for the development and testing of lifestyle intervention models.

In accordance with our findings, high levels of vigorous PA, rather than light/moderate PA, have shown to be associated with a lower total adiposity, estimated from skinfold thicknesses in children and adolescents.[36, 43, 135] These results, together with those found in this thesis (Paper III and IV), suggest that high intensity PA could play a key role in the associations between PA and adiposity, both total and abdominal, in young people.

The interactions between CVF and PA in relation to abdominal adiposity found in this study suggest that CVF modifies the associations between PA and abdominal adiposity. This is the first study examining how CVF can influence the associations between objectively measured PA and abdominal adiposity. The high CVF level group showed a lower prevalence of being overweight/obese, having an excess of total fat and having a high-risk waist circumference. Therefore, our hypothesis was that in this group of people with higher fitness and lower fatness, a weak or even non-existent association between

PA variables and abdominal adiposity would be observed. However, the results paradoxically showed that, in these individuals with high CVF levels, all PA variables were positively associated with abdominal adiposity. We checked whether this result could be due to the influence of some potential confounders, such as maternal educational level, maternal BMI, birth weight and TV viewing, but the associations were not affected by any of these factors. Other studies using objective measurements of PA should confirm or disprove these findings.

From an energy balance point of view, this finding would be inexplicable if all the factors that actually influence abdominal adiposity had been accounted for (something almost impossible to achieve in practical terms). Other confounding factors, such as genetic variation and energy intake or dietary patterns, could explain these observations. Only randomization within a trial can deal with issues of unmeasured confounding.

Finally, Paper IV also examined the associations between CVF and abdominal adiposity. The findings indicate that CVF is inversely associated with abdominal adiposity, independently of sex, age, height and all the PA variables. We observed similar results in Spanish adolescents from the AVENA study, but in that case PA was self-reported.[42] The fact that CVF is associated with abdominal adiposity in children and adolescents independently of their objectively measured PA levels suggests that genetics component may play an important role in these associations.

Sexual maturation status can modify the results and interpretations of analyses concerning CVF in young people,[158] so the analyses were also controlled for maturation. Cardiovascular fitness was still significantly associated with abdominal adiposity. Likewise, confounders such as maternal educational level, maternal BMI, birth weight or TV viewing did not substantially affect this association.

The inverse association between physical fitness and abdominal adiposity in young people has been consistently reported. It seems to be independent of the abdominal adiposity measurement method used (e.g. high technology methods or anthropometry), the method of physical fitness testing (e.g. running or biking tests), and the physical fitness components used (e.g. CVF, muscular fitness or speed/agility).[38, 42-45, 60, 159, 160] This study contributes to the previous literature by reporting that these associations seem also to be independent of total PA and different intensity levels of PA, as measured by objective methods.

5.2. Methodological considerations

5.2.1. Study design

The present work, as with any cross-sectional study, only provides suggestive evidence concerning the causal relationship between the exposures (e.g. PA) and the outcome variables (e.g. CVF and abdominal adiposity). The direction of the relationship can be suggested but never stated with certainty. Actually, it is likely that the relationships between PA and CVF or adiposity are reciprocal.

5.2.2. Physical activity measurement

Weaknesses and strengths of accelerometry

Some limitations must be recognized in any study involving accelerometry. The accelerometers do not compensate for the relative increase in energy expenditure by increase in body size.[161] Non-weight-bearing activities such as cycling are not properly measured by the accelerometers. Likewise, swimming is not usually assessed by accelerometry due to practical problems (i.e. not fully water-proof instruments) and the lack of detectable vertical movements while swimming.

Another critical issue in studies involving accelerometers is the choice of cut-off points for defining different activity intensities. There is no consensus on which cut-off points to use.[162] Likewise, several equations can be used for estimating PA energy expenditure. Among the equations most commonly used are those proposed by Trost et al.,[163] those proposed by Puyau et al.[164] and those proposed by Freedson et al.[104-106] In this thesis, Freedson's equations were used to estimate the PA energy expenditure (expressed in METs), and then time spent in moderate PA (3-6 METs) and in vigorous PA (>6 METs) were calculated.[106, 107] These equations have shown to be useful and valid for estimating participation in moderate and vigorous PA in children and adolescents, particularly time spent in vigorous PA.[165] In addition, a recently published review concluded that of those commercially available accelerometers, the CSA/MTI/Actigraph, the accelerometer used in this thesis, has proven the best correlation with doubly labeled water derived energy expenditure.[166]

Because of the limitation of self-report methods in young children and the high cost of techniques such as direct observation or doubly labeled water, accelerometry is nowadays considered the method of choice for objectively measuring PA in free-living children and adolescents.[10] The fact that more than one thousand individuals were assessed by means of accelerometry in relation to CVF and abdominal adiposity, taking into account a set of relevant confounders, including TV viewing, birth weight, maternal educational level and parental overweight is a notable strength of this study.

Considerations about total and vigorous physical activity variables

Total PA, also called average PA, is not only a measurement of how long the subjects are active, but also how intense that activity is. The unit of measure used in accelerometry is a "count". The counts are quantifiable digital signals resulting from the analogical acceleration records. The higher the acceleration, the more counts are recorded by the accelerometer. Total PA is usually expressed as counts/min, which means that if two people are moving for the same period of time, the one who is moving more vigorously will show a higher total PA. Therefore, although total PA and time spent in vigorous PA are two different variables, estimated and expressed in a different way, both have one component in common: the intensity. This can explain why vigorous PA and total PA have shown similar patterns of associations with different outcomes throughout this thesis. For instance, in paper III, high levels of both total and vigorous PA were associated with a lower risk of being overweight and having a high-risk waist circumference. Likewise, in paper IV, total PA and time spent in vigorous PA were associated with waist circumference in a similar way, even when controlling or stratifying for CVF.

5.2.3. Cardiovascular fitness

Running vs. cycling tests

The VO_{2max} attained during a graded maximal exercise to voluntary exhaustion has long been considered by the World Health Organization as the single best indicator of CVF.[167] The VO_{2max} can be estimated using maximal or sub-maximal tests, and by direct or indirect methods. The most commonly used tests are walking/running and cycling tests. In the AVENA study and in EYHS, a 20 m shuttle run test[113] and a maximal ergometer bike test,[109] respectively, were used to assess CVF.

It has been reported that running tests (particularly treadmill tests) give a higher VO_{2max} value than bike tests.[168, 169] Care must be taken when comparing CVF level in populations assessed by different protocol tests. Several reasons have been cited in the literature which may explain differences in the final VO_{2max} output between running *vs.* cycling tests when assessing CVF in young people:[170, 171] 1) On the cycling tests, the limiting influence of undeveloped knee extensor muscle mass induces local muscle fatigue, with subsequent early end of the test. 2) Youths may have difficulty maintaining the proper pedal rate. 3) Running is defined as a weight-bearing activity, what means it requires the person's body weight to be transported when it is being performed. Heavier people are more likely to perform worse than lighter people in running tests, but not necessarily in cycling tests.

A threshold for defining healthy cardiovascular fitness levels in young people

Given the importance of CVF as a powerful marker of health in childhood and adolescence, sex-specific cut-offs for a "Healthy Fitness Zone" in childhood and adolescence has been proposed by scientists and worldwide recognized organizations.[54, 110, 111, 172] The cut-off values proposed by the Cooper Institute were used in this thesis.[110, 111] These cut-off points were extrapolated from the thresholds established by Blair et al.,[31] for adult populations. The threshold for boys corresponds to a VO_{2max} of 42 ml/min/kg, for girls 14 y or older to 35 ml/min/kg and for younger than 14 y to 38 ml/min/kg.[110, 111]

Of note is that the health-related CVF thresholds suggested by the Cooper Institute are similar to those proposed by the European Group of Pediatric Work Physiology (40 and 35 ml/min/kg for boys and girls, respectively)[172] and also to those associated with an increased risk for metabolic disease, calculated by Ruiz et al. (42 and 37 ml/min/kg in boys and girls, respectively).[54]

The approaches used to calculate the CVF thresholds were different in the studies mentioned, as were the measured outcomes of age and cultural and social factors of the study subjects. However, the similarities among the results suggest the existence of a hypothetical health criterion value for CVF in young people, which seems to range between 40 and 42 ml/min/kg in boys and between 35 and 38 ml/min/kg in girls.

5.2.4. Adiposity measures

Body mass index

Body mass index is probably the anthropometric index most commonly used in the literature. Although it is has been criticized, BMI remains as a useful tool for obesity screening. In fact, pediatric overweight is internationally defined by sex- and age-specific cut-offs for BMI.[116].

The main drawback of BMI is that it does not distinguish between fat and lean mass, and the fact that increases in BMI during childhood are generally attributed to the lean rather than to the fat component of BMI.[173] Therefore, the use of BMI as the only measurement for body composition assessment does not seem a good choice, especially in young people, at an individual level. At a population level, BMI cut-offs are a good criterion for the screening of excess body fat in adolescents due to its high sensitivity and specificity (ranging from ~70% to ~90%). However, a significant percentage of subjects classified as overweight or obese do not really have excess adiposity (~30% of girls and ~40% of boys).[174-177] Moreno et al. have tried to improve the IOTF cut-off values, in terms of prediction of %BF assessed by DXA in adolescents.[120] They concluded that optimization of the IOTF BMI cut-off values, in terms of %BF, does not seem to be possible in adolescents. The IOTF criteria should be used for overweight and obesity screening; however, in clinical settings, a more accurate measure of body fat is recommended.

For the reasons discussed above, BMI was used as the main outcome for assessing overweight in this thesis. International standards for %BF in young people, estimated from skinfold thickness, have not yet been established. Nevertheless, whether or not the results differed when %BF (>85th percentile of body fat) is used instead of BMI, was also examined in this research.

Percentage body fat

Many reference methods are able to estimate body composition accurately at the individual level. Multicompartment models, underwater weighing, air displacement plethysmography, labeled water techniques and DXA are the most reliable methods to obtain accurate measures of total body fat.[178, 179] At present, reference methods are not always feasible for field and clinical use. Therefore, anthropometry is the most widely used method when the population to be measured is large, when economic resources are limited or when a quick measure is required.[180]

Body fat estimation from skinfold thicknesses is a well-established method. Skinfold thickness is accepted as a predictor of body fatness because subcutaneous fat (40-60% of total body fat) can be directly measured with a caliper. The selection of the appropriate equation to predict %BF from the initial measurements increases its accuracy.[122, 181] Rodríguez et al. studied the degree of agreement between the most commonly used equations for prediction of body fatness from skinfold thicknesses in young people, with %BF measured by DXA as the reference method.[182] The authors concluded that Slaughter's equations[119] resulted in the most accurate estimation of body fat from skinfold thickness in boys and girls. Consequently, these equations were used to calculate the %BF in this thesis.

Waist circumference

Waist circumference has shown to be an accurate marker of abdominal fat accumulation[95] and visceral adiposity[96] in young people. In addition, waist circumference seems to explain the variance in a range of CVD risk factors to a similar extent as measures derived from high-technology techniques, including DXA and Magnetic Resonance Imaging.[76] Therefore, the use of waist circumference as a surrogate of abdominal adiposity, and as a powerful index associated with metabolic risk in young people, seems to be appropriate for epidemiological studies.

In the absence of a recognized definition of high central adiposity in young people, the terms “overweight” and “obesity” when referring to central adiposity are currently being arbitrarily defined. Data from different cohorts and countries need to be reported in order to establish international criteria for determining central obesity based on these simple and valuable anthropometric measurements. In this regard, sex- and age-specific percentile values for waist circumference and other indices of central adiposity (i.e. waist-to-height ratio) have been provided for the children and adolescents involved in this thesis (Annex I).

In Papers III and IV, the age- and sex-specific waist circumference cut-off values for predicting risk factor clustering proposed by the Bogalusa Heart Study were used to classify the individuals as having a high or low metabolic risk. [117] Several percentile-based reference values for central obesity have been published elsewhere,[183, 184] however the sex- and age-specific cut-off values chosen for this study provide meaningful information about a waist circumference size associated with higher metabolic risk, and not just a high level of abdominal fatness.

5.2.5. Maturation assessment in young people

In this thesis, sexual maturation status was assessed by brief observation of the subject by a trained researcher in the EYHS and based on self-reports in the AVENA study, both according to Tanner and Whitehouse.[121] Although some differences may exist and the results from both methods are not directly comparable, a good concordance has been demonstrated between these methods.[185, 186] Biological maturation is most accurately assessed by measuring skeletal age; however, in large scale research this is not always possible.

6. PUBLIC HEALTH IMPLICATIONS

Paper II showed that nearly all the children involved in the Swedish part of the EYHS met the current PA recommendations (≥60 min of daily MVPA). Although this finding is in accordance with previously reported data from other European countries,[187] the question of whether the studied children are actually active enough or if the PA recommendations are appropriate for this population remain unanswered. Andersen et al. reported that 90 minutes or more of daily MVPA is associated with a lower likelihood of CVD risk factor clustering, including excess of fatness in children and adolescents.[188] Results from Paper III support the hypothesis that 60 minutes or more of daily PA could be sufficient, if enough vigorous PA is accumulated during such a period. Based on these findings, the following question is raised: How much vigorous PA is required to achieve a healthier adiposity status?

According to the results presented in this thesis, the levels of vigorous PA associated with a lower risk of being overweight and having a high-risk waist circumference were met at the third tertile. The sex- and age-specific values for the third tertile were: ≥40 min/day and ≥25 min/day for children (boys and girls, respectively), and ≥20 min/day and ≥15 min/day for adolescent boys and girls, respectively. Interestingly, further analyses using data from the Swedish and Estonian EYHS children aged 9-10 y showed a significant difference in total body fat, as measured by skinfold thicknesses, between those who accumulated more than 40 min/day of vigorous PA and those who accumulated 10-18 min/day.[36] Of note is that although different adiposity indices and statistical approaches were used in that study, the figures reported are identical to those found in the current study in boys aged 9-10 y. More than 40 min/day of vigorous PA seems to be associated with both a lower total and a lower abdominal adiposity.

The data also showed that the negative effect on central fatness of spending more than 2 hours per day viewing TV could be counteracted by having high levels of vigorous PA. This suggests that future public health interventions focused on increasing the time spent in vigorous PA may have a positive effect on central adiposity, even in those children and adolescents with high levels of sedentary behavior. Future randomized controlled trials should investigate these findings.

Results from Paper IV also highlight the importance of vigorous PA in those individuals with a low CVF. The fact that the low CVF group had a higher proportion of subjects with overweight, excess of total fatness and high-risk waist circumference, than the high CVF group, suggests that PA, in particular, vigorous PA, may be of greater value in this vulnerable group of individuals, rather than in high fit/low fat people.

It should be noted that an expert panel of scientists, on behalf of the American College of Sports Medicine and the American Heart Association, considered, for first time, the relevance of vigorous PA for health in their update (2007) of the PA recommendations for adults.[189] They stated that "to promote and maintain health, all healthy adults aged 18 to 65 y need moderate-intensity aerobic (endurance) PA for a minimum of 30 minutes on five days each week or vigorous-intensity aerobic PA for a minimum of minutes on three days each week".

Results from Paper II suggest that achieving 60 minutes or more of MVPA daily is appropriate in order to achieve a healthy CVF level. Nevertheless, data from epidemiological studies using objective methods for assessing PA, and findings from randomized controlled trials, support the idea that high intensity PA is associated with physical fitness,[36, 43, 135, 137, 145] and also that physical exercise programs, properly designed and controlled, improve physical fitness in children and adolescents, independently of age, maturation and sex.[36, 43, 135, 137, 145] High intensity PA seems to definitely be a key element for CVF enhancement.

New PA recommendations adapted to young people, most probably differing for children and adolescents, and taking into account the evidence-based importance of vigorous PA, are needed. The figures shown above (vigorous PA cut-offs associated with lower abdominal adiposity) will help to develop future randomized-controlled trials and useful intervention strategies based on lifestyle changes in children and adolescents.

7. CONCLUDING REMARKS

- When examining CVF in adolescents, sexual maturation status and %BF, as well as the units chosen to express CVF, should be taken into account.

- Adolescents who spend 60 minutes or more in MVPA daily are more likely to have a healthier CVF level, independently of their sexual maturation and adiposity status, according to the “Healthy fitness zone” proposed by the Cooper Institute. The stronger association seen in boys compared to girls seems to be due to the fact that adolescent boys spend a greater proportion of their PA time in vigorous PA compared to adolescent girls.

- Low levels of total PA and, in particular, of vigorous PA, may play an important role in the development of overweight and an excess of central adiposity in children and adolescents, independently of important determinant factors, such as TV viewing and birth weight. In addition, low levels of vigorous PA predicted the “risk” of being overweight independently of parental overweight, which is one of the stronger predictors for overweight and high-risk waist circumference in children and adolescents.

- Spending more than 2h/day viewing TV seems to be related to having a high-risk waist circumference. However, the data indicate that its effect on central fat deposition could be attenuated if enough vigorous PA is accumulated. Sex- and age-specific cut-off values for levels of vigorous PA associated with a lower total and central adiposity have been provided.

- Meeting the current PA recommendations, i.e. 60 min/day of MVPA, is associated with a healthier CVF level but not a lower risk of having high levels of total and central adiposity. However, additional analyses performed in those papers support the hypothesis that 60 minutes or more of daily PA could be enough to achieve a healthier fitness and adiposity status, if enough vigorous PA is accumulated during that period.

- Cardiovascular fitness is inversely associated with abdominal adiposity and seems to modify the associations between PA and abdominal adiposity. In children and adolescents with low fitness, time spent in vigorous PA seems to be the key component linked to abdominal adiposity. This finding should be considered in the further development of lifestyle intervention strategies. The results found in the high fitness group need to be confirmed. The paradoxically positive association between PA and abdominal adiposity found in highly fit individuals warrants further research.

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