THE EFFECTS OF PHYSICAL FITNESS AND FATNESS ON CARDIOVASCULAR DISEASE RISK FACTORS IN CHILDREN AND ADOLESCENTS:

THE UP&DOWN LONGITUDINAL STUDY

EFECTOS DE LA CONDICIÓN FÍSICA Y LA ADIPOSIDAD SOBRE INDICADORES DE SALUD CARDIOVASCULAR EN NIÑOS Y ADOLESCENTES: ESTUDIO LONGITUDINAL UP&DOWN

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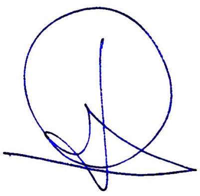

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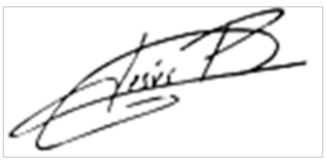

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ABREVIATIONS

AIDS: Acquired immunodeficiency syndrome

ALPHA: Assessing Levels of Physical Activity *health-related fitness test battery*

ANCOVA: Analysis of covariance

ANOVA: Analysis of variance

AUC: Area under the curve

BMI: Body mass index

BP: Blood pressure

CI: Confidence interval

CRF: Cardiorespiratory fitness

CPM: Counts per minute

CVD: Cardiovascular diseases

CVDRF-I: Cardiovascular disease risk factor index

CVDRF-II: Cardiovascular disease risk factor index excluding waist circumference

DALYs: Disability-adjusted life-years

DBP: Diastolic blood pressure

EDTA: Ethylenediaminetetraacetic acid

FMI: Fat mass index

HDL-c: High-density lipoprotein cholesterol

HIV: Human immunodeficiency virus

HOMA-IR: Homeostatic model assessment for insulin resistance

MAP: Mean arterial pressure

MF: Muscular fitness

NC: Neck circumference

NW: Normal weight including thinness grades 1, 2, and 3

OR: Odds ratio

OW: Overweight including obesity

PDAY: Pathobiological Determinants of Atherosclerosis in Youth Study

SBP: Systolic blood pressure

SD: Standard deviation

SES: Socioeconomic status

TC: Total cholesterol

VO_2max: Maximal oxygen uptake

WC: Waist circumference

ZBMI: Sex- and age-standardized values of body mass index

ZWHO: Standardized BMI according to reference data from the World Health Organization

%BF: Body fat percentage

ABSTRACT

Cardiovascular diseases (CVD) are the leading cause of global mortality. Normally, cardiovascular alterations become visible after the fifth decade of life, but increasing evidence suggest that their origin may occur in early ages. Thus, the identification of children and adolescents at higher risk is of vital importance for the prevention of CVD. In this sense, the definition of young people at risk of CVD is normally based on the levels of several factors, including waist circumference, triglycerides, high-density lipoprotein-cholesterol, blood pressure (BP), and glucose levels, among others. Nonetheless, the identification of those at risk using these markers would entail a high economic cost, in addition to subjecting young people to invasive measures. Physical fitness, specifically cardiorespiratory (CRF) and muscular fitness (MF), and fatness are considered key elements for the cardiovascular risk identification, given their close association with the previously mentioned markers. Both, fitness and fatness provide a quicker and relatively simple way to identify those at risk of future CVD. However, although their association with CVD risk factors has been previously examined, their independent and combined effect on CVD risk factors remain to be fully determined. More information is needed since fitness and fatness may lay in the same causal chain leading to future CVD. Thus, the main aim of the present International Doctoral Thesis was to study the independent and combined effects of different components of physical fitness and fatness on CVD risk factors levels, cross-sectionally and longitudinally (two-year follow-up), in a sample of Spanish children and adolescents.

The results of the eight studies included indicate that body mass index (BMI) is an independent predictor of CVD risk factors, and a mediator in the association of CRF **(Study I)** and MF **(Study II)** with clustered CVD risk factors. Different fitness cut-off points associated with reduced CVD risk two-years later have been identified for CRF in children **(Study III)** and for upper- and lower-body MF in children and adolescents **(Study IV)**. A bidirectional longitudinal association was observed between CRF and different fatness indicators in children and adolescents, but the associations between CRF as exposure and fatness weakened when fatness at baseline was considered **(Study V)**. The

bidirectional associations between CRF (Study VI) and MF (Study VII) with neck circumference (NC) were only observed cross-sectionally. Longitudinally, only NC showed an independent association with CRF and MF. Furthermore, BP measures seem to be longitudinally affected to a higher extent by NC than by CRF (Study VI) and MF (Study VII). Finally, waist circumference, but neither CRF nor MF, is independently associated with future BP and its changes over two years (Study VIII).

The results from the present thesis enhance our knowledge on the combined and independent effects of fitness and fatness on CVD risk factors. In addition, it provides fitness cut-off points for the identification of those children and adolescents at a higher risk of future CVD.

RESUMEN

Las enfermedades cardiovasculares (ECV) son la principal causa de mortalidad a nivel mundial. Normalmente, las alteraciones cardiovasculares se hacen patentes tras la quinta década de vida, pero cada vez existe más evidencia de que el origen de estas enfermedades puede ocurrir en edades tempranas. Así, la identificación de los niños y adolescentes en riesgo de ECV es de vital importancia para su prevención. En este sentido, la definición del estado de riesgo en jóvenes está normalmente basado en los niveles de diferentes factores, incluyendo el perímetro de cintura (PC), triglicéridos, lipoproteínas de alta densidad, tensión arterial (TA), y glucosa entre otros. Sin embargo, la identificación de aquellos en riesgo utilizando estos marcadores supondría un alto coste económico, además de ser una medida invasiva para los jóvenes. La condición física, específicamente la capacidad aeróbica (CA) y la fuerza muscular (FM), así como la adiposidad, son considerados elementos clave para la identificación del riesgo de ECV dada su estrecha asociación con los marcadores previamente señalados. Tanto la CA, FM y la adiposidad ofrecen una herramienta más rápida, barata, y sencilla de identificar a aquellos en riesgo de ECV futura. Sin embargo, aunque la asociación con los factores de riesgo de ECV ha sido previamente estudiada, el efecto independiente y combinado de estas variables (CA, FM y adiposidad) sobre los factores de riesgo de ECV, está aún por definirse. Más información es necesaria dado que la CA, la FM y la adiposidad pueden estar en la misma cadena causal que finaliza en ECV futura. Por

esto, el objetivo principal de esta Tesis Doctoral Internacional fue estudiar el efecto independiente y combinado de la CA, FM y adiposidad sobre los niveles de factores de riesgo de ECV, a nivel transversal y longitudinal (seguimiento de 2 años), en una muestra de niños y adolescentes españoles.

Los resultados de los ocho estudios incluidos indican que el índice de masa corporal (IMC) es un predictor independiente del riesgo de ECV, y un mediador en la asociación entre la CA **(Estudio I)** y la FM **(Estudio II)** y el riesgo de ECV. Se han identificado diferentes puntos de corte de CA en niños **(Estudio III)** y de FM de miembro superior e inferior en niños y adolescentes **(Estudio IV)** asociados con un menor nivel de factores de riesgo de ECV a los dos años. Se observó una asociación longitudinal bidireccional entre la CA y diferentes marcadores de adiposidad en niños y adolescentes, pero la asociación entre CA, como variable independiente, y la adiposidad futura se debilitó cuando se introdujo como covariable la adiposidad en el primer año de estudio **(Estudio V)**. Se observó una asociación transversal bidireccional entre la CA **(Estudio VI)** y la FM **(Estudio VII)** con el perímetro del cuello (PCU). A nivel longitudinal, solo el PCU mostró estar independientemente asociada con la CA y la FM. Además, La TA parece estar más afectada a nivel longitudinal por el PCU que por la CA **(Estudio VI)** y la FM **(Estudio VII)**. Finalmente, el PC, pero no la CA ni la FM, estuvo asociado de manera independiente con la TA futura, así como sus cambios a lo largo de los dos años de seguimiento **(Estudio VIII)**.

Los resultados de la presente tesis aumentan nuestro conocimiento sobre el efecto combinado e independiente de la condición física y la adiposidad sobre los factores de riesgo de ECV. Además, se ofrecen puntos de corte de condición física para la identificación de aquellos niños y adolescentes con un mayor riesgo de presentar ECV en edades futuras.

INTRODUCTION

GENERAL INTRODUCTION

Cardiovascular diseases

Global disease burden reports are crucial to elucidate which health conditions demand higher priority to focus society's resources on (1). In this sense, and given the attention that some conditions such as human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), malaria, tuberculosis, and diarrhoeal diseases have received, mortality rates due to communicable, maternal, neonatal, and nutritional diseases have decreased by 23.9% in the last decade (1). This implies a shift in the worldwide health burden from communicable to non-communicable diseases (2). According to the Global Burden of Disease Study 2016 (1), non-communicable diseases were responsible of 72.3% of global deaths, being cardiovascular diseases (CVD) the main contributor within this group. Indeed, CVD are nowadays the main source of mortality, being accountable for 17.6 million deaths in 2016 (1). In this sense, ischaemic heart disease and cerebrovascular disease (stroke), the two main forms of CVD, rank in the top five in terms of years of life lost in high to middle-low income countries (1). The CVD prevalence and incidence in 2016 were 469.5 and 54.1 millions, respectively, and an increase of 29.4% has been observed in the number of years lived with disability from 2006 to 2016 (3).

Importantly, and although genetic factors contribute to the disease risk definition (4), most of the CVD burden is attributable to behavioural, environmental, and metabolic risk factors (2,5). For instance, 93.3% of deaths and 94.4 of disability-adjusted life-years (DALYs) caused by ischaemic heart disease are attributable to specific factors addressed in the Global Burden of Disease Study 2016 (5), which does not include genetic variables. In this sense, high blood pressure (BP), total cholesterol (TC), fasting plasma glucose, body mass index (BMI), low levels of physical activity, and tobacco and alcohol use are recognized among the leading causes of CVD (6). Interestingly, CVD is a multifactorial condition and the definition of CVD risk differ from the simple addition of the individual effects of each factor. (6).

CVD risk in the pediatric population

CVD have traditionally been considered an issue in adult health since clinical CVD mainly occur after the fifth decade of life (3,7). Nonetheless, evidence on the childhood origin of these pathologies began to appear in the 1970s (8), when a wide range of serum cholesterol and triglyceride, BP, and obesity levels were observed in school children within the same age groups. The "Bogalusa Heart Study" (9) and the "Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Study" (10), offered the first solid evidence of the associations of some risk factors (i.e. high-density lipoprotein cholesterol (HDL-c), non-HDL-c, BMI, glycosylated hemoglobin, smoking, and hypertension) with a greater area of the coronary and aorta's intima surface affected by fatty streaks (considered the first step in the atherosclerotic process) (10). These observations were made directly on arteries, blood and selected tissues of cadavers of people aged 6-30 (Bogalusa Heart Study) and 15-34 (PDAY), autopsied after accidental injuries, homicide or suicide deaths. Further evidence support this notion, observing a positive association between exposure to different CVD risk factor in children and adolescents and coronary artery calcification (11), and carotid artery intima-media thickness (12). These studies showed the vital importance of elucidating the optimal age at which preventive strategies should be carried, and which behavioural aspects during childhood and adolescence have the potential to slow down the risk factor development, thus delaying or preventing the onset of clinical CVD (13). This promising results in terms of CVD prevention has led to an exponential increase of publications. Taking the PubMed database as an example, the number of publications on CVD risk factors in the pediatric population increased from approximately 37,500 in 2000 to 93,000 in 2019.

Although significant progress has been made in this subject, a clear and unified definition of CVD risk in the pediatric population has not been reached yet (7). In this regard, the adult definition of the metabolic syndrome comprises a cluster of several CVD risk factors. Although with sex- and ethnic-specific cut-offs, a single definition is valid in this age group (14). Contrary, reaching a single definition of metabolic syndrome in children and adolescents is problematic for two main reasons: first, the low prevalence of clinical CVD at early ages makes it difficult to accurately determine specific risk factor cut-offs values associated with the appearance of the disease; secondly, some key biological features change with age and pubertal development, including BP, lipid levels and anthropometric variables (7). Given this, sex- and age-standardized 90th, 95th, or 97th percentile values seem a more accurate approach rather than single cut-off points for all ages from childhood to adolescence (7). Nevertheless, no general agreement has been reached on which criteria should be used, and several proposals have been raised (15–19). To address this problem, the International Diabetes Federation developed a definition unifying all the available evidence which divided the pediatric population in three age groups: 6 to <10, 10 to <16, and ≥16 years. Specific considerations were defined for each group, but, as in adults, high waist circumference (WC) was considered a mandatory prerequisite, with triglycerides, HDL-c, BP, and glucose completing de definition (7).

A different approach to assess CVD risk is the clustering of the standardized values of individual CVD risk factors. This method has been mainly used from 2003, when Andersen et al. (20) observed that up to 4-5 CVD risk factors co-existed in the 5.4% of the children and adolescents studied. In fact, the clustering of CVD risk factors may be a better indicator of CVD risk at early ages for different reasons: (i) it uses a single continuous score in which all individual CVD risk factors are involved, what offers more information compared to dichotomization (i.e. having or not having metabolic syndrome); (ii) it can somehow neutralize the day-to-day fluctuations in single risk factors; and (iii) clustered CVD risk factors show a higher degree of stability from childhood to adolescence and adulthood compared to that observed for CVD risk factors individually (20–22). Nonetheless, this approach also presents some disadvantages: first, the risk is specific to the study sample, since there is no clear definition of 'being at risk'; and second, it implies that individual risk factors included in the score are equally important, which is probably not correct. However, no information on how individual risk factors should be weighted within a score exists to date (21).

Fitness and its association with CVD risk factors in children and adolescents

Physical fitness performance involve the correct functioning of most body systems, such as respiratory, circulatory, endocrine, nervous, and musculoskeletal (23). Thus, physical fitness testing can be seen as an examination of the correct functioning of all these systems (23). Given this, physical fitness is considered one of the main health markers already in children and adolescents (23–26). Physical fitness is a multifactorial feature, with genetics having an important role on physical performance phenotypes (27). However, evidence from large-scale epidemiological studies and randomized controlled trials suggest that high-intensity physical activity is a key element in its development, which makes this condition potentially improvable through well-designed interventions (23).

Cardiorespiratory (CRF) and muscular fitness (MF) are the fitness components that have shown to be more strongly related to cardiovascular health (24–26). Two systematic reviews of longitudinal studies have found moderate-to-strong evidence indicating that CRF in childhood and adolescence is associated with BMI, WC, body fatness, CVD risk factors and the metabolic syndrome at least two years later (24,25). Importantly, not only baseline CRF but also its changes over the years have been linked to an improved cardiovascular profile (24). For instance, Carnethon et al. (28) observed that improvements in CRF was associated with a 50% risk reduction of diabetes and metabolic syndrome development 15 years later. On the other hand, MF levels in children and adolescents have showed a consistent longitudinal association with BMI, body fatness, homeostatic model assessment of insulin resistance (HOMA-IR), triglycerides and CVD risk score (26). Importantly, Mintjens et al (25) in its recent systematic review concluded that the association observed between fitness and future CVD risk factors could be hampered by the lack of adjustment for potential confounders such as fatness. These adjustments, and given the important effects of both CRF and MF on fatness, are crucial to correctly understand the causal path that ends in future CVD.

Fatness and its association with CVD risk factors in children and adolescents

Obesity in adults is strongly associated with the prevalence of most CVD (29). Importantly, fatness levels track from childhood to adulthood and a high percent of children with obesity present this condition in their adult life (30). In this sense, the persistence of the overweight/obese condition from childhood to adulthood has been linked to an increased risk of type two diabetes, hypertension and atherosclerosis (30), which makes childhood obesity a public health concern. Accordingly, Goodman et al. (31) observed that adolescence obesity was the main contributor to cumulative CVD risk, compared to other CVD risk factors such as cholesterol, triglycerides, fasting insulin, glucose and BP. This evidence justifies that WC, an indicator of central fatness, is considered the 'sine qua non' (i.e. mandatory prerequisite) for the metabolic syndrome diagnosis (15). Several physiologic mechanisms can explain the deleterious effects of excess fatness on future cardiovascular health, including the effects on hemodynamics (increased blood volume, stroke volume, arterial pressure, etc.), cardiac structure (left ventricular remodeling and hypertrophy, left atrial enlargement, etc.), and function (left ventricular systolic and diastolic dysfunction, and right ventricular failure) (29). The etiology of obesity is very complex, with 30-50% of the variation in fatness being attributable to genetics. Importantly, easily modifiable environmental factors, such as diet and physical activity, have a major impact on fatness levels of children and adolescents (29). In this regard, physical fitness improvement, which is mainly driven by high-intensity physical activity levels (23), is an important factor for the obesity prevention. For instance, Ortega et al. (32) observed that the risk of developing overweight/obesity after six years follow-up was decreased by 10% every 1 ml/kg/min increment in maximum oxygen consumption (VO_2max). All this evidence, as previously mentioned, suggest that fitness and fatness are on the same causal path towards CVD. Nonetheless, their independence and how these features interact remains to be fully defined.

CHAPTER-SPECIFIC INTRODUCTION

Chapter I: mediation (studies I and II)

CRF and MF are associated with future fatness levels (25,26). This should be kept in mind when interpreting studies that analyze the effects of fitness on CVD risk factors in children and adolescents. In this sense, previous studies observed a reduction in the effect of CRF (25,33,34) and MF (35–37) on CVD risk factors when fatness was considered. This evidence suggest that fitness may exert an indirect effect on CVD risk by reducing fatness levels. Despite this, many previous studies did not include this potential confounder in their analyses, which limits the validity of their findings (25,26).

Mediation, compared to multivariate analyses, goes a step further, allowing the evaluation of the extent to which a potential mediator accounts for the relation between the exposure and the outcome variables (38). To our knowledge, only two studies have been conducted using this procedure, concluding that fatness acts as a mediator in the association of CRF (39) and MF (40) with CVD risk factors in children aged 8-11 years. Apart from the scarce evidence in children, whether fatness plays a mediator role in this association during adolescence remains unstudied.

Chapter II: fitness cut-off points (studies III and IV)

Given the close association between CRF (25) and MF (26) and future CVD risk factors, physical fitness testing provides a simple and non-invasive way to determine those children and adolescents at higher risk of CVD development. Thus, the implementation of CRF and MF cut-off points are needed for the detection of 'at-risk' individuals that would benefit from preventive strategies. Previous cut-off points have been proposed for CRF (41,42) and MF (43). Importantly, the previously proposed CRF cut-off values have been mainly based on data collected from adolescents (42) and, since the age-related development of VO_2max is a key feature during childhood growth (44), it would be desirable to extent these values to the early childhood. On the other hand, only upper MF cut-off points (derived from the handgrip test) have been proposed (43), with no information to date about the validity of lower MF cut-offs in the prediction of CVD risk. Furthermore, both the CRF and MF existing cut-offs are mainly based on cross-sectional data. Thus, more evidence on the predictive capacity of these values would be of great interest.

Chapter III: bidirectionality (studies V, VI, and VII)

Designing effective interventions for the prevention of CVD requires an understanding of how its determinants interact with each other, in order to focus such interventions on the key variables. In this sense, and besides the aforementioned effect that CRF (24,25) and MF (24,26) exerts on future fatness, there is also convincing evidence supporting an association between fatness with CRF (45) and MF (46,47). Nevertheless, the bidirectional association between these features has not been studied within the same study cohort. This knowledge will allow determining whether physical fitness, fatness, or both should be considered the target feature to act on at early ages for the prevention of future CVD.

Interestingly, fatness has been generally reported as BMI, WC, or skinfold thickness (48). Since not only overall fatness, but also its distribution is important in the CVD risk quantification in young people (49), it would be desirable to investigate the bidirectional associations between fitness and fatness measures including not only overall and central body fatness indices, but also an upper-body fatness indicator such as neck circumference (NC). This anthropometric measure is getting increasing attention due to its simplicity and its accuracy in the assessment of overweight and obesity in different age groups (50).

Chapter IV: independent effect of fitness and fatness on BP (studies VI, VII, and VIII)

High BP is recognize as the leading risk factor for CVD, and the global exposure to high BP continues to increase (5). Despite the non-modifiable genetic predisposition towards hypertension, it is considered a highly preventable condition given the influence of environmental and lifestyle factors (51).

Although the evidence of the association between CRF and MF with CVD risk factors is consistent (23–26), longitudinal studies have led to unconvincing results regarding the longitudinal effects of CRF and MF on BP measures (25,26). Contrary, a recent meta-analyses concluded that childhood fatness is associated with systolic (SBP) and diastolic (DBP) BP in adult life (48). Nonetheless, this study took adult fatness as the potential confounder and did not considered any fitness measure. Thus, the independent associations of CRF, MF, and fatness in their association with future BP remains unclear.

Similar to the most used fatness indices, NC has been associated with SBP and DBP, but this evidence comes from cross-sectional analyses, with longitudinal evidence being non-existent to date (52). Further evidence would therefore be needed to ascertain the independence of this fatness index in its association with future BP.

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AIMS

AIMS

General aim

The general aim of this International Doctoral Thesis was to study the independent and combined effects of different components of physical fitness and fatness on CVD risk factors levels, cross-sectionally and longitudinally (two-year follow-up), in a sample of Spanish children and adolescents. This overall aim was addressed through eight studies with the following specific objectives:

Chapter I: Mediation

Study I: To test the individual and combined associations of CRF and BMI with clustered CVD risk factors, and to analyze the mediator role of BMI in the association between CRF and clustered CVD risk factors.

Study II: To study the association of MF and BMI, individually and combined, with clustered CVD risk factors, and to test whether the association between MF and clustered CVD risk factors is mediated by BMI.

Chapter II: Fitness cut-off points

Study III: **(i)** to examine the cross-sectional and longitudinal associations between CRF and CVD risk factors, **(ii)** to examine whether there is a CRF cut-off point associated with CVD risk factors at 6 to 10 years (cross-sectional), **(iii)** to test whether the identified cut-off point is associated with CVD risk factors two years later, and **(iv)** to study whether changes with age (from 6-10 years to 8-12 years) in CRF are associated with CVD risk factors in 8-12 years children.

Study IV: **(i)** to study the cross-sectional and longitudinal associations between MF and CVD risk factors, **(ii)** to examine whether there are MF cut-off points associated with CVD risk factors (cross-sectional), and **(iii)** to test whether the identified cut-off points at baseline are associated with CVD risk two years later in children and adolescents.

Chapter III: Bidirectionality

Study V: to study the cross-sectional and longitudinal bidirectional association between CRF and fatness indicators in children and adolescents.

Study VI (part I): to analyze the bidirectionality in the cross-sectional and longitudinal association between CRF and NC.

Study VII (part I): to examine the bidirectionality in the cross-sectional and longitudinal association between MF and NC.

Chapter IV: Independent effect of fitness and fatness on BP

Study VI (part II): to determine the independence of CRF and NC in their cross-sectional and longitudinal association with BP measures.

Study VII (part II): to establish the independent associations of MF and NC at baseline with BP measures at baseline and at two-year follow-up.

Study VIII: to determine the independent associations of CRF, MF, and WC with BP levels over two years in children and adolescents.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present International Doctoral Thesis is composed of eight studies derived from the UP&DOWN Study (1). In brief, this is a two-year longitudinal multi-center study aimed to assess the diagnostic properties of sedentary behavior, physical activity, and fitness on CVD risk factors and other health issues of public interest in the pediatric population. It included a convenience sample of 2225 healthy children (aged 6-11.9 years) and adolescents (aged 12-17.9 years) from schools in the cities of Cádiz and Madrid (Spain), respectively. Baseline and follow-up data were collected from September 2011 to June 2012, and from September 2013 to June 2014, respectively. Only those participants with complete data on the analyzed variables were included in each study (see table 1).

Written informed consents were collected from parents and school supervisors after being informed about the study protocols, which were approved by the ethics committee of the Hospital Puerta de Hierro (Madrid, Spain), the bioethics committee of the Spanish National Research Council (Madrid, Spain), and the committee for research involving human subjects of the University of Cádiz (Cádiz, Spain). The methods complied with the Declaration of Helsinki.

Measurements

Cardiorespiratory fitness

CRF was assessed through the 20-m shuttle run test, according to the Assessing Levels of Physical Activity (ALPHA) health-related fitness test battery protocol (2). In short, participants run between two lines 20 m apart at the pace of a pre-recorded audio. The initial speed is 8.5 km/h, increasing by 0.5 km/h each minute (each stage lasts for one minute). Participants were instructed to run in straight line and to pass the line with both feet in each shuttle. The test finished with the exhaustion of the participant or when they could not reach the line twice in a row at the time of the acoustic signal. The last half stage completed was recorded and used for analyses. Alternatively, the last half stage completed was used to estimate maximal oxygen uptake (VO_2max [ml/kg/min]) through the Léger's equation (3).

Muscular fitness

Upper-body MF was assessed through the handgrip strength test. In brief, after the grip spam was adapted to the hand size of each participant (4,5), they were asked to squeeze a validated dynamometer (TKK 5101 Grip D; Takey, Tokyo, Japan) (6) gradually, by exerting as much pressure as possible with the tested hand, and to hold this pressure for approximately two seconds. The test was performed twice with each hand and the mean value from the highest scores attained with each hand was calculated. Finally, this result was normalized by body weight to preclude body size influences (7).

Lower-body MF was assessed using the standing long jump test (8). Participants were told to jump as far horizontally as possible from a standing position with feet shoulders' wide apart and just behind a starting line. The test was performed twice, and the highest score was retained in centimeters.

A global MF index was created from the mean of the sex- and age-standardized values of both tests (i.e. handgrip/weight and standing long jump).

Anthropometry

Harmonization and standardization of the different anthropometric measures included in the UP&DOWN study were comprehensively controlled (1).

Weight was measured using an electronic scale (Type SECA 861; range, 0.05 to 130 kg; precision, 0.05 kg; Hamburg, Germany). Height was measured in the Frankfort plane with a telescopic stature-measuring instrument (Type SECA 225; range, 60 to 200 cm; precision, 1 mm; Hamburg, Germany). BMI was calculated as weight/height squared (kg/m^2). We also computed BMI sex- and age-standardized values (ZBMI). Furthermore, we computed a new standardized BMI variable considering the growth reference standards provided by the World Health Organization (ZWHO) (9). Alternatively, BMI was categorized as normal weight (NW), including thinness grades 1, 2 and 3, and overweight (OW), including obesity, according to the sex- and age-specific BMI cut-off points previously proposed (10,11).

WC was measured at the narrowest part of the torso. When this point could not be clearly detected, measurements were performed at the midpoint between the lower rib and the upper border of the iliac crest. NC was measured with the participant standing, with arms relaxed at both sides of the body, and the head aligned in the Frankfort horizontal plane. The measurements were performed just below the laryngeal prominence, perpendicular to the long axis of the neck (12). Both WC and NC were assessed using a non-elastic tape (SECA 200; range, 0 to 150 cm; precision, 1 mm; Hamburg, Germany).

Triceps and subscapular skinfolds were assessed according to standardized procedures (13). Skinfolds were taken on the non-dominant side of the body using a Holtain calliper (range, 0 to 40mm; precision, 0.2 mm). Body fat percentage (%BF) was calculated using the Slaughter equation (14). Subsequently, fat mass index (FMI) was calculated by first estimating fat mass (derived from body weight and the %BF value) and then dividing it by height squared (kg/m2).

All anthropometric measurements were performed twice and not consecutively, and the mean values were retained and used for analyses. A third measure was carried when the results of the first two measures differed by more than 1 kg (weight), 1 cm (height, WC, and NC), and 1 mm (skinfolds). Then, the mean of the nearest two measures were used for further analyses.

Blood pressure

SBP and DBP were measured according to the standardized protocol of the European Society of Hypertension using a validated digital automatic BP monitor (OMROM M6; OMROM Health Care Co., Ltd., Kyoto, Japan) (15). Participants remained seated for five minutes before measurements, which were taken in the left arm in an extended but relaxed position. Two measurements were carried out one to two minutes apart. When SBP or DBP results differed by ≥ 10 mmHg a third measure was performed and the mean of the two closest values were retained for analyses. Mean arterial pressure (MAP) was calculated as (SBP+(DBPx2))/3. Given the influence of sex and age on BP, the sex- and age-standardized values of SBP, DBP, and MAP were used for the analyses (16).

Blood sampling

After an overnight fast, 13.5 ml of blood were extracted from the cubital vein of each participant, of which, 3.5 ml were collected in ethylenediaminetetraacetic acid (EDTA) and analyzed to acquire hemogram data. The remaining blood was collected in dried gel and sodium citrate and centrifuged to remove both serum and plasma. Finally, it was frozen al -80°C for future analyses. Enzymatic colorimetric methods (Olympus AU2700 Analyser; Olympus UK Ltd, Watford, UK) were used to analyze serum lipid triglycerides, total cholesterol, HDL-c, and fasting plasma glucose. Analyte profiling xMAP technology (Luminex Corp) was used to quantify insulin. Furthermore, we calculated insulin resistance through the homeostasis model assessment for insulin resistance (HOMA-IR) score as (insulin [µIU/mL) x glucose [mmol/L])/22.5 (17).

Clustered CVD risk factors

A CVD risk factor index (CVDRF-I) was created from the mean of the standardized values of each individual CVD risk factor (i.e. WC, SBP, triglycerides, HDL-c and glucose) by sex (males and females) and age groups (children and adolescents). This index includes the variables used for the metabolic syndrome definition by the International Diabetes Federation (18). The standardized value for HDL-c was multiplied by (-1) since higher HDL-c levels represent lower CVD risk. Alternatively, in studies III and IV we constructed a CVD cluster as the mean of the sex- and age-standardized values of the following single CVD risk factors: sum of two skinfolds, SBP, HOMA-IR, triglycerides, and total cholesterol (TC)/HDL-c (19). Those participants with values >1.0 standard deviation (SD) in the risk score were defined as 'at risk' (20).

Pubertal development

Explicative drawings of genital and breast development for males and females, respectively, were given to participants for self-classification in one of the five stages of pubertal development according to Tanner & Whitehouse (21).

Socioeconomic status

Socioeconomic status (SES) was determined through the Health Behavior in School-Aged Children Family Affluence Scale (22). This scale includes items related to vehicle belonging, availability of own bedroom, frequency of holidays during the last year, and the number of computers at home. We categorized the final score as low (0-3 points), medium (4-6 points) or high SES (≥7 points).

Physical activity

Physical activity was assessed using Actigraph accelerometer models GT1M, GT3X and GT3X+ (Actigraph TM, LLC, Pensacola, FL, US). Participants wore the accelerometer attached tightly to the lower back, with the notch faced upwards, for seven consecutive days except during sleep hours and water-based activities. ActiLife software (v.6.6.2) was used to analyze accelerometry data, which was integrated into 10-second epochs. Non-wear time was defined as a period of 60 minutes of zero counts and an allowance of two consecutive minutes with <100 counts per minute (cpm). These periods of non-wear time were not accounted in further analyses. The following cut-off points were used to defined physical activity intensities according to previous studies in European children and adolescents (23): light between 100-2000 cpm; moderate between 2001-4000 cpm; and vigorous >4000 cpm. Moderate-to-vigorous physical activity was calculated as the sum of moderate and vigorous periods in minutes. Only those with at least three valid days (≥8 hours per day of wear time and including at least one weekend day) were considered.

Mother's education level

The highest education level of the participants' mothers was reported and coded as: 1 = unschooled; 2 = school graduate; 3 = high school graduate; 4 = mid-level studies; 5 = higher education.

Statistical analyses

Data analysis was conducted with the Statistical Package for Social Sciences (IBM SPSS Statistic for Windows, Version 21.0 for Windows and 22.0 for MacOS. Armonk, New York: IBM Corp). The significance was set at $p<0.05$.

Chapter I: Mediation

Study I: The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of body mass index in youth: The UP&DOWN Study

Due to significant interactions between sex (males and females) and age groups (children and adolescents) in the studied associations (all $p<0.01$), all computations and analyses were performed separately for sex and age groups. All biochemical variables were checked for normality and triglycerides was transformed to the natural logarithm due to its skewed distribution.

Descriptive statistics are presented as mean ± SD. Sex differences were examined by one-way analysis of variance (ANOVA).

Linear regression models controlling for age (model 1) were used to assess the individual association of CRF (stages) and BMI with CVDRF-I. Further adjustment by CRF or BMI (model 2), depending on the fixed factor (i.e. BMI and CRF, respectively), were conducted to examine the independent association of each variable with CVDRF-I.

To perform combined analyses participants were first categorized as “Low CRF” or “High CRF” depending on whether their CRF were below or above their population- and sex-specific 50th percentile, respectively. Then, sex and age-specific BMI cut-off points previously proposed (10,11) were used to categorize participants as NW (including thinness grades 1, 2 and 3) or OW (including obesity). Finally, a new variable with 4 categories was computed: 1=“NW+Low CRF”, 2=“NW+High CRF”, 3=“OW+Low CRF” and 4=“OW+High CRF”. Analysis of covariance (ANCOVA) was used to examine differences in CVDRF-I between groups, adjusting for age. Bonferroni’s correction was employed to check these differences reducing the possibility of type I error.

Mediation analyses were conducted according to Baron and Kenny procedures (24). To do this, the three following regression equations were fitted: equation 1 regressed the mediator (BMI) on the independent variable (CRF); equation 2 regressed the dependent variable (CVDRF-I) on the independent variable; and equation 3 regressed the dependent variable on both the mediator (equation 3) and the independent variable (equation 3’). To accept mediation hypotheses, regression equations 1, 2 and 3 must be significant and the regression coefficient between the independent and dependent variables in equation 3’ must be attenuated compared to equation 2. To test whether this attenuation was significant the Sobel test was performed (25). Finally, the percentage of the total effect (equation 2) that is mediated by BMI (indirect effect) was estimated by multiplying the unstandardized coefficients of equations 1 and 3 and dividing it by the unstandardized coefficient of equation 2. All regression equations were adjusted by age.

Given the close relationship between BMI and WC, all the aforementioned analyses were also performed using a CVDRF-I excluding WC from the index (CVDRF-II) (supplementary analyses).

Study II: The role of adiposity in the association between muscular fitness and cardiovascular disease

Preliminary analyses showed significant interactions between sex and age groups and independent variables; therefore, all analyses were performed separately for males and females and for children and adolescents.

Descriptive statistics are shown as mean ± SD. ANOVA was used to examine differences between males and females.

To examine the association of MF (i.e. upper-body, lower-body, and global MF) with CVDRF-I, we used linear regression models where handgrip/weight, standing long jump and the MF index were individually introduced as independent variables and CVDRF-I as the dependent variable. In model 1 we adjusted by age, and in model 2 we included age and BMI as covariates. The same analyses were made to examine the association of BMI with CVDRF-I. In this case we controlled for age (model 1); age + handgrip/weight (model 2); age + standing long jump (model 3); and age + MF index (model 4) to test the independence of BMI in this association.

To study the combined effects of MF and BMI with CVDRF-I we created various groups. Firstly, participants were categorized as "low MF" or "high MF" depending on whether their MF indexes were below or above their population- and sex-specific 50th percentile. Secondly, and as in study I, participants were categorized as NW (including thinness grades 1, 2 and 3), or OW (including obesity) according to sex and age-specific cut-off points previously defined (10,11). Finally, we created a new variable with 4 categories: 1 = "NW+Low MF", 2 = "NW+High MF", 3 = "OW+Low MF" and 4 = "OW+High MF". Differences in CVDRF-I between groups were tested using ANCOVA, controlling for age. Pairwise post hoc differences were tested using the Bonferroni's correction.

As in the previous study, the Baron and Kenny procedures (24) were performed to examine the mediator role of BMI in the association between MF and CVDRF-I. The global MF index, BMI, and CVDRF-I were considered the independent, mediator, and dependent variables, respectively. Likewise, the Sobel test (25) was performed to test whether the attenuation of the association between the independent and dependent variables, after accounting for the mediator, was significant. All regression equations were performed controlling by age. In addition, as confirmatory analyses, mediation hypothesis was also tested by the bootstrap method (26).

Owing to the close relation between BMI and WC, the aforementioned analyses were performed using

CVDRF-II as the dependent variable (supplementary analyses).

Chapter II: fitness cut-off points

Study III: Cardiorespiratory fitness cut-off points for early detection of present and future cardiovascular risk in children: a 2-year follow-up study

Descriptive data are shown as mean ± SD unless otherwise indicated. The Student t-test was used to test differences in characteristics of the study sample by sex at baseline and follow-up, except for the tanner stage, which was analyzed by the chi-square test.

We performed multiple linear regression analyses to examine the association between CRF (VO_2max) at baseline (6-10 years-old) and CVD risk in 6-10 years-old children (cross-sectional) and 8-12 years-old children (longitudinal). We entered each single CVD risk factor and the CVD risk score at baseline (6-10 years) and follow-up (8-12 years-old) as dependent variables, CRF at baseline as the independent variable, and age as a covariate in separate models. In addition, we controlled longitudinal analyses for the corresponding baseline value of the dependent variable.

To determine whether there is a CRF cut-off point associated with CVD risk at 6-10 years of age (cross-sectional), we determined the CRF cut-off point at baseline associated with CVD risk (CVD risk score >1.0 SD) at baseline by using the receiver operating characteristic curve (27). The area under the curve (AUC) and 95% confidence intervals (CI) were calculated. Binary logistic regression was also used to study the relationship between the CRF cut-off point (high vs low) and CVD risk (≤1 SD vs >1 SD).

We conducted a binary logistic regression analysis using a CRF cut-off point (high vs low) at baseline and CVD risk (≤1 SD vs >1 SD) at follow-up, adjusting for age at follow-up to study whether the health-related CRF cut-off point identified in 6-10 years-old children is associated with CVD risk two years later (longitudinal).

Moreover, we assessed differences on single CVD risk factors and CVD risk score at follow-up by CRF levels at baseline (high vs low) by ANCOVA. We entered single CVD risk factors and the CVD risk score at follow-up as dependent variables, CRF (high vs low) as the independent variable, and sex and age at follow-up as covariates. In addition, each CVD risk factor and the CVD risk score were adjusted for their corresponding baseline levels. We also analyzed the association between CRF at baseline and CRF at follow-up by means of linear regression analysis.

To determine whether changes in CRF with age (from 6-10 to 8-12 years-old) is associated with CVD risk in children aged 8-12, we computed a CRF variable as follows: children who were in the low fitness category at both baseline and follow-up were classified as "persistent low fitness," and those who dropped from the high fitness category at baseline to the low fitness category at follow-up were classified as "decreasing fitness." The other categories were "persistent high fitness" (high fitness category at both baseline and follow-up) and "increasing fitness" (those who changed from the low fitness category at baseline to the high fitness category at follow-up). The association of CRF change categories (persistent low, decreasing, persistent high, and increasing) with CVD risk score at follow-up was assessed by ANCOVA. We entered the CVD risk score at follow-up as the dependent variable, CRF-change categories as the independent variable, and sex, age at follow-up, and CVD risk score at baseline as covariates.

We repeated all the analyses after adjusting for pubertal development instead of age but results did not materially change (data not shown).

Study IV: Muscle fitness cut points for early assessment of cardiovascular risk in children and adolescents

Descriptive data are shown as mean ± SD unless otherwise stated. The Student t-test was used to test differences in characteristics of the study sample by sex at baseline and follow-up, except for the tanner stage, which was analyzed by the chi-square test.

Multiple linear regression analyses were performed to examine the cross-sectional and longitudinal association between MF (i.e. upper-body, and lower-body MF) and CVD risk factors in children and adolescents. Single CVD risk factors and the CVD risk score at baseline and follow-up were entered, respectively, as dependent variables, handgrip/weight and the standing long jump tests at baseline as independent variables, and age as covariate in separate models. Longitudinal analyses were further adjusted by the baseline value of the corresponding dependent variable.

Receiver operating characteristic curve (27) was used to determine MF cut-off points for each test (i.e. handgrip/weight and standing long jump) associated with CVD risk (CVD risk score >1.0 SD) at baseline in children and adolescents. We calculated the AUC, 95% CI, sensitivity, specificity, the Youden index, positive and negative predictive value, and likelihood ratio of a positive and negative test results. Binary logistic regression was used to study whether the stablished cut-off points (high vs low MF) were associated with the CVD risk score (≤1 SD vs >1 SD).

Furthermore, binary logistic regression was performed to analyze the association between the MF cut-off points identified at baseline (independent variable) and CVD risk two-years later (≤1.0 SD vs >1.0 SD [dependent variable]), adjusting by age at follow-up. ANCOVA was also used to study the differences in the CVD risk score (in its continuous form) at follow-up according to MF groups (high vs low) at baseline. CVD risk score was introduced as the dependent variable, MF tests as the independent variable in separate models, and CVD risk score at baseline, and sex and age at follow-up as covariates. The association between MF at baseline and MF at follow-up was analyzed by means of linear regression analysis.

Chapter III: Bidirectionality

Study V: Bidirectional associations between fitness and fatness in youth: A longitudinal study

Significant interactions by sex (males and females) and age groups (children and adolescents) in the studied associations were observed. Consequently, all analyses were performed by sex and age groups. Descriptive statistics are presented as mean ± standard deviation. Paired t-tests were used to check differences in variables of interest between baseline and follow-up measurements.

Since participants of the UP&DOWN study were recruited from different schools, the multilevel approach was considered by following the procedures proposed by Heck, Thomas, & Tabata (28). Thus, we first estimated the intraclass correlation coefficients in order to evaluate how much of the variance of each outcome lies in belonging to a specific school. Given the low intraclass correlation coefficients obtained (i.e. ranging between 1-6%), the multilevel approach was not warranted, and the analyses were reduced to the student level.

Linear regression models were used to assess the associations between CRF

(VO_2max) and body fatness indices (i.e. BMI, ZBMI, ZWHO, %BF and FMI). First, we analyzed the baseline cross-sectional association between CRF as exposure and each body fatness index, separately, as outcomes, after controlling for age, educational centre, SES, mother's education level, and moderate-to-vigorous physical activity at baseline. Second, to examine the longitudinal associations, we used CRF fitness at baseline and body fatness indices at follow-up as exposure and outcomes, respectively. Age, educational centre, SES, and mother's education level at follow-up and moderate-to-vigorous physical activity at baseline were entered as covariates in model 1. Further adjustment by the corresponding body fatness index at baseline were performed (model 2). Finally, we examined the association between changes in CRF (exposure), computed as CRF at follow-up – CRF at baseline, and fatness at follow-up (outcome). Age, educational centre, SES, and mother's education level at follow-up and moderate-to-vigorous physical activity at baseline were entered as covariates (model 1). Further adjustment by the corresponding body fatness index at baseline were performed (model 2).

To test whether there exists bidirectionality in the associations between CRF and body fatness indices, we performed the same analyses but modelling body fatness indices levels at baseline as exposures, and CRF levels at baseline and follow-up as outcomes. The same covariates were introduced in model 1 for cross-sectional and longitudinal analyses. Prospective analyses were further adjusted by baseline CRF levels (model 2).

Supplementary analyses were performed to test whether changes in CRF and fatness over the 2-years period predict changes in fatness and CRF levels, respectively. To do this, different linear regression models were fitted, introducing changes in CRF and fatness as exposures and outcomes alternatively. These analyses were adjusted by age, educational centre, SES, and mother's education level at follow-up, and moderate-to-vigorous physical activity at baseline. Finally, to study the probability of being OW at baseline and at follow-up according to CRF values at baseline, and the probability of having an unhealthy CRF at baseline and at follow-up according to baseline BMI values, we first performed the following categorizations: CRF was divided in two groups (i.e. healthy and unhealthy) using <41.8 ml·kg-1·min-1 and <34.6 ml·kg-1·min-1 as cut-off points for males and females, respectively, as previously suggested (29). Moreover, the study sample was categorised by their BMI levels as NW (including thinness grades 1, 2 and 3) and OW (including obesity) according to international cut-off values (10,11). Subsequently, we fitted binary logistic regression models. CRF (healthy vs. unhealthy) and BMI (NW vs OW) groups at baseline, were alternatively entered as exposures (healthy CRF and NW were taken as the reference groups; i.e. OR=1.0). BMI and CRF groups at baseline and at follow-up were entered as the outcomes, respectively. Cross-sectional analyses were adjusted by age, educational centre, SES, mother's education level, and moderate-to-vigorous physical activity at baseline. Prospective analyses were adjusted by age, educational centre, SES, and mother's education level at follow up, and moderate-to-vigorous physical activity at baseline (model 1). Model 2 was adjusted by model 1 plus baseline BMI or CRF values (model 2), depending on the exposure.

Study VI (part I): The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study

Significant interactions were found for sex and age groups in the studied associations (all $p<0.05$) and all analyses were performed differentiating by sex (i.e. males and females) and population (i.e. children and adolescents). Descriptive statistics are presented as mean (SD). Dependent samples t-test were used to analyze the differences in the studied variables at baseline and at two-year follow-up.

A cross-lagged panel design was employed to assess the tracking in CRF (stages) and NC, and the bidirectional association between these variables (30). Nonetheless, in order to account for different covariates, multiple regression models were used instead of bivariate correlations. The longitudinal associations to assess the stability in CRF and NC (auto-associations) were adjusted by age at baseline. The cross-sectional bidirectional associations between CRF and NC at both time points (synchronous associations) were adjusted by age. The longitudinal bidirectional associations between

CRF and NC (cross-lags) were adjusted by age and the baseline value of the outcome.

Study VII (part I): Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis. The UP&DOWN study

Analyses were performed separating by sex and age groups due to significant interactions observed in the studied associations (all $p<0.05$). Descriptive statistics are presented as means (standard deviation). Differences between baseline and follow-up values were tested through paired t-test.

As in study VI, a cross-lagged panel design was used to assess the stability of MF (global MF) and NC over the two-year period, as well as the cross-sectional and longitudinal bidirectional association between these variables (30). Likewise, multiple regression models were fitted instead of the originally used bivariate correlations. The longitudinal associations to assess the stability in MF and NC (auto-associations) were controlled by age at baseline. The cross-sectional bidirectional associations between MF and NC at both time points (synchronous associations) were adjusted by age. Finally, the longitudinal bidirectional associations between MF and NC (cross-lags) were adjusted by age and the outcome values at baseline.

Chapter IV: Independent effect of fitness and fatness on BP

Study VI (part II): The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study

Multiple regression models were fitted to assess the independence of CRF and NC in the cross-sectional and longitudinal associations with BP measures (i.e. SBP, DBP, and MAP). To analyze the cross-sectional associations, CRF and NC at baseline were included in the same model as exposures, BP measures at baseline as outcomes, and age as covariate. To analyze the longitudinal associations, CRF and NC at baseline were included as exposures in the same model, BP measures at follow-up as outcomes and age and the baseline of the outcome as covariates.

Study VII (part II): Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis. The UP&DOWN study

Multiple regression models were fitted to assess the independent associations between MF and NC at baseline and BP values at baseline and follow-up. Cross-sectional analyses were performed by introducing MF and NC in the same model as exposures; SBP, DBP, and MAP, separately, as outcomes; and age as covariate. Longitudinal associations were tested by introducing MF and NC at baseline in the same model as exposures; BP variables at follow-up, separately, as outcomes; and age and the corresponding outcome value at baseline as covariates.

Study VIII: Fitness, fatness and their association with future blood pressure in youth: The UP&DOWN longitudinal study

Interaction analyses were carried out by multiplying the independent variables (i.e. MF, CRF, and WC) by sex (i.e. males=1, females=2). Then, the exposures (individually), sex and the interaction variables were included in the same regression model for children and adolescents separately. Since no significant interactions were observed between sexes for the studied associations ($p>0.05$), analyses were performed controlling for sex, but separately by age groups (i.e. children and adolescents). Descriptive statistics are presented as the mean (SD) or frequency (percentage) as stated. Paired t-tests were performed to analyze differences between baseline and two-year follow-up.

Multiple linear regression models were fitted to assess the associations between MF variables (i.e. upper-body, lower-body, and global MF), CRF (VO_2max), and WC at baseline and SBP, DBP, and MAP at two-year follow-up. First, we included each exposure (i.e. MF variables, CRF, and WC) and outcome (i.e. SBP, DBP, and MAP) variables individually. These analyses were adjusted by sex, age, SES, and the baseline level of the outcome (model 1). To analyze the independent associations for upper-body, lower-body, and global MF, CRF, and WC with BP variables, we fitted joint models including one of the MF variables, (repeated including each variable once) CRF, and WC, in addition to the covariates in model 1, as independent variables (model 2).

The aforementioned analyses were repeated including changes (i.e. follow-up – baseline) in MF variables, CRF, and WC as the exposures and SBP, DBP, and MAP at two-year follow-up as the outcomes. The same covariates were used in the models. Finally, changes in MF variables, CRF, and WC were introduced as exposures and changes in SBP, DBP, and MAP as the outcomes (supplementary results). These analyses were adjusted by sex, age, and SES at baseline (model 1), and introducing the change values of the main exposures simultaneously (i.e. MF variables, CRF, and WC) (model 2).

Participants were additionally categorized in two groups according to their baseline WC values (i.e. low and high) using previously determined cut-off points (31). ANCOVA was then used to examine differences in SBP, DBP, and MAP at follow-up according to their baseline WC groups (supplementary results). These analyses were adjusted by sex, age, SES, global MF, CRF, and the outcome variable at baseline. All these analyses were repeated using BMI instead of WC (sensitivity analyses).

General overview of the studies' methodology

A summary of the methodologies of the included studies is shown in table 1.

Table 1. General overview of the methods followed in the studies included.

Chapter/Study	Design	Participants	Independent (exposure) variables	Dependent (outcome) variables
Chapter 1: Mediation				
Study I	Cross-sectional	237 children (111 females) 260 adolescents (120 females)	CRF (stages) BMI (mediator)	CVDRF-I
Study II	Cross-sectional	239 children (113 females) 270 adolescents (128 females)	Upper-body MF Lower-body MF Global MF BMI (mediator)	CVDRF-I
Chapter 2: Fitness cut-off points				
Study III	Cross-sectional Longitudinal	236 children (109 females)	CRF (VO_2max)	Sum of two skinfolds SBP HOMA-IR Triglycerides TC/HDL-c CVD risk score
Study IV	Cross-sectional Longitudinal	237 children (110 females) 274 adolescents (131 females)	Upper-body MF Lower-body MF	Sum of two skinfolds SBP HOMA-IR Triglycerides TC/HDL-c CVD risk score
Chapter 3: Bidirectionality				
Study V	Cross-sectional Longitudinal	1082 children (512 females) 727 adolescents (342 females)	CRF (VO_2max) BMI ZBMI ZWHO %BF FMI	CRF (VO2max) BMI ZBMI ZWHO %BF FMI
Study VI (part I)	Cross-sectional Longitudinal	1070 children (507 females) 716 adolescents (337 females)	CRF (stages) NC	CRF (stages) NC
Study VII (part I)	Cross-sectional Longitudinal	1070 children (507 females) 716 adolescents (337 females)	Global MF NC	Global MF NC

Chapter IV: Independent effects of fitness and fatness on BP				
Study VI (part II)	Cross-sectional Longitudinal	1070 children (507 females) 716 adolescents (337 females)	CRF (stages) NC	SBP DBP MAP
Study VII (part II)	Cross-sectional Longitudinal	1070 children (507 females) 716 adolescents (337 females)	Global MF NC	SBP DBP MAP
Study VIII	Longitudinal	1089 children (517 females) 787 adolescents (378 females)	Upper-body MF Lower-body MF Global MF CRF (VO_2max) WC	SBP DBP MAP

BMI: body mass index; CRF: cardiorespiratory fitness; CVDRF-I: cardiovascular disease risk factor index; FMI: fat mass index; HDL-c: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment for insulin resistance; MF: muscular fitness; NC: neck circumference; SBP: systolic blood pressure; TC: total cholesterol; VO_2max: maximal oxygen uptake; WC: waist circumference; ZBMI: sex- and age-standardized values of body mass index; ZWHO: standardized BMI according to reference data from the World Health Organization; %BF: body fat percentage

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RESULTS

RESULTS

The results of the individual studies included in the present International Doctoral Thesis are presented below.

Supplementary tables and figures of the included studies are enclosed at the end of the results section.

Chapter I: Mediation

Study I: The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of body mass index in youth: The UP&DOWN Study

Participant's characteristics are depicted in table 2. In children, males reached higher scores in the 20-m shuttle run test than females ($p<0.001$) and females had higher levels of triglycerides than males ($p=0.037$). In adolescents, males were significantly heavier and taller than females ($p=0.005$ and $p<0.001$, respectively) and presented higher levels of WC, SBP (both $p<0.001$) and glucose ($p=0.029$). Besides, males performed better in the 20-m shuttle run test compared to females ($p<0.001$).

Associations between CRF and CVDRF-I are presented in table 3. CRF was significantly associated with CVDRF-I in male ($\beta=-0.437$; $p<0.001$) and female ($\beta=-0.305$; $p=0.001$) children, and in male ($\beta=-0.404$; $p<0.001$) and female ($\beta=-0.191$; $p=0.048$) adolescents when adjusting by age (model 1). However, these associations were no longer significant after including BMI in the model (model 2). The results were similar when using CVDRF-II as the dependent variable (table S1 in supplementary content).

Associations between BMI and CVDRF-I are shown in table 4. BMI showed a positive association with CVDRF-I in male and female children ($\beta=0.688$ and $\beta=0.709$, respectively) and in male and female adolescents ($\beta=0.654$ and $\beta=0.509$, respectively) (all $p<0.001$) in model 1. When CRF level was added in model 2, these associations remained in all sex and age groups (all $p<0.001$). The same pattern was observed when using CVDRF-II (table S2 in supplementary content).

Combined effects of CRF and BMI on CVDRF-I are displayed in figure 1. In children, those participants categorized in the NW groups (i.e. NW+Low CRF or NW+High CRF) had significantly lower CVDRF-I levels than the OW+Low CRF group in males and females and the OW+High CRF group in females (all $p<0.05$).

Table 2. Participants' characteristics by sex and age groups.

Variable	Children			Adolescents		
	Males (n=126)	Females (n=111)	p	Males (n=140)	Females (n=120)	p
Age (years)	8.1 (1.5)	8.0 (1.5)	0.843	14.1 (1.7)	13.9 (1.5)	0.185
Weight (kg)	30.6 (8.3)	31.0 (11.0)	0.765	56.7 (14.4)	52.3 (10.4)	**0.005**
Height (cm)	128.8 (9.9)	129.2 (12.1)	0.801	163.1 (12.4)	157.2 (7.0)	**<0.001**
Body mass index (kg/cm²)	18.2 (2.8)	18.1 (3.6)	0.799	21.0 (3.6)	21.1 (3.4)	0.942
Waist circunference (cm)	59.4 (7.1)	58.0 (8.6)	0.166	70.3 (8.3)	65.9 (7.2)	**<0.001**
Systolic blood pressure (mmHg)	101.7 (11.0)	99.6 (11.49)	0.144	112.6 (15.5)	105.9 (9.9)	**<0.001**
Glucose (mg/dl)	61.9 (18.1)	63.3 (16.5)	0.537	81.1 (16.6)	76.6 (16.1)	**0.029**
Triglycerides (mg/dl)*	41.0 (20.5)	46.3 (17.9)	**0.037**	54.2 (36.7)	58.1 (30.1)	0.361
High-density lipoprotein cholesterol (mg/dl)	40.7 (17.6)	42.0 (15.7)	0.556	47.8 (15.2)	48.7 (15.5)	0.627
20-m shuttle run test (stages)	3.3 (1.7)	2.3 (1.6)	**<0.001**	7.0 (2.5)	4.3 (1.8)	**<0.001**

Values are presented as mean (standard deviation).

*Values were natural log-transformed before analysis, but non-transformed values are presented.

Statistically significant differences between sexes are highlighted in bold.

Table 3. Associations between cardiorespiratory fitness and the cardiovascular disease risk factor index (CVDRF-I) in children and adolescents.

	Children							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
20-m shuttle run test (stages)								
Model 1	-0.114	-0.158;-0.070	-0.437	**<0.001**	-0.095	-0.150;-0.039	-0.305	**0.001**
Model 2	-0.024	-0.065;0.017	-0.091	0.254	0.001	-0.043;0.046	0.004	0.956
	Adolescents							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
20-m shuttle run test (stages)								
Model 1	-0.089	-0.129; -0.049	-0.404	**<0.001**	-0.045	-0.090; 0.000	-0.191	**0.048**
Model 2	-0.017	-0.051; 0.016	-0.077	0.315	-0.020	-0.061; 0.021	-0.085	0.334

Model 1: analyses controlled for age. Model 2: analyses controlled for model 1 + body mass index. B: unstandardized coefficient; β: standardized coefficient; CI, confidence interval. Statistically significant values are highlighted in bold.

Table 4. Associations between body mass index and the cardiovascular disease risk factor index (CVDRF-I) in children and adolescents.

	Children							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Body mass index								
Model 1	0.108	0.087;0.128	0.688	**<0.001**	0.097	0.079;0.114	0.709	**<0.001**
Model 2	0.101	0.077;0.125	0.641	**<0.001**	0.097	0.077;0.117	0.711	**<0.001**
	Adolescents							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Body mass index								
Model 1	0.101	0.084; 0.118	0.654	**<0.001**	0.062	0.041; 0.083	0.509	**<0.001**
Model 2	0.097	0.078; 0.115	0.628	**<0.001**	0.060	0.038; 0.081	0.490	**<0.001**

Model 1: analyses controlled for age. Model 2: analyses controlled for model 1 + 20-m shuttle run test (stages). B: unstandardized coefficient; β: standardized coefficient; CI, confidence interval. Statistically significant values are highlighted in bold.

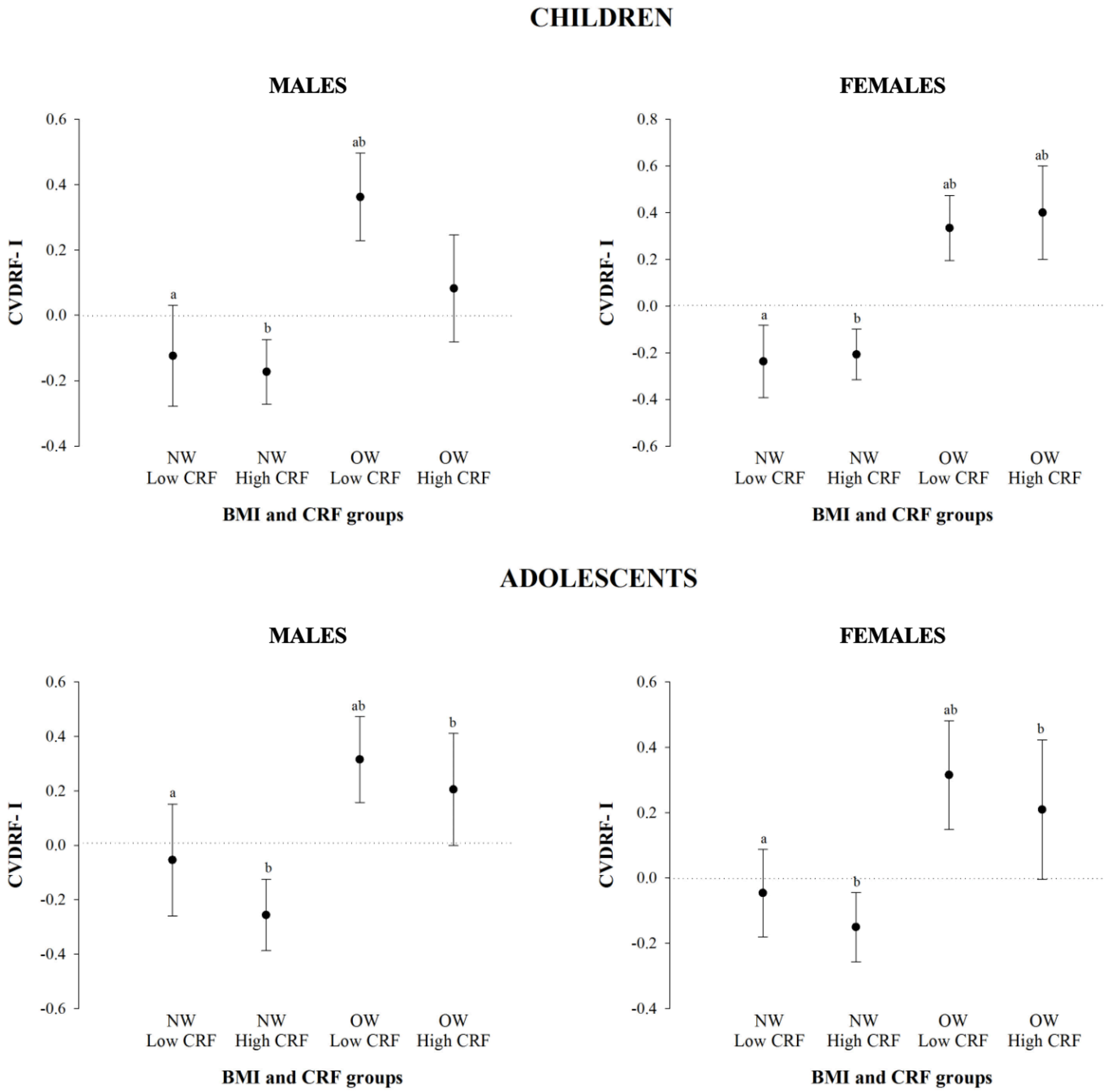

Figure 1. Combined effects of body mass index (BMI) and cardiorespiratory fitness (CRF) on cardiovascular disease risk factor index (CVDRF-I) in children and adolescents. Dots and error bars indicate estimated means and 95% CI, respectively, after adjustment for age. NW: normal weight including thinness grades 1, 2 and 3; OW: overweight including obesity. Common superscripts denote statistically significant differences at level $p<0.05$ after Bonferroni's correction.

In adolescents, the NW+High CRF group displayed lower CVDRF-I than their OW counterparts regardless of CRF levels ($p<0.05$). Moreover, the NW+Low CRF group presented lower CVDRF-I compared to the OW+Low CRF group ($p<0.05$). When CVDRF-II was used in the analyses instead of CVDRF-I, the pattern in female children remained the same. However, significant differences only persisted between NW+High CRF and OW+Low CRF groups in male children and male adolescents ($p<0.05$) (figure S1 in supplementary content).

Results of mediation analyses are depicted in figure 2. Regression equations 1, 2 and 3 were significant in male (all $p<0.001$) and female (all $p<0.01$) children, and in male (all $p<0.001$) and female (all $p<0.05$) adolescents. Regression coefficients in equation 3' were attenuated compared to equation 2 and they were no longer significant either in children and adolescents of both sexes. The percentage of the total effect of CRF on CVDRF-I that is carried by BMI was 79.5% for male ($z=-5.287$, $p<0.001$) and 100% for female ($z=-4.437$, $p<0.001$) children, and 81.2% for male ($z=-4.772$, $p<0.001$) and 55.7% for female ($z=-2.231$, $p<0.05$) adolescents. When CVDRF-II was entered as the dependent variable (figure S2 in supplementary content), results of the different regression equations were similar in male and female children and male adolescents. In contrast, the Sobel test for female adolescents was not significant ($z=-1.655$, $p>0.05$).

All the aforementioned analyses were repeated adjusting for pubertal development instead of age, and using %BF instead of BMI, and the results did not materially change (data not shown).

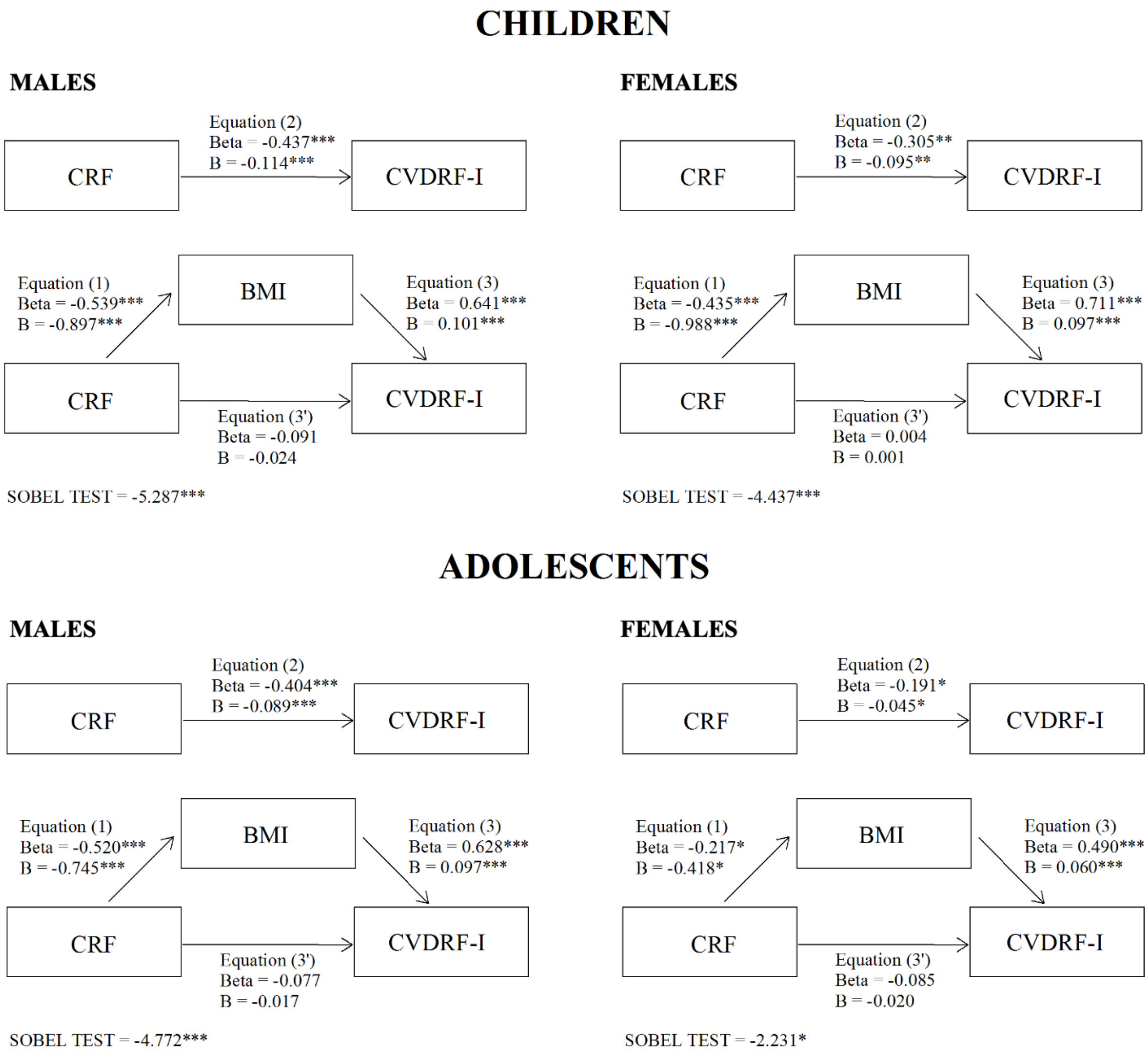

Figure 2. Mediating effect of body mass index (BMI) in the association between cardiorespiratory fitness (CRF) and cardiovascular disease risk factor index (CVDRF-I) in children and adolescents. Standardized (Beta) and unstandardized (B) regression coefficients are shown for equation (1) (involving the independent and mediator variables), equation (2) (involving the independent and dependent variables), equation (3) (involving the mediator and dependent variables) and equation (3') (the direct effect of the independent variable on the dependent variable). All analyses were adjusted by age. * p<0.05; ** p<0.01; *** p<0.001.

Table S1. Associations between cardiorespiratory fitness and the cardiovascular disease risk factor index (CVDRF-II) in children and adolescents.

	Children							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
20-m shuttle run test (stages)								
Model 1	-0.080	-0.124;-0.037	-0.341	**<0.001**	-0.057	-0.111;-0.003	-0.200	**0.040**
Model 2	-0.036	-0.085;0.012	-0.154	0.142	0.001	-0.054;0.056	0.002	0.980

	Adolescents							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
20-m shuttle run test (stages)								
Model 1	-0.066	-0.105; -0.027	-0.321	**0.001**	-0.050	-0.093; -0.006	-0.214	**0.027**
Model 2	-0.023	-0.063; 0.016	-0.114	0.241	-0.038	-0.082; 0.006	-0.165	0.090

Model 1: analyses controlled for age. Model 2: analyses controlled for model 1 + body mass index. B: unstandardized coefficient; β: standardized coefficient; CI, confidence interval. Statistically significant values are highlighted in bold.

Table S2. Associations between body mass index and the cardiovascular disease risk factor index (CVDRF-II) in children and adolescents.

	Children							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Body mass index								
Model 1	0.060	0.036;0.084	0.425	**<0.001**	0.058	0.037;0.080	0.465	**<0.001**
Model 2	0.049	0.021;0.077	0.347	**0.001**	0.058	0.034;0.083	0.466	**<0.001**
	Adolescents							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Body mass index								
Model 1	0.062	0.042; 0.083	0.435	**<0.001**	0.031	0.009; 0.054	0.262	**0.008**
Model 2	0.057	0.035; 0.079	0.397	**<0.001**	0.027	0.004; 0.050	0.225	**0.024**

Model 1: analyses controlled for age. Model 2: analyses controlled for model 1 + 20-m shuttle run test (stages). B: unstandardized coefficient; β: standardized coefficient; CI, confidence interval. Statistically significant values are highlighted in bold.

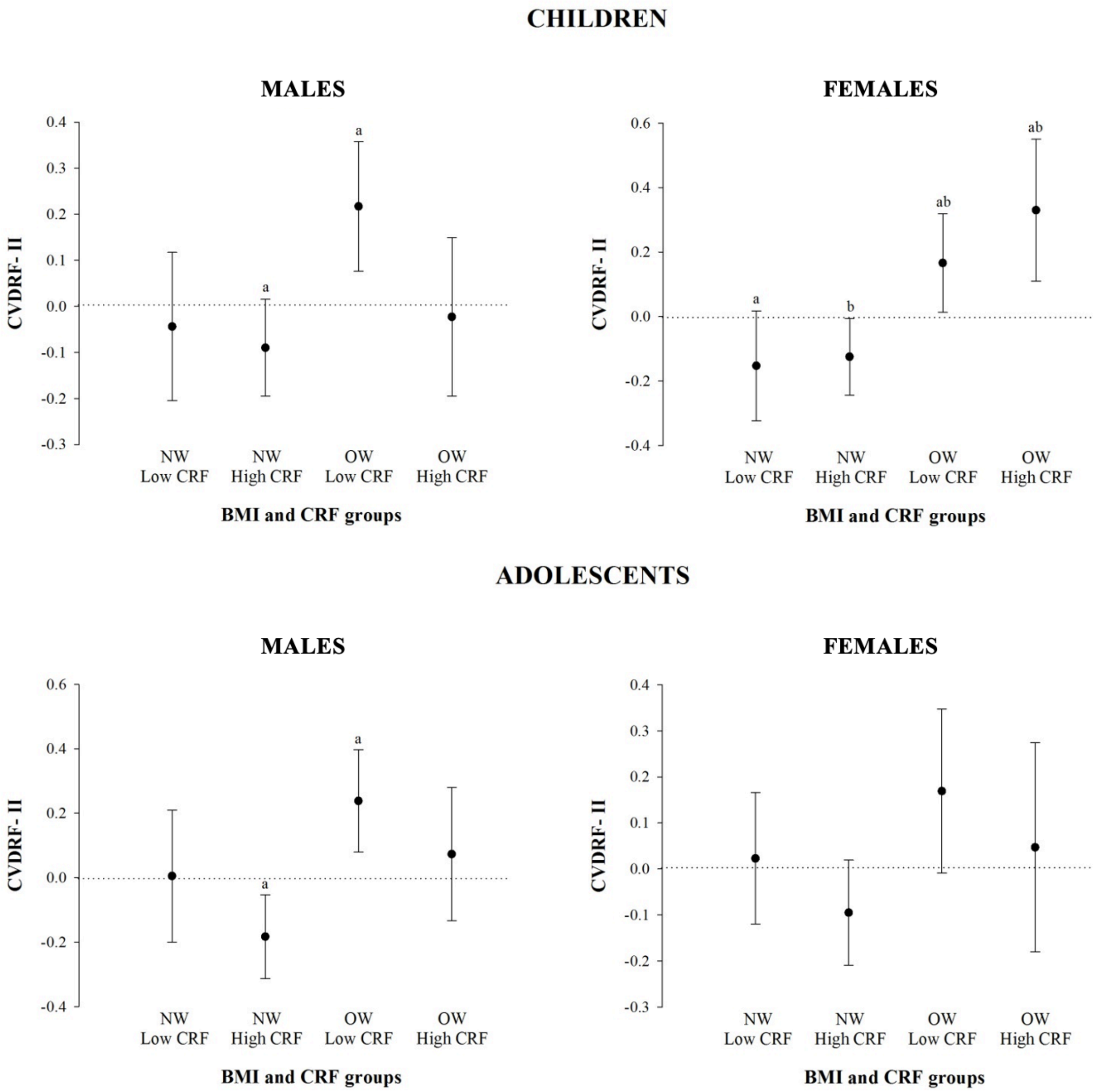

Figure S1. Combined effects of body mass index (BMI) and cardiorespiratory fitness (CRF) on cardiovascular disease risk factor index excluding waist circumference (CVDRF-II) in children and adolescents. Dots and error bars indicate estimated means and 95% CI, respectively, after adjustment for age. NW: normal weight including thinness grades 1, 2 and 3; OW: overweight including obesity. Common superscripts denote statistically significant differences at level $p<0.05$ after Bonferroni's correction.

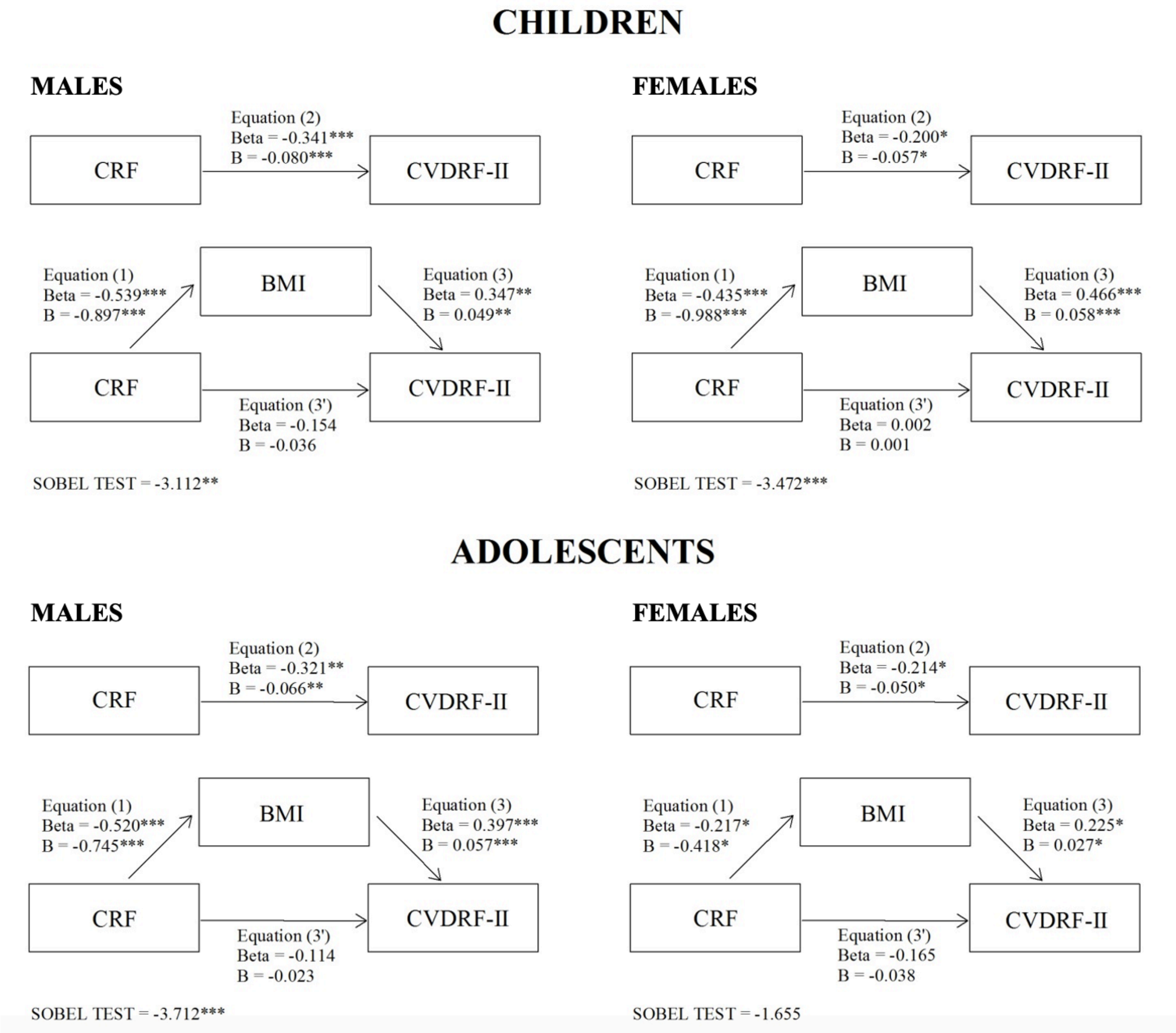

Figure S2. Mediating effect of body mass index (BMI) in the association between cardiorespiratory fitness (CRF) and cardiovascular disease risk factor index excluding waist circumference (CVDRF-II) in children and adolescents. Standardized (Beta) and unstandardized (B) regression coefficients are shown for equation (1) (involving the independent and mediator variables), equation (2) (involving the independent and dependent variables), equation (3) (involving the mediator and dependent variables) and equation (3') (the direct effect of the independent variable on the dependent variable). All analyses were adjusted by age. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Study II: The role of adiposity in the association between muscular fitness and cardiovascular disease

Participant's characteristics are shown in table 5. In children, males presented higher levels of handgrip/weight ($p=0.023$) and standing long jump ($p=0.004$) than females, while females' triglyceride and %BF levels were higher than in males ($p=0.037$ and $p=0.035$, respectively). In adolescents, males had higher levels of WC, SBP (both $p<0.001$) and glucose ($p=0.044$) than females. Moreover, males presented higher scores in MF tests (all $p<0.001$). Females had higher %BF levels than males ($p<0.001$).

The association of MF with CVDRF-I is presented in table 6. In children, there was an inverse association between the different MF tests and the global MF index with CVDRF-I (all $p<0.05$) in model 1. These associations disappeared when we adjusted by BMI (model 2) in both sexes. In male adolescents, higher scores in the handgrip/weight test ($\beta=-0.395$; $p<0.001$) and in global MF ($\beta=-0.416$; $p<0.001$) were negatively associated with CVDRF-I (model 1). However, these associations were no longer significant after further adjustment for BMI (model 2). The standing long jump test was not significantly associated with CVDRF-I. In female adolescents, only handgrip/weight was associated with CVDRF-I ($\beta=-0.177$; $p=0.045$), but the association did not persist after adjusting by BMI in model 2. Similar results were found for male children and male and female adolescents when using CVDRF-II as the dependent variable. Nonetheless, there were no association between MF tests and global MF in female children in model 1 (table S3 in supplementary content).

The association between BMI and CVDRF-I is depicted in table 7. BMI was positively associated with CVDRF-I in children and adolescents of both sexes (all $p<0.001$) (model 1). This association remained significant after further adjustment for handgrip/weight (model 2), standing long jump (model 3) and global MF (model 4) (all $p<0.001$). The same results were obtained when we used CVDRF-II in the analyses (table S4 in supplementary content).

The combined effect of MF and BMI on CVDRF-I is shown in figure 3. In children, OW/Low MF males had significantly higher levels of CVDRF-I compared with the rest of groups (all $p<0.05$).

Table 5. Participants' characteristics by sex.

Variable	Children			Adolescents		
	Males (n=126)	Females (n=113)	p	Males (n=142)	Females (n=128)	p
Age (years)	7.6 (1.5)	7.5 (1.5)	0.662	13.6 (1.7)	13.5 (1.6)	0.464
Weight (kg)	30.6 (8.3)	30.9 (10.9)	0.810	56.4 (13.9)	52.8 (10.2)	**0.015**
Height (cm)	128.8 (9.9)	129.0 (12.2)	0.908	163.0 (12.1)	157.4 (6.6)	**<0.001**
Body mass index (kg/cm²)	18.2 (2.8)	18.1 (3.6)	0.821	21.0 (3.5)	21.2 (3.5)	0.569
Normal weight, n (%)	77 (61.1)	70 (61.9)		83 (58.5)[a]	91 (71.1)[a]	
Overweight, n (%)	49 (38.9)	43 (38.1)		59 (41.5)[b]	37 (28.9)[b]	
Waist circumference (cm)	59.4 (7.1)	58.0 (8.5)	0.165	70.2 (8.2)	66.2 (7.0)	**<0.001**
Body fat (%)	19.3 (7.7)	21.5 (8.3)	**0.035**	19.6 (11.2)	25.3 (7.2)	**<0.001**
Systolic blood pressure (mmHg)	101.7 (11.0)	99.4 (11.6)	0.106	112.8 (15.5)	106.8 (10.4)	**<0.001**
Glucose (mg/dl)	61.9 (18.1)	63.1 (16.5)	0.609	80.9 (16.5)	76.8 (16.1)	**0.040**
Triglycerides (mg/dl)	41.0 (20.5)	46.3 (17.9)	**0.037**	54.5 (36.5)	58.0 (29.4)	0.391
High-density lipoprotein cholesterol (mg/dl)	40.7 (17.6)	41.6 (15.8)	0.666	47.5 (15.1)	48.8 (15.4)	0.485
Handgrip strength (kg)	12.4 (3.6)	11.6 (3.6)	0.083	28.5 (9.4)	22.7 (4.9)	**<0.001**
Handgrip strength/weight	0.41 (0.08)	0.39 (0.08)	**0.023**	0.50 (0.11)	0.43 (0.08)	**<0.001**
Standing long jump (cm)	114.8 (21.2)	106.5 (22.9)	**0.004**	175.3 (33.2)	141.1 (21.3)	**<0.001**

Values are presented as mean (standard deviation) or frequency (percentage). Statistically significant differences between sexes are highlighted in bold. Superscripts with the same letter indicate statistically significant differences at the level p<0.05 in the proportions of males and females.

Table 6. Associations between muscular fitness and the cardiovascular disease risk factor index (CVDRF-I) in children and adolescents.

	Children							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Upper muscular fitness (handgrip strength / weight)								
Model 1	-2.115	-3.061;-1.169	-0.357	**<0.001**	-2.001	-3.034;-0.968	-0.319	**<0.001**
Model 2	-0.069	-0.942;0.805	-0.012	0.877	0.580	-0.341;1.500	0.093	0.215
Lower muscular fitness (standing long jump)								
Model 1	-0.007	-0.011;-0.003	-0.332	**<0.001**	-0.005	-0.009;0.000	-0.212	**0.038**
Model 2	0.000	-0.003;0.003	-0.003	0.970	0.002	-0.001;0.006	0.103	0.180
Muscular fitness (upper + lower; z-score)								
Model 1	-0.211	-0.298;-0.124	-0.406	**<0.001**	-0.183	-0.286;-0.079	-0.310	**0.001**
Model 2	-0.005	-0.089;0.079	-0.010	0.904	0.071	-0.019;0.161	0.120	0.120

	Adolescents							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Upper muscular fitness (handgrip/weight)								
Model 1	-1.986	-2.899;-1.073	-0.395	**<0.001**	-0.960	-1.896;-0.023	-0.177	**0.045**
Model 2	0.176	-0.627;0.980	0.035	0.665	0.257	-0.696;1.210	0.047	0.594
Lower muscular fitness (standing long jump)								
Model 1	-0.004	-0.007;0.000	-0.225	0.060	-0.001	-0.004;0.003	-0.026	0.779
Model 2	0.001	-0.002;0.004	0.073	0.415	0.002	-0.001;0.006	0.120	0.156
Global muscular fitness (upper + lower; z-score)								
Model 1	-0.239	-0.365;-0.113	-0.416	**<0.001**	-0.062	-0.151;0.027	-0.124	0.168
Model 2	0.04	-0.067;0.147	0.070	0.460	0.055	-0.034;0.143	0.109	0.224

Model 1: analyses controlled for age. Model 2: analyses controlled for model 1 + body mass index. B: unstandardized coefficient; β: standardized coefficient. Statistically significant values are highlighted in bold.

Table 7. Associations between body mass index and the cardiovascular disease risk factor index (CVDRF-I) in children and adolescents.

	Children							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Body mass index								
Model 1	0.110	0.090;0.131	0.684	**<0.001**	0.099	0.081;0.116	0.704	**<0.001**
Model 2	0.109	0.085;0.134	0.678	**<0.001**	0.106	0.085;0.128	0.760	**<0.001**
Model 3	0.110	0.087;0.134	0.683	**<0.001**	0.104	0.085;0.123	0.740	**<0.001**
Model 4	0.109	0.084;0.135	0.679	**<0.001**	0.108	0.087;0.129	0.771	**<0.001**
	Adolescents							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Body mass index								
Model 1	0.100	0.084;0.117	0.663	**<0.001**	0.061	0.041;0.082	0.502	**<0.001**
Model 2	0.103	0.083;0.122	0.678	**<0.001**	0.064	0.041;0.087	0.526	**<0.001**
Model 3	0.102	0.085;0.120	0.677	**<0.001**	0.066	0.044;0.087	0.538	**<0.001**
Model 4	0.104	0.085;0.123	0.686	**<0.001**	0.068	0.045;0.091	0.555	**<0.001**

Model 1: analyses controlled for age. Model 2: analyses controlled for model 1 + handgrip strength / weight. Model 3: analyses controlled for model 1 + standing long jump. Model 4: analyses controlled for model 1 + muscular fitness index. B: unstandardized coefficient; β: standardized coefficient. Statistically significant values are highlighted in bold.

In females, those in the NW groups (i.e. NW+Low MF and NW+High MF) had lower levels of CVDRF-I than their OW counterparts regardless of MF level (all p<0.05). In adolescents, males who presented a favourable weight status (i.e. NW+Low MF or NW+High MF groups) displayed significantly lower CVDRF-I than those with an unfavourable weight status (i.e. OW+Low MF or OW+High MF groups) independently of their MF level (all p<0.05). In females, the group with the best profile (i.e. NW+High MF) showed lower CVDRF-I than the OW groups, regardless of the level of MF (all p<0.05). Furthermore, among females with low MF levels, those who presented a better weight status (i.e. NW+Low MF group) had significantly lower CVDRF-I than their OW counterparts (p=0.003). When using CVDRF-II as the dependent variable, the results remained similar in female children. Apart from this, significant differences were only observed between the NW+High MF and OW+Low MF groups in male children (p=0.003) and between the NW+High MF and OW+High MF groups in male adolescents (p=0.011) (figure S3 in supplementary content).

The mediating effect of BMI in the association between global MF and CVDRF-I is presented in figure 4. In children, a negative association was found between MF and BMI in equation 1 in both sexes (both p<0.001). In equation 2, MF was negatively associated with CVDRF-I in males (p<0.001) and females (p=0.001). In equation 3, using both MF and BMI as independent variables, a positive association was observed between BMI and CVDRF-I in males and females (both p<0.001), and the regression coefficient between MF and CVDRF-I (equation 3') were attenuated compared with equation 2 in both males (β=-0.010 vs. β=-0.406, respectively) and females (β=-0.120 vs. β=-0.310, respectively) and these associations were no longer significant. The Sobel test confirmed the mediating role of BMI in the association between MF and CVDRF-I in males (z=-5.664, p<0.001) and females (z=-5.705, p<0.001). In male adolescents, equations 1, 2 and 3 were significant (all p<0.001) and followed the same direction as in children. The regression coefficient between MF and CVDRF-I (equation 3') was not significant and it was attenuated compared with equation 2 (β=-0.070 vs. β=-0.416, respectively). The Sobel test was significant (z=-5.525, p<0.001). In female adolescents, since there was not a significant association between

MF and CVDRF-I in equation 2, no further mediation role of BMI was studied. Similar results were found when we performed analyses with CVDRF-II, except for female children because of the lack of association between MF and CVDRF-II (figure S4 in supplementary content).

Mediation analyses performed by the bootstrap method showed similar results on the mediating role of BMI in the association between global MF and CVDRF-I (data not shown). We also repeated all analyses using %BF instead of BMI as a measure of fatness and the results persisted (data not shown).

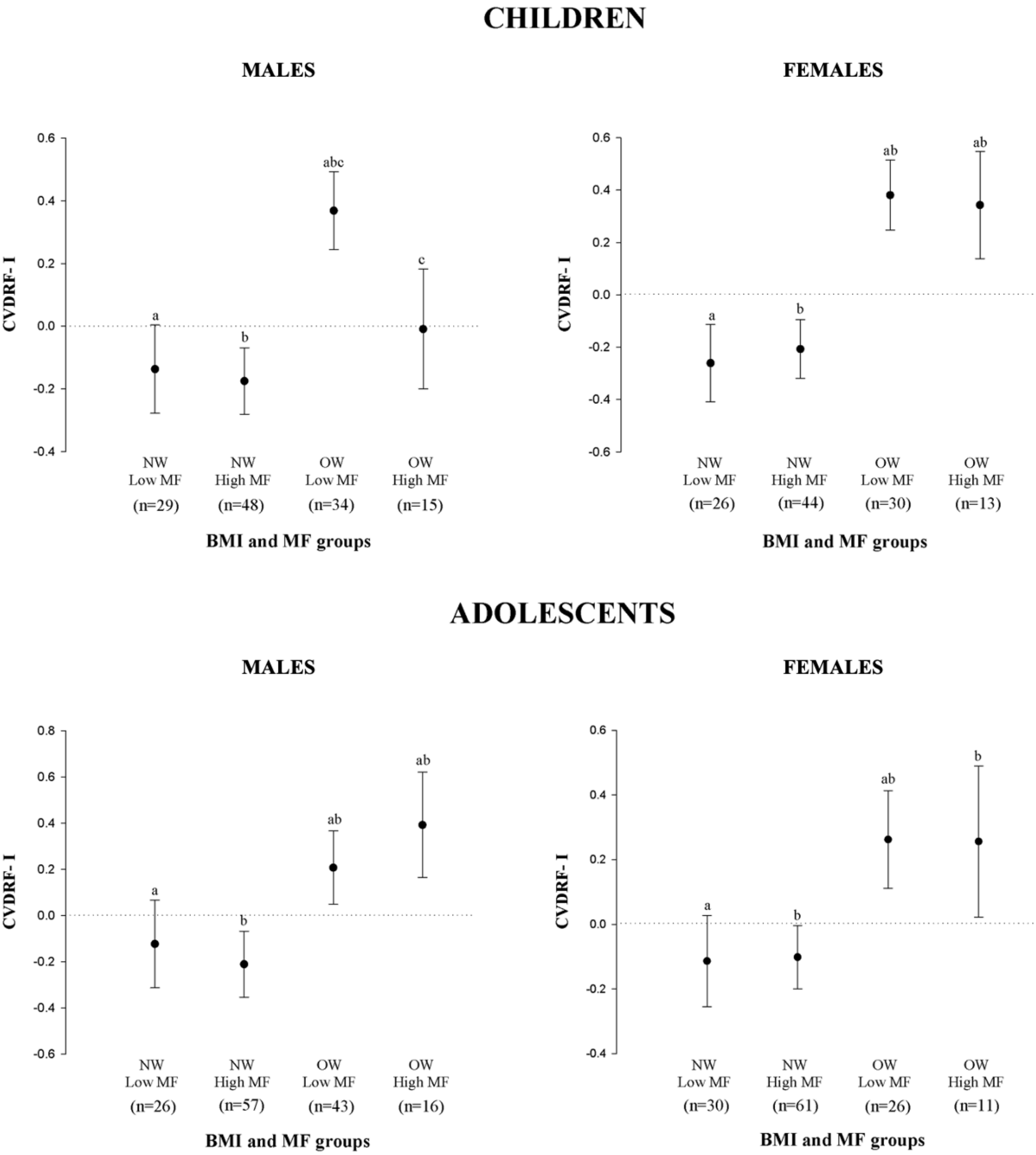

Figure 3. Combined effects of muscular fitness (MF) and body mass index (BMI) on cardiovascular disease risk factors index (CVDRF-I) in children and adolescents. Results are presented as estimated mean (dots) and 95% CI (error bars) after adjustment for age. NW: normal weight including thinness grades 1, 2 and 3; OW: overweight including obesity. Common superscripts indicate statistically significant differences between groups at level $p < 0.05$ after Bonferroni's correction.

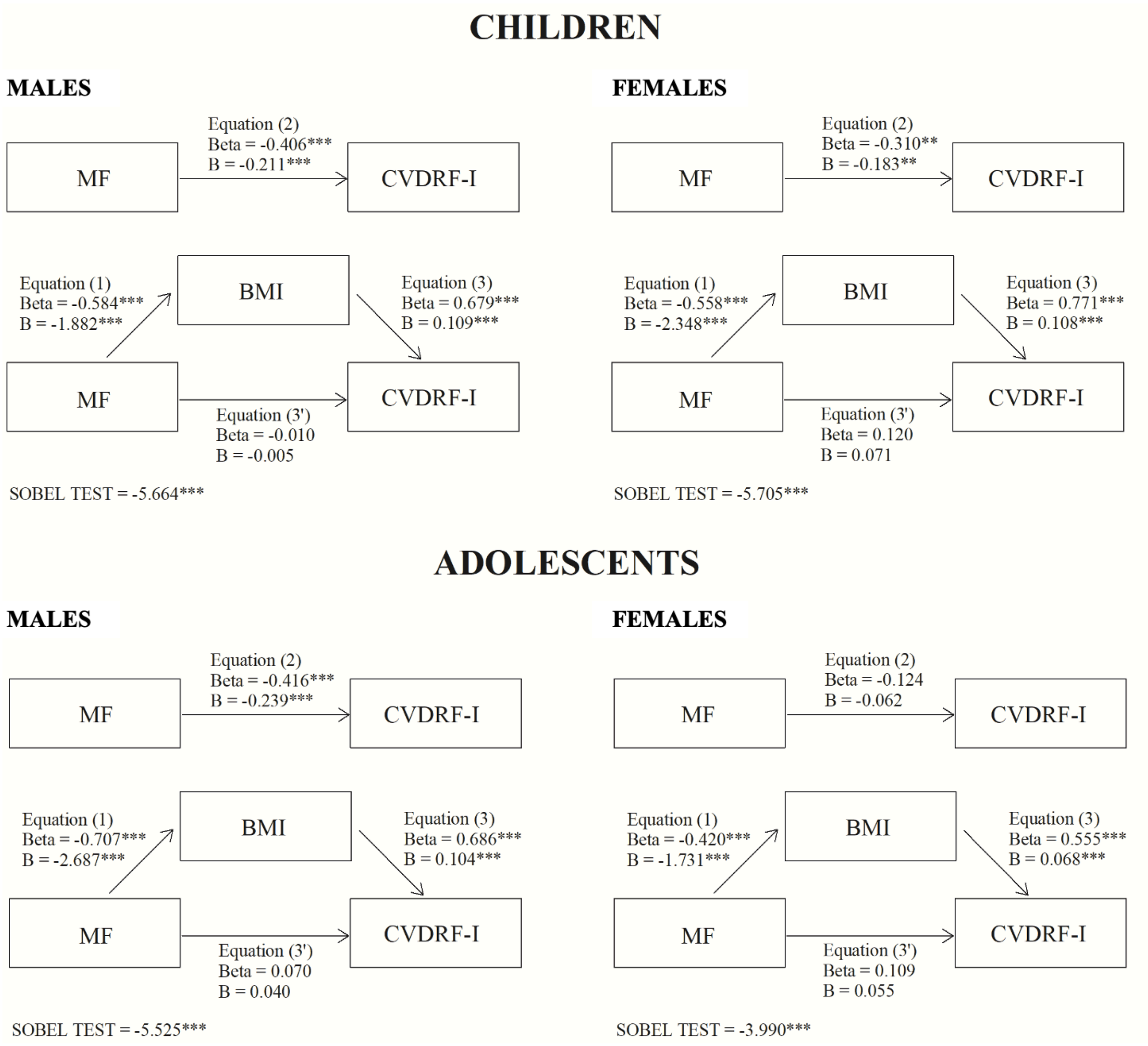

Figure 4. Mediating effect of body mass index (BMI) in the association between muscular fitness (MF) and cardiovascular disease risk factors index (CVDRF-I) in children and adolescents. Results are presented as standardized (Beta) and unstandardized (B) regression coefficients. All analyses were adjusted by age. Equation (1): the effect of MF on BMI; Equation (2): the effect of MF on CVDRF-I; Equation (3): the effect of BMI on CVDRF-I; Equation (3'): the direct effect of MF on CVDRF-I. * $p < 0.05$; ** $p < 0.01$; ***; $p < 0.001$.

Table S3. Associations between muscular fitness and the cardiovascular disease risk factor index (CVDRF-II) in children and adolescents.

	Children							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Upper muscular fitness (handgrip strength / weight)								
Model 1	-1.152	-2.089;-0.214	-0.216	**0.016**	-0.971	-2.003;0.061	-0.168	0.065
Model 2	0.043	-0.987;1.073	0.008	0.934	0.727	-0.398;1.853	0.126	0.203
Lower muscular fitness (standing long jump)								
Model 1	-0.004	-0.008;0.000	-0.214	**0.031**	-0.002	-0.006;0.002	-0.093	0.384
Model 2	0.000	-0.004;0.004	-0.006	0.955	0.003	-0.002;0.007	0.125	0.222
Muscular fitness (upper + lower; z-score)								
Model 1	-0.118	-0.205;-0.032	-0.253	**0.008**	-0.084	-0.186;0.019	-0.154	0.109
Model 2	0.001	-0.098;0.100	0.002	0.986	0.084	-0.026;0.194	0.155	0.133
	Adolescents							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Upper muscular fitness (handgrip strength / weight)								
Model 1	-1.098	-1.994;-0.202	-0.235	**0.017**	-0.221	-1.172;0.729	-0.041	0.646
Model 2	0.263	-0.688;1.214	0.056	0.585	0.449	-0.597;1.494	0.084	0.397
Lower muscular fitness (standing long jump)								
Model 1	-0.002	-0.006;0.002	-0.134	0.272	0.000	-0.004;0.003	-0.012	0.900
Model 2	0.001	-0.002;0.004	0.063	0.578	0.001	-0.002;0.005	0.061	0.517
Muscular fitness (upper + lower; z-score)								
Model 1	-0.132	-0.254;-0.010	-0.247	**0.034**	-0.016	-0.105;0.073	-0.032	0.724
Model 2	0.042	-0.084;0.169	0.079	0.507	0.046	-0.052;0.143	0.092	0.358

Model 1: analyses controlled for age. Model 2: analyses controlled for model 1 + body mass index. B: unstandardized coefficient; β: standardized coefficient. Statistically significant values are highlighted in bold.

Table S4. Associations between body mass index and the cardiovascular disease risk factor index (CVDRF-II) in children and adolescents.

	Children							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Body mass index								
Model 1	0.063	0.039;0.088	0.436	**<0.001**	0.060	0.039;0.082	0.468	**<0.001**
Model 2	0.064	0.035;0.093	0.440	**<0.001**	0.070	0.044;0.096	0.543	**<0.001**
Model 3	0.063	0.035;0.090	0.433	**<0.001**	0.066	0.042;0.089	0.511	**<0.001**
Model 4	0.063	0.033;0.093	0.437	**<0.001**	0.071	0.045;0.097	0.554	**<0.001**
	Adolescents							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Body mass index								
Model 1	0.061	0.041;0.081	0.435	**<0.001**	0.030	0.008;0.053	0.251	**0.009**
Model 2	0.065	0.041;0.088	0.459	**<0.001**	0.035	0.010;0.061	0.294	**0.007**
Model 3	0.063	0.042;0.084	0.447	**<0.001**	0.032	0.009;0.056	0.270	**0.008**
Model 4	0.065	0.042;0.088	0.462	**<0.001**	0.036	0.010;0.061	0.296	**0.006**

Model 1: analyses controlled for age. Model 2: analyses controlled for model 1 + handgrip strength / weight. Model 3: analyses controlled for model 1 + standing long jump. Model 4: analyses controlled for model 1 + muscular fitness index. B: unstandardized coefficient; β: standardized coefficient. Statistically significant values are highlighted in bold.

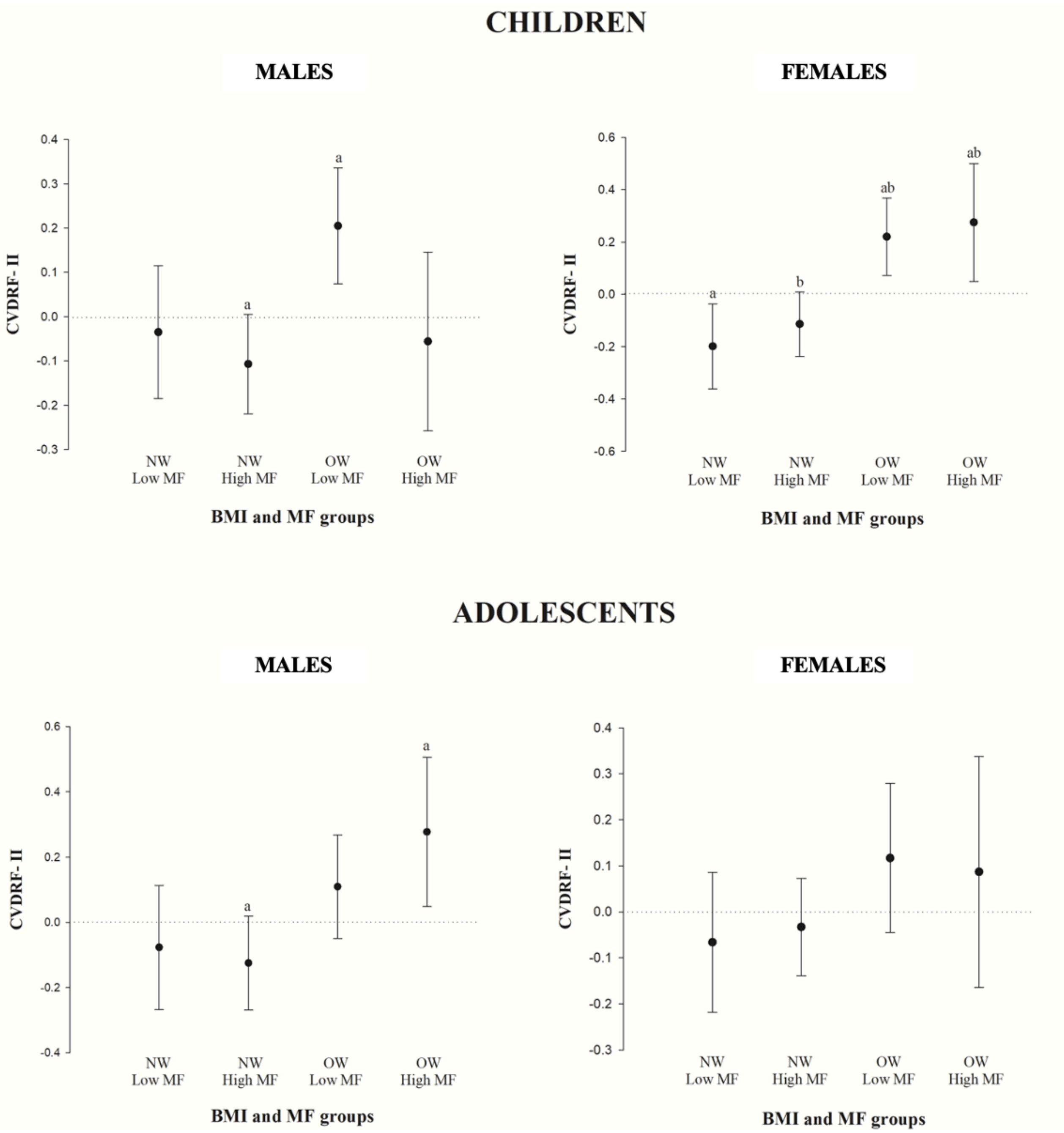

Figure S3. Combined effect of muscular fitness (MF) and body mass index (BMI) on cardiovascular disease risk factors index excluding waist circumference (CVDRF-II), in children and adolescents. Results are presented as estimated mean (dots) and 95% CI (error bars) after adjustment for age. NW: normal weight including thinness grades 1, 2 and 3; OW: overweight including obesity. Common superscripts denote statistically significant differences at level $p<0.05$ after Bonferroni's correction.

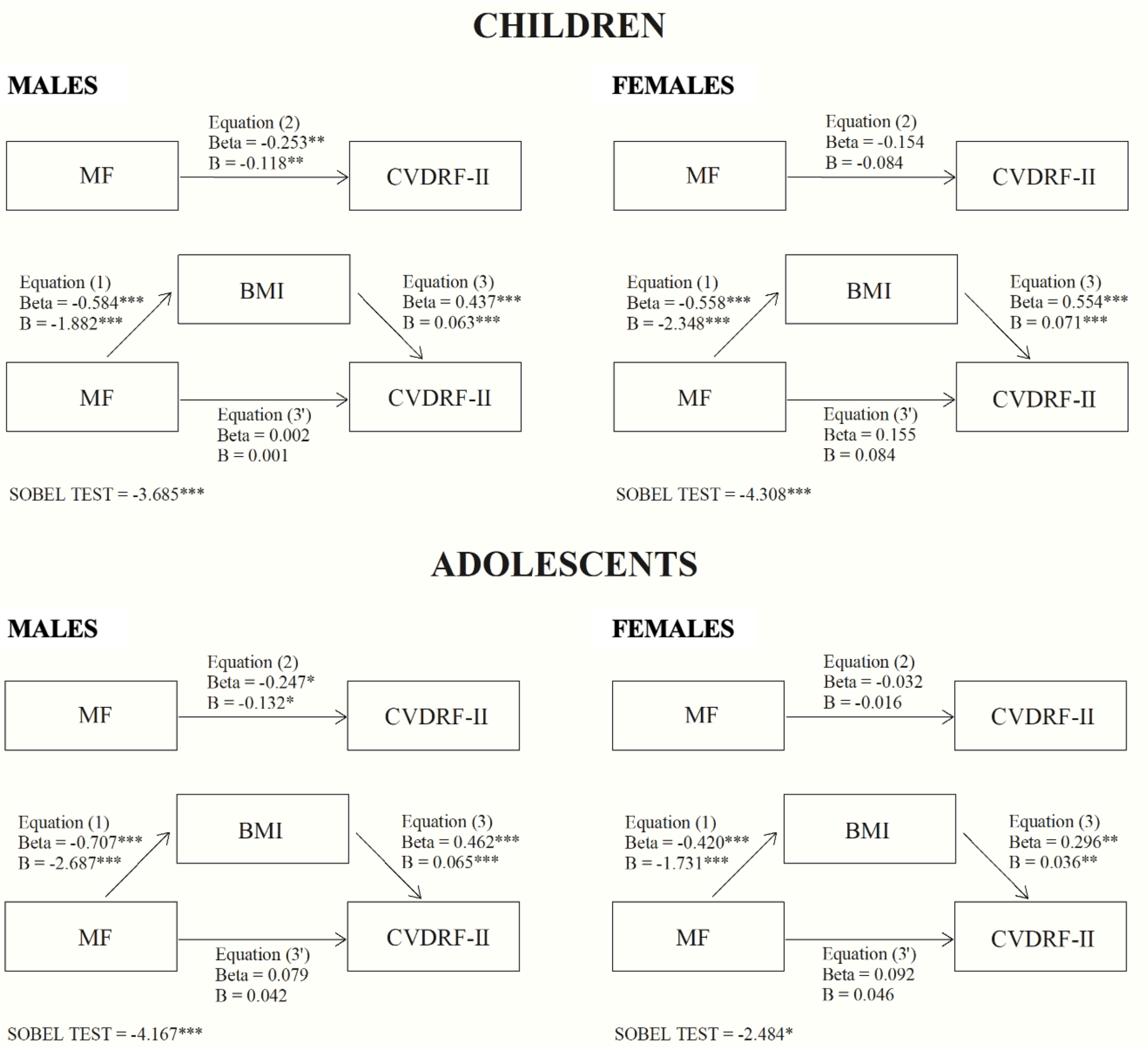

Figure S4. Mediating effect of body mass index (BMI) in the association between muscular fitness (MF) and cardiovascular disease risk factors index excluding waist circumference (CVDRF-II) in children and adolescents. Results are presented as standardized (Beta) and unstandardized (B) regression coefficients. All analyses were adjusted by age. Equation (1): the effect of MF on BMI; Equation (2): the effect of MF on CVDRF-II; Equation (3): the effect of BMI on CVDRF-II; Equation (3'): Direct effect of MF on CVDRF-II. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Chapter II: fitness cut-off points

Study III: Cardiorespiratory fitness cut-off points for early detection of present and future cardiovascular risk in children: a 2-year follow-up study

The characteristics of the study sample are presented in table 8. Single CVD risk factor values significantly increased from baseline to follow-up in males and females (all $p<0.05$) except for triglycerides and TC/HDL-c in both sexes and HOMA-IR in females. Likewise, the CVD risk score increased only in males significantly from baseline to follow-up. However, VO_2max significantly decreased in both males and females from baseline to follow-up ($p<0.001$).

Table 9 shows the association of CRF at baseline with single CVD risk factors and the CVD risk score at baseline and follow-up. CRF at baseline was inversely associated with single CVD risk factors and the CVD risk score at baseline and follow-up (all $p<0.05$ and $p<0.001$, respectively) in both males and females, except for SBP and HOMA-IR at follow-up.

The receiver operating characteristic curve analysis showed a significant discriminating accuracy of CRF in identifying CVD risk at baseline in males (AUC 0.852, 95% CI 0.741-0.964, $p<0.001$) and in females (AUC 0.866, 95% CI 0.795-0.938, $p<0.001$) (figure 5). The CRF cut-off point associated with CVD risk was 39.0 ml/kg/min (optimal sensitivity, specificity, and accuracy 70.0, 88.9 and 80.2, respectively) in males, and 37.5 ml/kg/min (optimal sensitivity, specificity, and accuracy 75.0, 87.0 and 81.0, respectively) in females. Logistic regression analyses showed that males and females with levels of CRF ≥39.0 and ≥37.5 ml/kg/min at baseline, respectively, were more likely to have a favorable CVD risk score at baseline compared with those with CRF levels below these values (males: odds ratio [OR], 12.51; 95% CI, 1.44-109.34; p=0.01; females: OR, 11.182; 95% CI 1.969-64.642; p=0.002).

We observed that males and females with CRF levels ≥39.0 and ≥37.5 ml/kg/min at baseline, respectively, were more likely to have a favorable CVD risk score at follow-up compared with those with cardiorespiratory fitness levels below these values (males: OR, 7.117; 95% CI 1.300-39.970; p=0.02; females: OR, 4.439; 95% CI 0.857-23.006; p=0.03).

Table 8. Baseline and two-year follow-up characteristics of the study sample by sex.

Variables	Males					Females				
	Baseline (n=127)		Follow-up (n=115)		p	Baseline (n=109)		Follow-up (n=98)		p
Pubertal development, n (%)					**<0.001**					**<0.001**
1	62	(48.8)	6	(5.2)		72	(65.2)	34	(33.3)	
2	56	(44.1)	73	(63.5)		28	(27.7)	27	(26.5)	
3	9	(7.1)	33	(28.7)		8	(7.3)	30	(29.4)	
4	-	-	3	(2.6)		2	(1.8)	11	(10.8)	
Age (years)	8.1	(1.5)	10.2	(1.5)	**<0.001**	8.0	(1.5)	10.2	(1.5)	**<0.001**
Sum of two skinfolds (mm)	21.7	(9.7)	26.7	(13.5)	**<0.001**	25.0	(12.8)	28.1	(14.3)	**0.003**
Systolic blood pressure (mmHg)	101.9	(11.0)	105.0	(9.1)	**<0.001**	99.4	(11.6)	104.8	(10.6)	**<0.001**
HOMA-IR	0.8	(0.8)	1.1	(1.0)	**0.001**	1.1	(1.2)	1.3	(1.0)	0.98
Triglycerides (mg/dL)	41.1	(20.5)	41.0	(19.2)	0.28	46.3	(27.8)	48.3	(14.3)	0.07
TC/HDL	3.0	(0.6)	3.1	(0.6)	0.62	3.2	(0.7)	3.0	(0.7)	0.61
20-m shuttle run (stage)	3.3	(1.7)	4.4	(1.8)	**<0.001**	2.3	(1.6)	3.4	(2.0)	**<0.001**
$VO_{2\,max}$ (ml/kg/min)	40.1	(5.0)	38.1	(6.2)	**<0.001**	37.6	(5.4)	35.0	(6.0)	**<0.001**
CVD risk score	-0.1	(0.5)	0.1	(0.5)	**0.008**	-0.06	(0.6)	0.03	(0.6)	**0.67**

Values represent mean (standard deviation), unless otherwise indicated. HOMA-IR indicates homeostasis model assessment for insulin resistance; TC/HDL, total cholesterol/ high-density lipoprotein cholesterol; VO_2max, maximum oxygen consumption; CVD, cardiovascular disease. Significant differences between baseline and two-year follow-up are highlighted in bold.

Table 9. Association of cardiorespiratory fitness at baseline (6-10 years old) with cardiovascular disease (CVD) risk factors at baseline and at two-year follow-up (8-10 years old).

	Males						Females					
	B	95% CI			β	p	B	95% CI			β	p
Baseline												
Sum of two skinfolds (mm)	-1.113	-1.428	;	-0.798	-0.565	**<0.001**	-1.335	-1.768	;	-0.903	-0.565	**<0.001**
Systolic blood pressure (mmHg)	-0.240	-0.084	;	-0.003	-0.107	**0.03**	-2.51	-0.161	;	-0.002	-0.119	**0.04**
HOMA-IR	-0.054	-0.101	;	-0.008	-0.311	**0.02**	-0.085	-0.158	;	-0.013	-0.227	**0.02**
Triglycerides (mg/dL)	-1.067	-1.858	;	-0.275	-0.258	**0.009**	-0.848	-1.676	;	-0.021	-0.187	**0.04**
TC/HDL	-0.035	-0.058	;	-0.013	-0.300	**0.003**	-0.037	-0.067	;	-0.007	-0.275	**0.01**
CVD risk score	-0.054	-0.073	;	-0.035	-0.502	**<0.001**	-0.048	-0.072	;	-0.024	-0.424	**<0.001**
Follow-up												
Sum of two skinfolds (mm)	-1.433	-1.593	;	-1.274	-0.619	**<0.001**	-1.521	-1.749	;	-1.292	-0.557	**<0.001**
Systolic blood pressure (mmHg)	-0.092	-0.224	;	0.039	-0.057	0.17	-0.158	-0.350	;	0.034	-0.071	0.11
HOMA-IR	-0.021	-0.079	;	0.038	-0.089	0.48	-0.024	-0.058	;	0.010	-0.164	0.15
Triglycerides (mg/dL)	-0.929	-1.538	;	-0.320	-0.297	**0.003**	-1.292	-2.240	;	-0.344	-0.282	**0.008**
TC/HDL	-0.044	-0.065	;	-0.023	-0.420	**<0.001**	-0.043	-0.066	;	-0.021	-0.377	**<0.001**
CVD risk score	-0.047	-0.066	;	-0.028	-0.553	**<0.001**	-0.043	-0.064	;	-0.021	-0.426	**<0.001**

B indicates unstandardized coefficient; β, standardized coefficient; HOMA-IR, homeostasis model assessment for insulin resistance; TC/HDL, total cholesterol/ high-density lipoprotein cholesterol.

All analyses were controlled for age. Additionally, for the two-year follow-up, cardiovascular risk factor and cardiovascular risk scores were adjusted for levels at baseline. Statistically significant values are highlighted in bold.

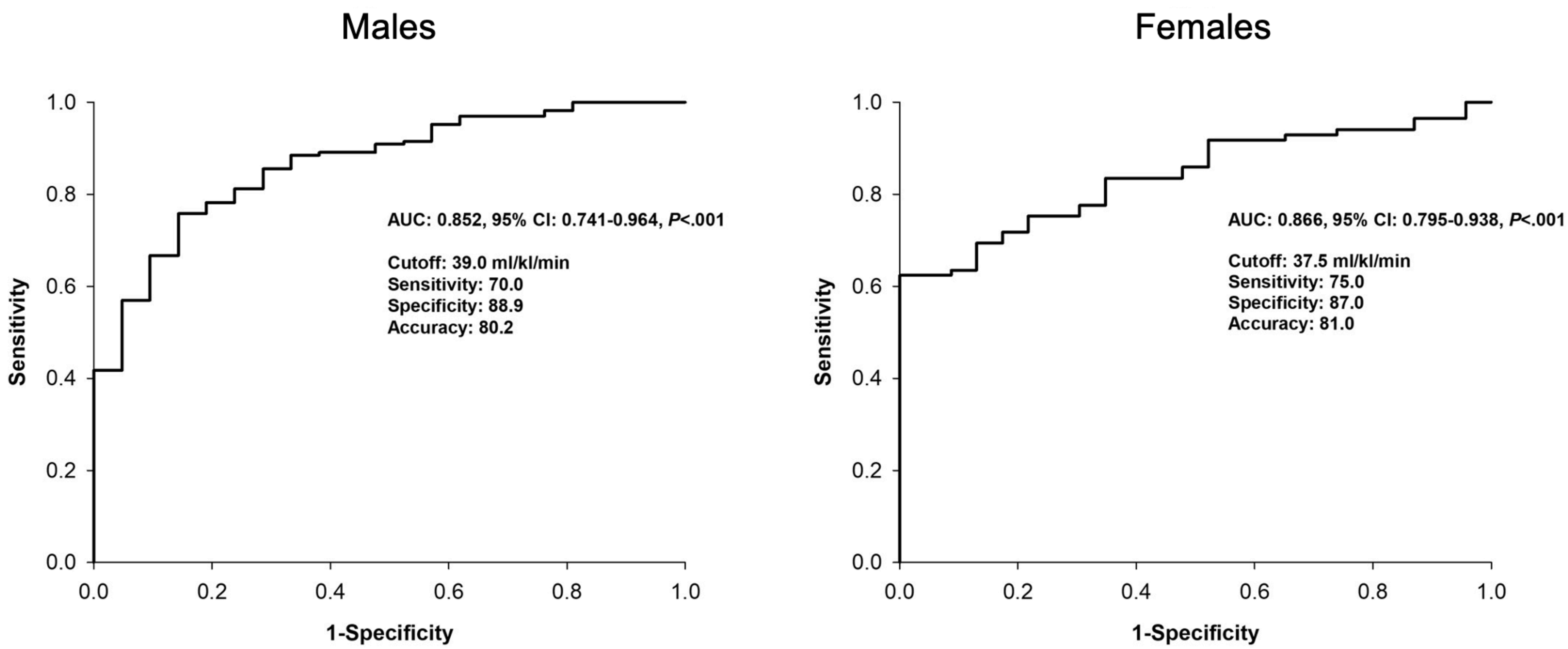

Figure 5: Receiver operating characteristic curves summarizing the potential of cardiorespiratory fitness to identify cardiovascular health status in males and females. AUC: area under the curve.

Figure 6 depicts a positive association between CRF level at baseline and CRF level at follow-up in males (R^2=0.81; p<0.001) and females (R^2=0.85; p<0.001). Figure 7 shows the differences in single CVD risk factors and CVD risk score at follow-up with CRF levels at baseline. Children with high CRF at baseline (≥39.0 and ≥37.5 ml/kg/min in males and females, respectively) presented lower concentrations of HOMA-IR (p=0.03), triglycerides (p=0.01), TC/HDL ratio (p=0.01) and CVD risk score (p=0.004) at follow-up.

Figure 8 shows the association between CRF change categories (persistent low, decreasing, persistent high, and increasing) and the CVD risk score at follow-up. Children in the persistent high fitness category had lower CVD risk score at follow-up than those in the persistent low and decreasing fitness categories (all p<0.001). Similarly, children in the increasing fitness category had lower levels of CVD risk score than those in the persistent low fitness category (p<0.001).

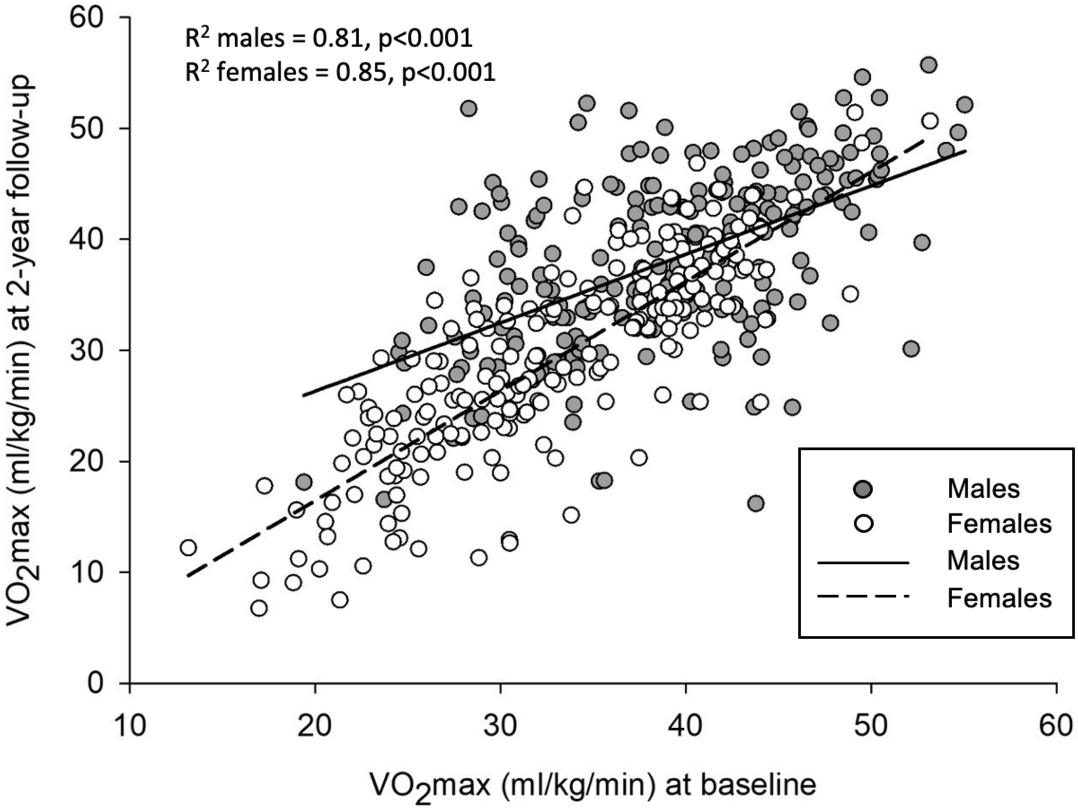

Figure 6: Scatterplot of cardiorespiratory fitness levels at baseline and at two-year follow-up in males and females. VO_2max: maximum oxygen consumption.

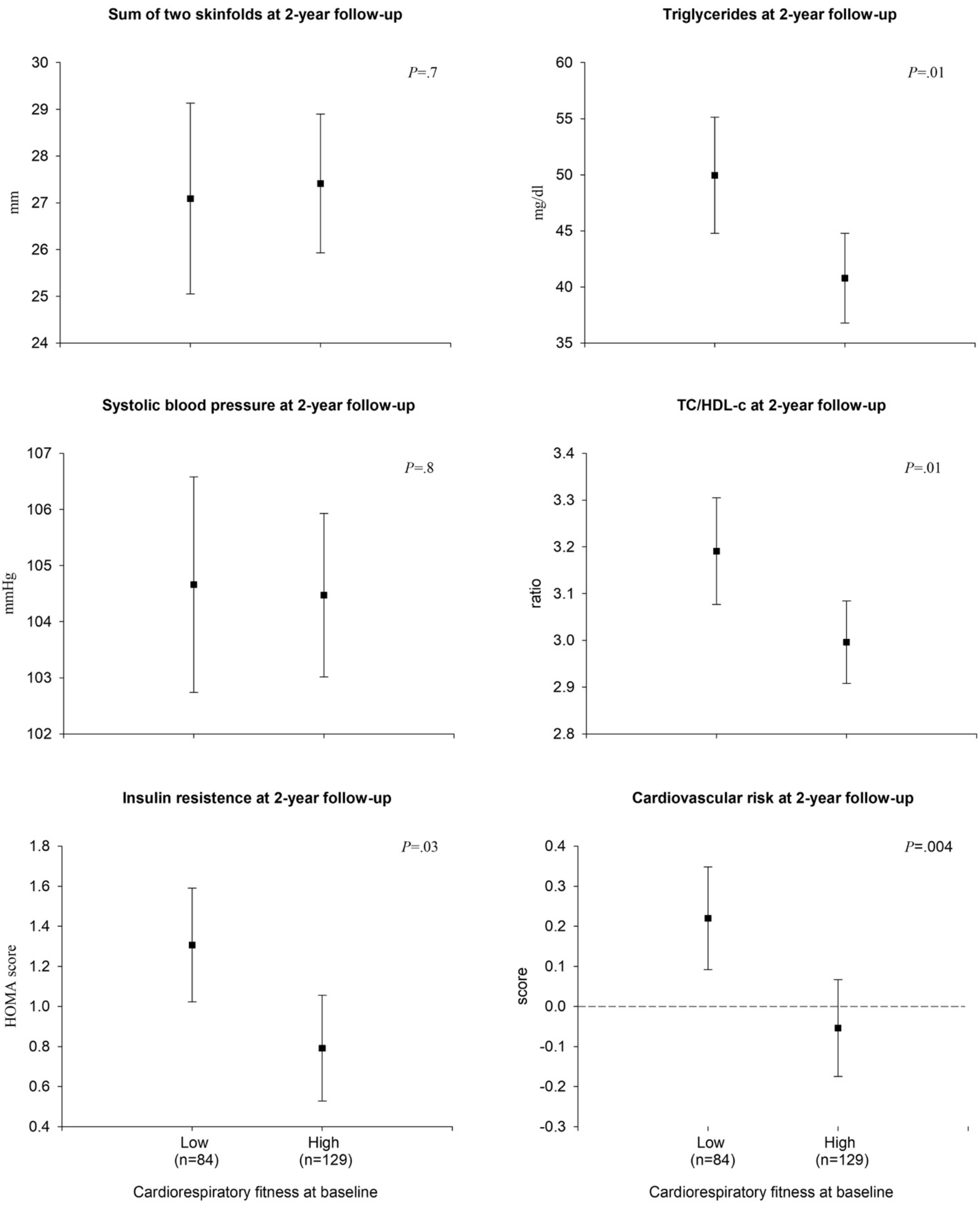

Figure 7: Association between cardiorespiratory fitness at baseline and cardiovascular risk factors at two-year follow-up. Analyses were adjusted for sex and age at follow-up. In addition, each cardiovascular risk factor and the cardiovascular risk score were adjusted for their baseline levels. HOMA-IR: homeostasis model assessment for insulin resistance.

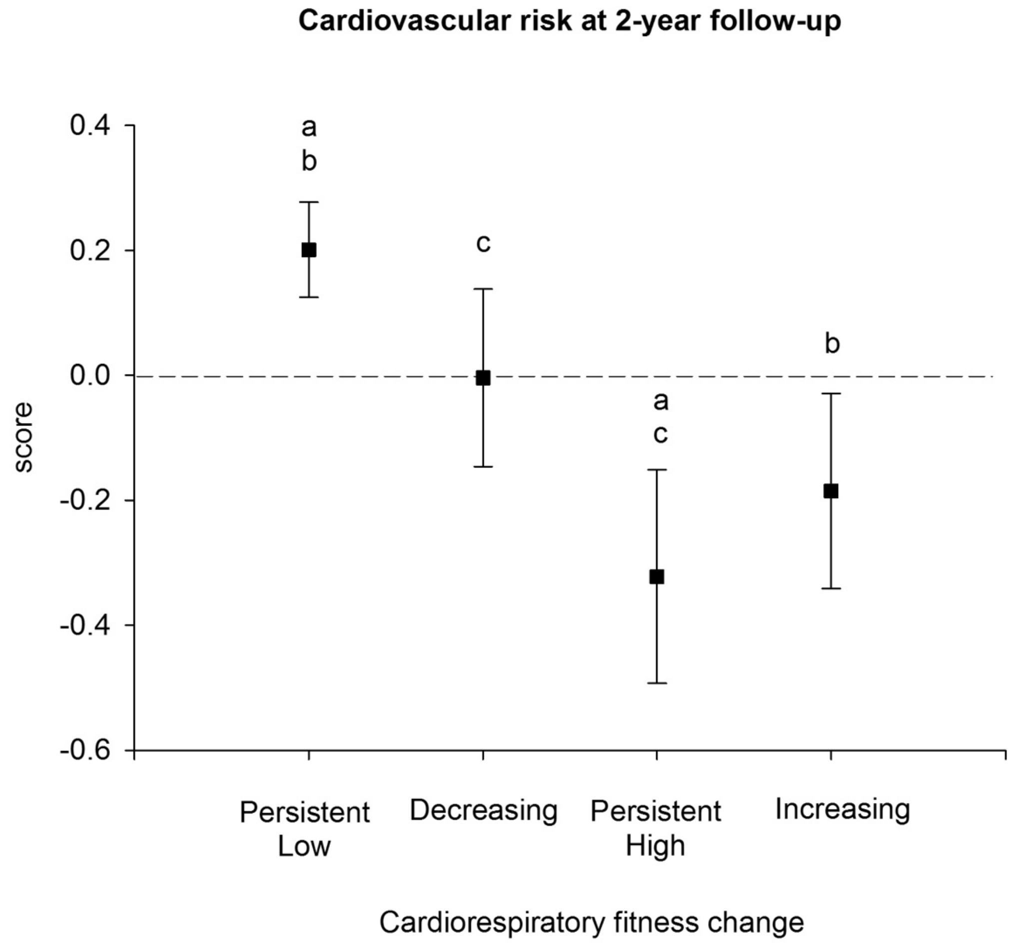

Figure 8: Association between cardiorespiratory fitness change categories and the cardiovascular risk score at two-year follow-up. The analysis was adjusted for sex, age at follow-up, and the cardiovascular risk score at baseline. Common superscripts indicate significant differences between groups (all $p<0.001$).

Study IV: Muscle fitness cut points for early assessment of cardiovascular risk in children and adolescents

The characteristics of the study sample are shown in table 10. Overall, single CVD risk factors values significantly increased from baseline to follow-up in both sexes and age groups (all p<0.05) except for triglycerides in children, both males and females, and TC/HDL-C ratio in male children, and male and female adolescents. In contrast, HOMA-IR significantly decreased from baseline to follow-up in male and female adolescents. Levels of handgrip/weight and standing long jump significantly increased (all p≤0.005), except for handgrip/weight in male children and the standing long jump in female adolescents.

Levels of handgrip/weight at baseline were inversely associated with single CVD risk factors and CVD risk score at baseline and follow-up (β from −0.002 to −0.753; all p<0.05 and p<0.01, respectively), except for HOMA-IR at baseline and follow-up, SBP at follow-up in male children and male and female adolescents; and TC/HDL-C ratio at follow-up in male adolescents (table 11). Levels of standing long jump performance were inversely associated with the sum of 2 skinfolds (β from −0.346 to −0.698; all p<0.001) and the CVD risk score (β from −0.216 to −0.467; all p<0.05) at baseline and follow-up in children and adolescents of both sexes; with SBP at baseline in children in both sexes (β=−0.118; p=0.007 in males and β=−0.230; p=0.020 in females), TC/HDL-C ratio at baseline in children and adolescents of both sexes (β from −0.221 to −0.239; all p<0.05), and at follow-up in children of both sexes (β=−0.289; p=0.005 in males, and β=−0.386; p=0.001 in females), triglycerides at follow-up in children and adolescents of both sexes (β from −0.202 to −0.299; all p<0.05), and HOMA-IR at baseline in male children (β=−0.279; p=0.048) and at follow-up in male and female adolescents (β=−0.239; p=0.040 in males, and β=−0.271; p=0.017 in females) (table 12).

Table 10. Baseline and two-year follow-up characteristics of the study sample by sex.

	Children					
	Males			Females		
	Baseline (n=127)	Follow-up (n=115)	p	Baseline (n=110)	Follow-up (n=98)	p
Pubertal development, n (%)			**<0.001**			**<0.001**
1	62 (48.8)	6 (5.2)		72 (65.2)	34 (33.3)	
2	56 (44.1)	73 (63.5)		28 (27.7)	27 (26.5)	
3	9 (7.1)	33 (28.7)		8 (7.3)	30 (29.4)	
4	-	3 (2.6)		2 (1.8)	11 (10.8)	
5	-	-		-	-	
Age (years)	7.6 (1.5)	10.1 (1.5)	**<0.001**	7.5 (1.6)	10.1 (1.6)	**<0.001**
Sum of two skinfolds (mm)	21.7 (9.7)	26.7 (13.6)	**<0.001**	25.0 (12.8)	28.1 (14.9)	**<0.001**
Systolic blood pressure (mmHg)	101.8 (11.0)	105.0 (9.3)	**0.001**	99.4 (11.6)	104.8 (10.8)	**<0.001**
HOMA-IR	0.8 (0.8)	1.1 (1.3)	**0.021**	1.2 (1.4)	1.1 (0.9)	0.63
Triglycerides (mg/dL)	41.1 (20.5)	40.8 (19.1)	0.906	46.3 (17.9)	48.3 (27.4)	0.248
TC/HDL-C ratio	3.0 (0.6)	3.1 (0.6)	0.223	3.2 (0.7)	3.0 (0.7)	**0.022**
Handgrip/weight	0.41 (0.1)	0.41(0.1)	0.921	0.39 (0.1)	0.40 (0.1)	**0.005**
Standing long jump (cm)	114.8 (21.1)	129.8(25.3)	**<0.001**	106.5 (22.9)	122.9 (24.1)	**<0.001**
	Adolescents					
	Males			Females		
	Baseline (n=143)	Follow-up (n=127)	p	Baseline (n=131)	Follow-up (n=118)	p
Pubertal development, n (%)			**<0.001**			**<0.001**
1	-	-		1 (0.8)	-	
2	16 (11.2)	-		11 (8.4)	-	
3	46 (32.2)	7 (5.5)		61 (46.6)	26 (20.0)	
4	49 (34.3)	53 (41.7)		46 (35.1)	70 (59.3)	
5	32 (22.4)	67 (52.8)		12 (9.2)	22 (18.6)	
Age (years)	13.6 (1.7)	16.0 (1.6)	**<0.001**	13.5 (1.6)	15.9 (1.5)	**<0.001**
Sum of two skinfolds (mm)	23.7 (13.7)	25.3 (10.5)	**<0.001**	30.3 (11.9)	30.5 (13.4)	0.457
Systolic blood pressure (mmHg)	112.9 (15.6)	114.7 (13.2)	0.071	106.8 (10.5)	104.6 (10.8)	**0.002**
HOMA-IR	2.8 (2.2)	0.9 (1.6)	**<0.001**	3.2 (2.7)	0.6 (0.5)	**<0.001**
Triglycerides (mg/dL)	54.38 (36.4)	67.8 (31.1)	**<0.001**	57.8 (29.6)	63.7 (24.2)	**<0.001**
TC/HDL-C ratio	2.8 (0.5)	2.9 (0.6)	0.245	2.8 (0.6)	2.7 (0.6)	0.162
Handgrip strength/body mass	0.50 (0.1)	0.56 (0.1)	**<0.001**	0.43 (0.1)	0.46 (0.1)	**<0.001**
Standing long jump (cm)	175.3 (33.2)	190.2 (38.9)	**<0.001**	141.1 (21.2)	143.8 (28.3)	0.196

Values represent mean (standard deviation), unless otherwise indicated. HOMA-IR indicates homeostasis model assessment for insulin resistance; TC/HDL-C ratio, total cholesterol/ high-density lipoprotein cholesterol ratio; VO_2max, maximum oxygen consumption.

Significant differences between baseline and two-year follow-up are highlighted in bold.

Table 11. Association of handgrip/weight at baseline in children (6-10 years old) and adolescents (12-16 years old) with cardiovascular disease (CVD) risk factors at baseline and at two-year follow-up (8-10 and 14-16 years old, respectively).

	Children													
	Males						Females							
	B	95% CI			β	p	B	95% CI			β	p		
Baseline														
Sum of two skinfolds (mm)	-82.396	-92.008	;	-872,783	-0.565	**<0.001**	-94.443	-104.618	;	-84.269	-0.585	**<0.001**		
Systolic blood pressure (mmHg)	-12.865	-22.606	;	-2.230	-0.102	**0.001**	-20.072	-31.113	;	-9.030	-0.137	**0.006**		
HOMA-IR	-2.526	-5.435	;	0.384	-0.235	0.088	-4.243	-9.471	;	0.985	-0.220	0.109		
Triglycerides (mg/dL)	-59.351	-105.905	;	-12.798	-0.224	**0.013**	-44.107	-86.465	;	-1.748	-0.195	**0.041**		
TC/HDL-C ratio	-3.708	-5.736	;	-2.219	-0.396	**<0.001**	-3.515	-5.114	;	-1.919	-0.384	**<0.001**		
CVD risk score	-2.935	-0.4.084	;	-1.785	-0.424	**<0.001**	-3.810	-5.146	;	-2.475	-0.484	**<0.001**		
Follow-up														
Sum of two skinfolds (mm)	-91.180	-103.815	;	-78.454	-0.525	**<0.001**	-100.740	-115.404	;	-86.076	-0.516	**<0.001**		
Systolic blood pressure (mmHg)	-5.389	-14.827	;	4.048	-0.046	0.262	-19.647	-32.007	;	-7.288	-0.123	**0.002**		
HOMA-IR	-0.034	-4.286	;	4.219	-0.002	0.987	-1.422	-3.993	;	1.149	-0.127	0.274		
Triglycerides (mg/dL)	-118.702	-179.357	;	-58.761	-0.368	**<0.001**	-126.636	-191.628	;	-61.644	-0.382	**<0.001**		
TC/HDL-C ratio	-1.934	-3.581	;	-2.880	-0.225	**0.022**	-3.922	-5.428	;	-2.417	-0.471	**<0.001**		
CVD risk score	-2.331	-3.830	;	-0.831	-0.362	**0.003**	-4.087	-5.620	;	-2.554	-0.540	**<0.001**		

	Adolescents											
	Males						Females					
	B	95% CI			β	p	B	95% CI			β	p
Baseline												
Sum of two skinfolds (mm)	-95.935	-103.509	;	-86.360	-0.753	**<0.001**	-73.638	-84.620	;	-62.777	-0.515	**<0.001**
Systolic blood pressure (mmHg)	-16.071	-27.765	;	-4.376	-0.118	**0.007**	-14.164	-26.097	;	-0.002	-0.230	**0.020**
HOMA-IR	-0.021	-0.079	;	0.038	-0.089	0.321	-4.483	-10.886	;	1.921	-0.133	0.168
Triglycerides (mg/dL)	-50.289	-108.903	;	-7.844	-0.226	**0.021**	-43.702	-88.241	;	-0.021	-0.186	**0.045**

TC/HDL-C ratio	-1.039	-2.030 ; -0.048	-0.210	0.040	-1.386	-2.272 ; -0.051	-0.181	**0.041**
CVD risk score	-2.541	-3.533 ; -1.549	-0.485	<0.001	-1.987	-3.066 ; -0.914	-0.311	**<0.001**
Follow-up								
Sum of two skinfolds (mm)	-71.983	-82.813 ; -61.154	-0.615	<0.001	-85.928	-100.632 ; -71.225	-0.516	**<0.001**
Systolic blood pressure (mmHg)	-3.449	-15.414 ; 8.516	-0.029	0.571	-14.620	-26.352 ; -1.113	-0.101	**0.035**
HOMA-IR	-3.702	-7.584 ; 0.181	-0.233	0.061	-1.239	-2.766 ; 0.287	-0.181	0.110
Triglycerides (mg/dL)	-84.042	-135.640 ; -3.586	-0.218	0.032	-62.083	-122.827 ; -1.339	-0.195	**0.045**
TC/HDL-C ratio	-0.951	-2.159 ; 0.258	-0.161	0.122	-2.236	-3.617 ; -0.856	-0.296	**0.002**
CVD risk score	-2.099	-3.250 ; -0.948	-0.430	<0.001	-3.114	-4.490 ; -1.738	-0.460	**<0.001**

B indicates unstandardized coefficient; β, standardized coefficient; HOMA-IR, homeostasis model assessment for insulin resistance; TC/HDL-C ratio, total cholesterol/ high-density lipoprotein cholesterol ratio.

All analyses were controlled for age. Additionally, for the two-year follow-up analyses, cardiovascular risk factors and cardiovascular risk scores were adjusted for levels at baseline. Statistically significant values are highlighted in bold.

Table 12. Association of the standing long jump test at baseline in children (6-10 years old) and adolescents (12-16 years old) with cardiovascular disease (CVD) risk factors at baseline and at two-year follow-up (8-10 and 14-16 years old, respectively).

	Children							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Baseline								
Sum of two skinfolds (mm)	-0.258	-0.398 ; -0.207	-0.437	<0.001	-0.279	-0.328 ; -0.229	-0.461	<0.001
Systolic blood pressure (mmHg)	-16.071	-27.765 ; -4.376	-0.118	0.007	-14.164	-26.097 ; -0.002	-0.230	0.020
HOMA-IR	-0.019	-0.039 ; -0.003	-0.279	0.048	-0.003	-0.021 ; 0.016	-0.045	0.773
Triglycerides (mg/dL)	-0.155	-0.341 ; 0.031	-0.60	0.101	-0.130	-0.300 ; 0.046	-0.167	0.133
TC/HDL-C ratio	-0.015	-0.028 ; -0.002	-0.235	0.037	0.008	-0.015 ; -0.001	-0.239	0.032
CVD risk score	-0.010	-0.014 ; -0.005	-0.380	<0.001	-0.007	-0.013 ; -0.001	-0.268	0.017
Follow-up								
Sum of two skinfolds (mm)	-0.280	-0.346 ; -0.214	-0.394	<0.001	-0.324	-0.391 ; -0.257	-0.444	<0.001
Systolic blood pressure (mmHg)	0.020	-0.024 ; 0.063	0.042	0.372	-0.012	-0.063 ; 0.041	-0.020	0.656
HOMA-IR	-0.001	-0.016 ; 0.015	-0.006	0.963	-0.008	-0.018 ; 0.002	-0.211	0.094
Triglycerides (mg/dL)	-0.145	-0.321 ; -0.030	-0.219	0.029	-0.353	-0.631 ; -0.075	-0.299	0.013
TC/HDL-C ratio	-0.009	-0.015 ; -0.003	-0.289	0.005	-0.011	-0.018 ; -0.005	-0.386	0.001
CVD risk score	-0.008	-0.014 ; -0.002	-0.359	0.006	-0.012	-0.019 ; -0.006	-0.467	<0.001
	Adolescents							
	Males				Females			
	B	95% CI	β	p	B	95% CI	β	p
Baseline								
Sum of two skinfolds (mm)	-0.285	-0.325 ; -0.245	-0.698	<0.001	-0.188	-0.231 ; -0.144	-0.363	<0.001
Systolic blood pressure (mmHg)	-0.008	-0.036 ; 0.051	-0.018	0.724	-0.013	-0.056 ; 0.051	-0.027	0.561
HOMA-IR	-0.007	-0.026 ; 0.013	-0.094	0.513	-0.017	-0.041 ; 0.007	-0.138	0.159
Triglycerides (mg/dL)	-0.009	-0.272 ; 0.291	-0.008	0.950	-0.173	-0.421 ; 0.075	-0.124	0.171

TC/HDL-C ratio	-0.009	-0.014 ; -0.001	-0.221	**0.046**	-0.011	-0.019 ; -0.002	-0.230	**0.039**
CVD risk score	-0.007	-0.011 ; -0.003	-0.397	**0.002**	-0.005	-0.009 ; -0.001	-0.216	**0.017**
Follow-up								
Sum of two skinfolds (mm)	-0.230	-0.272 ; -0.188	-0.631	**<0.001**	-0.203	-0.260 ; -0.145	-0.346	**<0.001**
Systolic blood pressure (mmHg)	0.005	-0.039 ; 0.049	-0.013	0.822	-0.045	-0.091 ; -0.002	-0.202	**0.047**
HOMA-IR	-0.012	-0.027 ; -0.002	-0.239	**0.040**	-0.007	-0.012 ; -0.001	-0.271	**0.017**
Triglycerides (mg/dL)	-0.140	-0.289 ; -0.023	-0.202	**0.038**	-0.258	-0.478 ; -0.039	-0.228	**0.022**
TC/HDL-C ratio	0.001	-0.004 ; 0.005	0.042	0.750	-0.003	-0.008 ; 0.003	-0.101	0.305
CVD risk score	-0.006	-0.011 ; -0.002	-0.426	**0.004**	-0.006	-0.011 ; -0.001	-0.243	**0.028**

B indicates unstandardized coefficient; β, standardized coefficient; HOMA-IR, homeostasis model assessment for insulin resistance; TC/HDL-C ratio, total cholesterol/ high-density lipoprotein cholesterol ratio.

All analyses were controlled for age. Additionally, for the two-year follow-up analyses, cardiovascular risk factors and cardiovascular risk scores were adjusted for levels at baseline. Statistically significant values are highlighted in bold.

Handgrip/weight cut-off points associated with CVD risk were 0.367 and 0.306 kg/kg body mass in male and female children, respectively, and 0.473 and 0.423 kg/kg body mass in male and female adolescents, respectively (figure 9). The standing long jump cut-off points associated with CVD risk were 104.5 and 81.5 cm in male and female children, respectively, and 140.5 and 120.5 cm in male and female adolescents, respectively. Children and adolescents, both males and females, with levels of handgrip/weight ≥0.367 kg/kg body mass, ≥0.306 kg/kg body mass, ≥0.473 kg/kg body mass, and ≥0.423 kg/kg body mass at baseline, respectively, were at greater odds for a favorable CVD risk score at baseline than their peers with lower levels of handgrip/weight (table S5 in supplementary content). Likewise, children and adolescents, both males and females, with levels of standing long jump ≥104.5 cm, ≥81.5 cm, ≥140.5 cm, and ≥120.5 cm at baseline, respectively, were at greater odds for a favorable CVD risk score at baseline than their peers with lower levels of standing long jump. The AUC, sensitivity, specificity (figure 9), and Youden Index, positive and negative predictive values, and likelihood ratio of a positive and negative test results (table S6 in supplementary content) are the diagnostic variables that have allowed determining the appropriated health-related MF cut-off points.

Similarly, logistic regression analysis showed that children and adolescents, both males and females, with handgrip/weight levels at or above the determined cut-off points were at greater odds for a favorable CVD risk score at follow-up compared with those with relative handgrip/weight levels below the cut-off points (all $p<0.05$) (table S5). Children and adolescents with levels of standing long jump at or above the determined cut-off points at baseline were at greater odds for a favorable CVD risk score at follow-up compared with those with standing long jump levels below the cut-off points (all $P < .05$).

Figure S5 (supplementary content) shows a positive association between levels of handgrip/weight and standing long jump at baseline and follow-up in both sex and age groups (R^2 ranging from 0.49 to 0.63; all $p<0.001$). Children and adolescents, both males and females, with high levels of handgrip/weight or standing long jump at baseline had lower levels of CVD risk score (all $p<0.01$ and all $p<0.001$, respectively) at follow-up (figure 10).

We repeated all analyses after adjusting for pubertal development instead of age, and after further adjusting by CRF (stages) and the results did not change (data not shown).

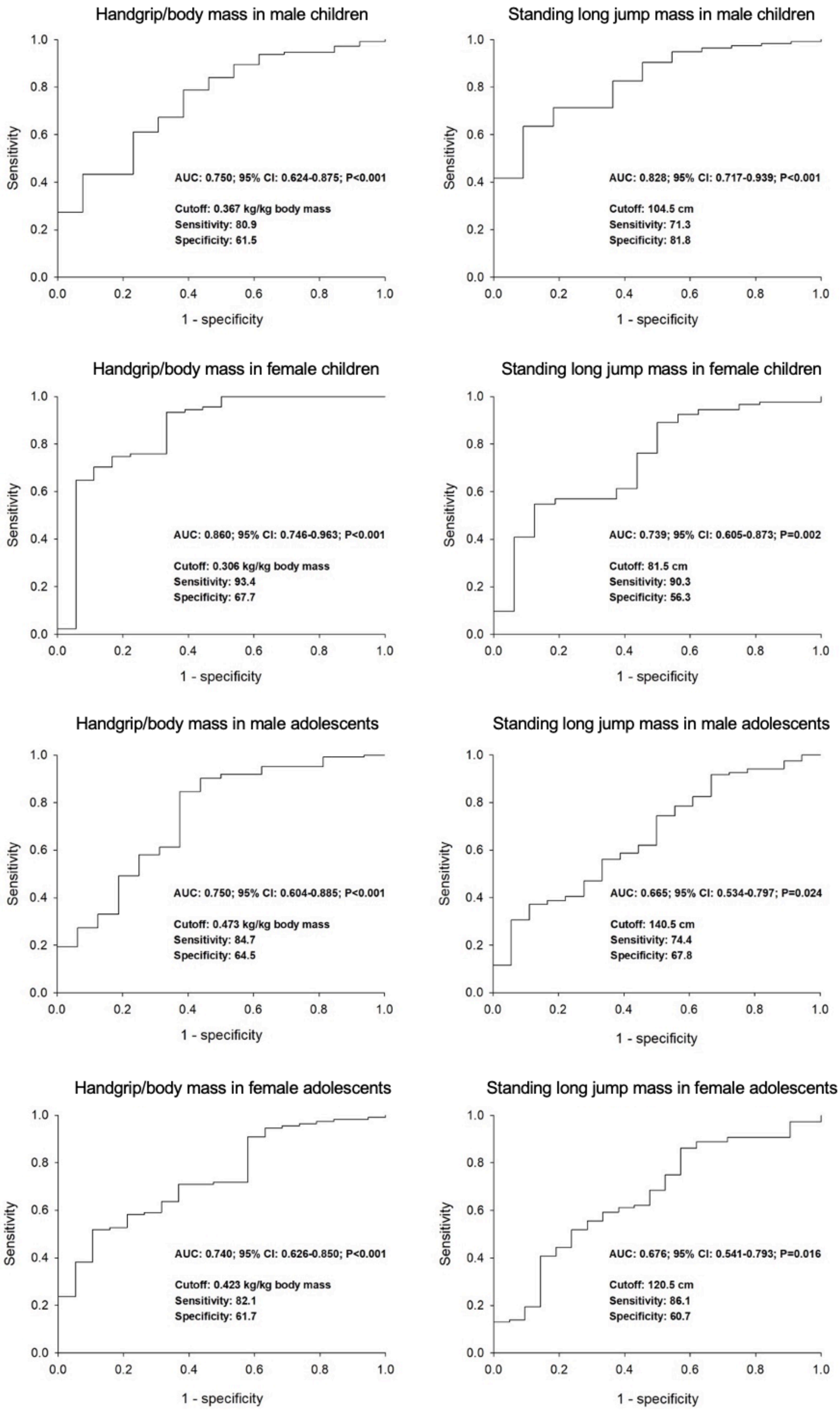

Figure 9. Receiver operating characteristic curves summarizing the potential of muscular fitness to identify cardiovascular health status in children and adolescents of both sexes.

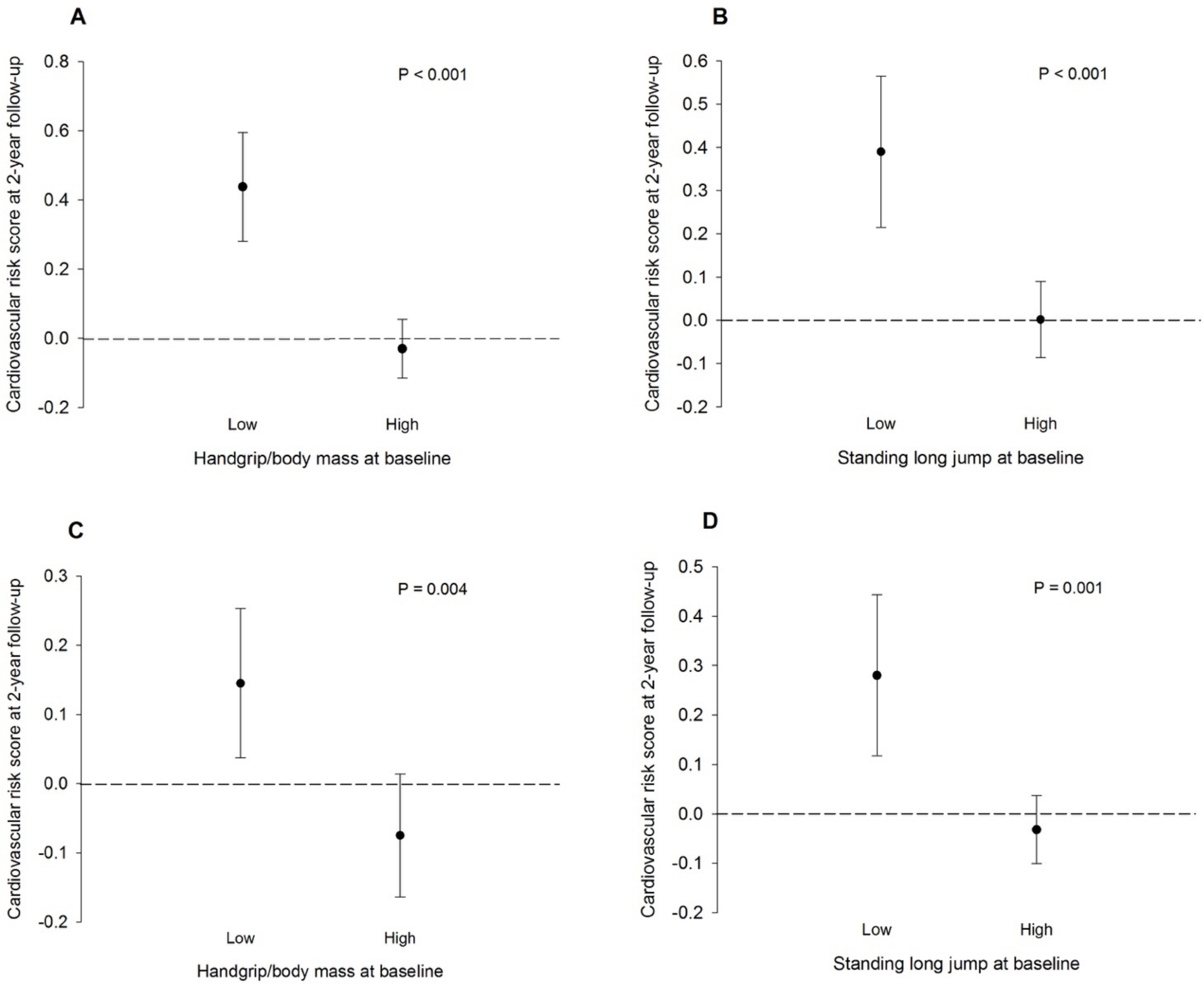

Figure 10. Association between muscular fitness levels at baseline and the cardiovascular risk score at two-year follow-up in children (A and B) and adolescents (C and D) including both sexes. Analysis was adjusted for the cardiovascular risk score at baseline, and sex, and age at two-year follow-up.

Table S5. Binary logistic regression statistics testing the predictive capacity of the muscle fitness testing thresholds derived from the ROC curve analysis for having higher CVD risk score at baseline and two-year follow-up.

	Children							
	Males				Females			
	β	95% CI	OR	p	β	95% CI	OR	p
Baseline								
Handgrip/body mass	1.833	1.859 ; 21.036	6.254	**0.003**	3.799	9.455 ; 211.125	44.68	**<0.001**
Standing long jump	2.579	2.497 ; 69.629	13.184	**0.002**	2.520	2.706 ; 57.034	12.424	**0.001**
Follow-up								
Handgrip/body mass	1.739	1.373 ; 23.582	5.691	**0.017**	2.083	2.023 ; 31.867	8.029	**0.003**
Standing long jump	1.857	1.478 ; 27.739	6.403	**0.013**	2.491	2.696 ; 54.052	12.072	**0.001**

	Adolescents							
	Males				Females			
	β	95% CI	OR	p	β	95% CI	OR	p
Baseline								
Handgrip/body mass	3.043	4.538 ; 96.867	20.970	**<0.001**	2.179	1.931 ; 40.440	8.837	**0.005**
Standing long jump	2.131	1.735 ; 40.397	8.427	**0.008**	1.832	2.060 ; 18.943	6.246	**0.001**
Follow-up								
Handgrip/body mass	1.989	1.701 ; 27.434	6.421	**0.010**	1.644	1.235 ; 23.146	4.666	**0.029**
Standing long jump	1.918	1.260 ; 36.787	6.809	**0.026**	1.661	1.629 ; 16.995	5.262	**0.006**

β indicates standardized coefficient.

All analyses were controlled for age. Additionally, for the two-year follow-up, cardiovascular risk scores were adjusted for levels at baseline. Statistically significant values are highlighted in bold.

Table S6. Diagnostic variables for muscular fitness tests to determine elevated CVD risk score.

	Children									
	Males					Females				
Muscular fitness test	Youden index	PPV	NPV	LR+	LR-	Youden index	PPV	NPV	LR+	LR-
Handgrip/weight	0.42	5.00	75.00	2.10	0.31	0.61	3.40	75.70	2.90	0.10
Standing long jump	0.53	37.50	97.60	3.92	0.35	0.47	43.80	90.30	2.07	0.17
	Adolescents									
	Males					Females				
	Youden index	PPV	NPV	LR+	LR-	Youden index	PPV	NPV	LR+	LR-
Handgrip/weight	0.49	5.40	65.50	2.39	0.24	0.44	3.40	75.70	2.14	0.29
Standing long jump	0.42	37.5	90.20	2.31	0.38	0.47	38.00	88.10	2.19	0.23

PPV: positive predictive value; NPV: negative predictive value; LR+: likelihood ratio of a positive test result; LR-: likelihood ratio of a negative test result.

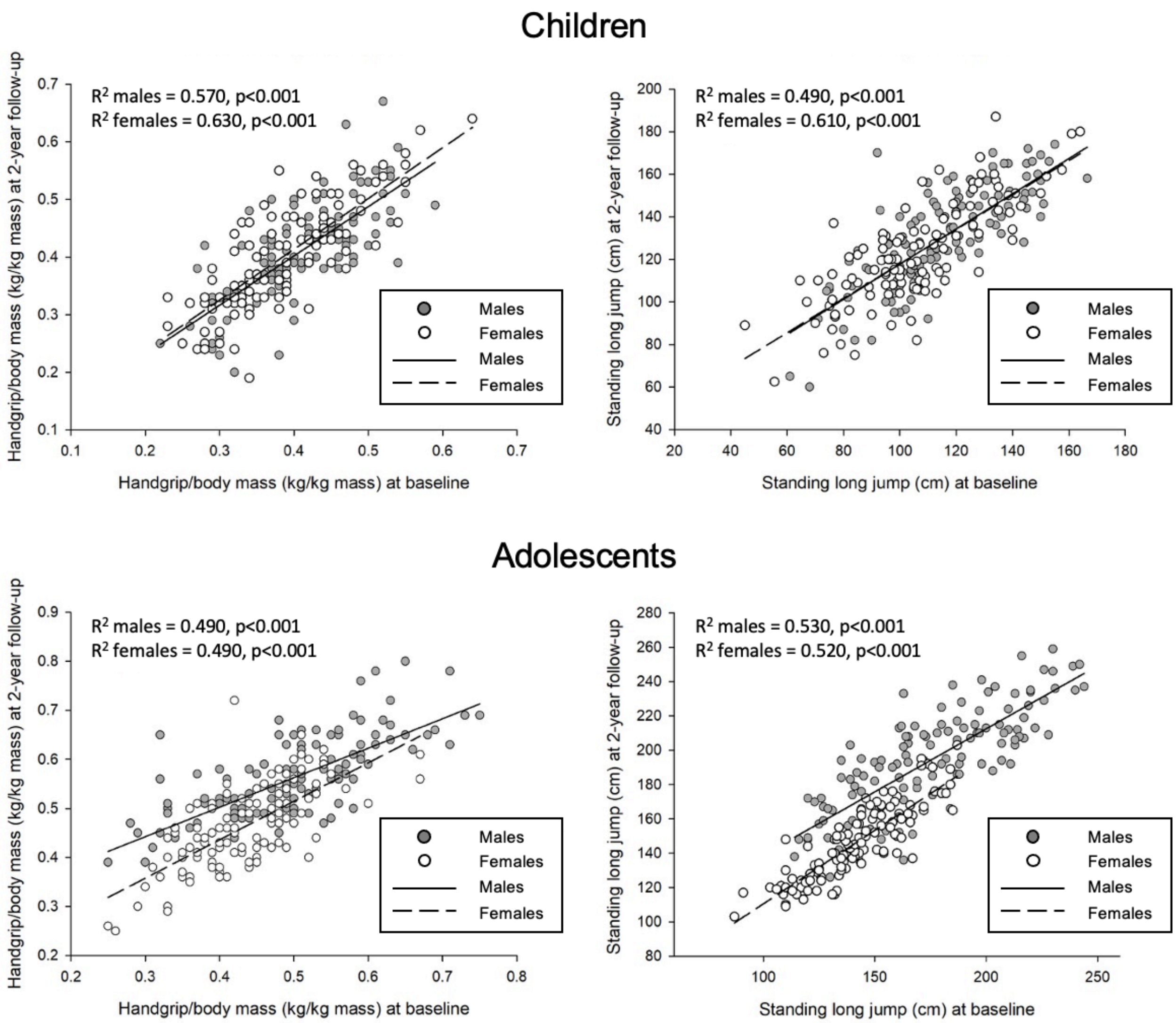

Figure S5. Scatter-plot of muscular fitness at baseline and at two-year follow-up in children and adolescents, both males and females.

Study V: Bidirectional associations between fitness and fatness in youth: A longitudinal study

Participant characteristics are shown in **table 13**. CRF levels (VO_2max) decreased from baseline to follow-up in children of both sexes and in female adolescents (all p<0.01). Body fatness indices increased from baseline to follow-up in children of both sexes (all p<0.001). In male adolescents, BMI levels at follow-up were higher than at baseline (p<0.001). However, %BF and FMI decreased over two years (both p<0.001). In female adolescents, BMI and FMI increased from baseline to follow-up (both p<0.001).

Associations between CRF at baseline (exposure) and BMI, ZBMI, ZWHO, %BF and FMI at baseline and at follow-up (outcomes) are depicted in **table 14**. CRF at baseline was negatively associated with all body fatness indices at baseline in all sex and age groups (β ranging from -0.236 to -0.575; all p<0.001), after controlling for sociodemographic and lifestyle confounders (i.e. age, educational centre, SES, mother's education level, and moderate-to-vigorous physical activity at baseline). Similar results were observed when body fatness indices values at follow-up were included as outcomes (model 1). However, after accounting for the respective baseline values of the body fatness indices (model 2), these associations only persisted in male and female children for %BF (β=-0.168; β=-0.134, respectively; both p<0.05) and in male children for FMI (β=-0.152; p=0.002).

Table 15 shows the associations between body fatness indices at baseline (exposures) and CRF at baseline and at follow-up (outcomes). There were significant associations between each body fatness index and CRF at baseline in both sex and age groups (β ranging from -0.194 to -0.523; all p<0.001), after accounting for sociodemographic and lifestyle confounders. Similarly, body fatness indices at baseline were significantly associated with CRF at follow-up in children and adolescent of both sexes (all p<0.001) (model 1). When CRF at baseline was included in the model (model 2), these associations remained significant in children of both sexes and in female adolescents (β ranging from -0.085 to -0.180; all p<0.05).

Table 13. Baseline and two-year follow-up characteristics of the study sample.

	Children					
Variables	Males			Females		
	Baseline (n=609)	Follow-up (n=570)	p	Baseline (n=564)	Follow-up (n=512)	p
Age (years)	8.1 (1.6)	10.1 (1.6)	-	8.2 (1.6)	10.3 (1.6)	-
Tanner stage (%)						
1	51.9	8.4	-	57.0	28.5	-
2	41.4	53.5	-	35.2	33.2	-
3	6.5	34.0	-	7.6	30.9	-
4	0.2	3.9	-	0.2	7.2	-
5	0.0	0.2	-	0.0	0.2	-
Weight (kg)	30.7 (9.0)	39.0 (11.5)	**<0.001**	31.1 (10.0)	40.1 (12.8)	**<0.001**
Height (cm)	129.3 (10.4)	141.1 (10.5)	**<0.001**	129.5 (11.3)	142.4 (11.8)	**<0.001**
Body mass index (kg/m²)	18.0 (3.0)	19.3 (3.8)	**<0.001**	18.1 (3.4)	19.3 (4.0)	**<0.001**
Percentage of body fat (%)	19.6 (8.9)	22.5 (11.6)	**<0.001**	22.3 (7.8)	24.6 (9.4)	**<0.001**
Fat mass index (kg/m²)	3.8 (2.5)	4.7 (3.4)	**<0.001**	4.3 (2.4)	5.1 (3.0)	**<0.001**
20m shuttle run test (stages)	3.2 (1.7)	4.3 (1.9)	**<0.001**	2.3 (1.3)	3.2 (1.6)	**<0.001**
VO2max (ml/kg/min)	39.9 (4.9)	37.7 (6.3)	**<0.001**	37.0 (5.0)	34.0 (5.6)	**<0.001**
	Adolescents					
Variables	Males			Females		
	Baseline (n=505)	Follow-up (n=385)	p	Baseline (n=460)	Follow-up (n=342)	p
Age (years)	13.8 (1.5)	15.8 (1.5)	-	13.8 (1.4)	15.7 (1.4)	-
Tanner stage (%)						
1	5.0	0.0	-	1.2	0.0	-
2	10.6	0.0	-	8.8	1.5	-
3	33.8	8.8	-	45.0	22.5	-
4	35.1	36.4	-	38.9	57.9	-
5	20.0	54.8	-	6.1	18.1	-
Weight (kg)	55.6 (13.8)	64.5 (12.8)	**<0.001**	51.8 (9.9)	56.3 (9.6)	**<0.001**
Height (cm)	161.7 (11.2)	171.6 (7.8)	**<0.001**	157.6 (6.8)	161.8 (5.8)	**<0.001**
Body mass index (kg/m²)	21.0 (3.6)	21.8 (3.5)	**<0.001**	20.7 (3.2)	21.4 (3.3)	**<0.001**
Percentage of body fat (%)	20.2 (11.0)	17.5 (10.3)	**<0.001**	24.6 (7.0)	24.9 (7.7)	0.122
Fat mass index (kg/m²)	4.5 (3.4)	4.1 (3.3)	**<0.001**	5.3 (2.4)	5.6 (2.7)	**<0.001**
20-m shuttle run test (stages)	6.6 (2.4)	7.8 (2.2)	**<0.001**	4.1 (1.6)	4.5 (1.7)	**<0.001**
VO2max (ml/kg/min)	36.9 (7.7)	37.1 (9.1)	0.674	27.7 (6.3)	23.9 (8.0)	**<0.001**

Values are presented as mean (standard deviation) or percentage. Statistically significant differences in variables at baseline and two-year follow-up are highlighted in bold.

Table 14. Linear regression models examining the associations between cardiorespiratory fitness at baseline (exposure) and body fatness indices at baseline and at two-year follow-up (outcomes).

		Children									
		Males					Females				
		r	R^2	β	95% CI	p	r	R^2	β	95% CI	p
Cross-sectional analysis Baseline											
CRF-BMI		0.566	0.321	-0.489	-0.372 ; -0.218	<0.001	0.516	0.267	-0.518	-0.508 ; -0.304	<0.001
CRF-ZBMI		0.563	0.316	-0.478	-0.104 ; -0.060	<0.001	0.510	0.260	-0.511	-0.138 ; -0.082	<0.001
CRF-ZWHO		0.545	0.297	-0.450	-0.128 ; -0.071	<0.001	0.526	0.276	-0.547	-0.172 ; -0.106	<0.001
CRF-%BF		0.593	0.351	-0.525	-1.281 ; -0.788	<0.001	0.591	0.350	-0.575	-1.304 ; -0.846	<0.001
CRF-FMI		0.557	0.310	-0.492	-0.384 ; -0.224	<0.001	0.562	0.315	-0.540	-0.391 ; -0.244	<0.001
Longitudinal analysis Follow-up											
CRF-BMI	Model 1	0.586	0.343	-0.492	-0.440 ; -0.264	<0.001	0.489	0.239	-0.491	-0.546 ; -0.318	<0.001
	Model 2	0.936	0.877	-0.043	-0.074 ; 0.013	0.169	0.936	0.877	-0.034	-0.082 ; 0.022	0.258
CRF-ZBMI	Model 1	0.571	0.326	-0.468	-0.103 ; -0.060	<0.001	0.485	0.236	-0.491	-0.128 ; -0.074	<0.001
	Model 2	0.928	0.861	-0.027	-0.016 ; 0.006	0.400	0.933	0.871	-0.047	-0.022 ; 0.003	0.126
CRF-ZWHO	Model 1	0.549	0.301	-0.420	-0.127 ; -0.068	<0.001	0.491	0.241	-0.507	-0.167 ; -0.099	<0.001
	Model 2	0.912	0.832	-0.003	-0.017 ; 0.015	0.926	0.927	0.859	-0.034	-0.026 ; 0.008	0.301
CRF-%BF	Model 1	0.587	0.344	-0.528	-1.471 ; -0.914	<0.001	0.551	0.304	-0.525	-1.414 ; -0.870	<0.001
	Model 2	0.813	0.662	-0.168	-0.619 ; -0.145	0.002	0.807	0.651	-0.134	-0.517 ; -0.065	0.012
CRF-FMI	Model 1	0.592	0.350	-0.528	-0.439 ; -0.274	<0.001	0.522	0.272	-0.493	-0.446 ; -0.262	<0.001
	Model 2	0.849	0.720	-0.152	-0.167 ; -0.040	0.002	0.859	0.738	-0.080	-0.121 ; 0.006	0.074
		Adolescents									
		Males					Females				
		r	R^2	β	95% CI	p	r	R^2	β	95% CI	p
Cross-sectional analysis Baseline											

CRF-BMI		0.550	0.303	-0.532	-0.290 ; -0.200	**<0.001**	0.345	0.119	-0.266	-0.191 ; -0.074	**<0.001**
CRF-ZBMI		0.528	0.278	-0.554	-0.084 ; -0.058	**<0.001**	0.313	0.098	-0.302	-0.065 ; -0.028	**<0.001**
CRF-ZWHO		0.513	0.263	-0.519	-0.087 ; -0.059	**<0.001**	0.283	0.080	-0.236	-0.054 ; -0.018	**<0.001**
CRF-%BF		0.591	0.349	-0.574	-0.943 ; -0.678	**<0.001**	0.376	0.142	-0.355	-0.519 ; -0.264	**<0.001**
CRF-FMI		0.568	0.322	-0.572	-0.287 ; -0.204	**<0.001**	0.367	0.135	-0.340	-0.168 ; -0.082	**<0.001**
Longitudinal analysis Follow-up											
CRF-BMI	Model 1	0.488	0.238	-0.458	-0.248 ; -0.158	**<0.001**	0.245	0.060	-0.220	-0.173 ; -0.049	**<0.001**
	Model 2	0.860	0.739	-0.015	-0.037 ; 0.024	0.665	0.901	0.812	0.014	-0.021 ; 0.036	0.627
CRF-ZBMI	Model 1	0.441	0.194	-0.465	-0.073 ; -0.046	**<0.001**	0.237	0.056	-0.225	-0.053 ; -0.016	**<0.001**
	Model 2	0.869	0.755	0.017	-0.006 ; 0.011	0.618	0.869	0.754	0.027	-0.006 ; 0.014	0.409
CRF-ZWHO	Model 1	0.430	0.185	-0.444	-0.075 ; -0.046	**<0.001**	0.232	0.054	-0.186	-0.048 ; -0.010	**0.003**
	Model 2	0.883	0.780	0.024	-0.005 ; 0.012	0.459	0.878	0.771	0.012	-0.008 ; 0.011	0.695
CRF-%BF	Model 1	0.504	0.254	-0.510	-0.815 ; -0.547	**<0.001**	0.305	0.093	-0.274	-0.457 ; -0.179	**<0.001**
	Model 2	0.828	0.686	-0.036	-0.153 ; 0.057	0.369	0.840	0.706	0.016	-0.066 ; 0.103	0.668
CRF-FMI	Model 1	0.491	0.241	-0.500	-0.252 ; -0.167	**<0.001**	0.294	0.086	-0.276	-0.161 ; -0.063	**<0.001**
	Model 2	0.836	0.699	-0.027	-0.044 ; 0.020	0.479	0.870	0.756	0.010	-0.023 ; 0.031	0.758

BMI: body mass index; ZBMI: sex- and age- standardized body mass index values; ZWHO: sex- and age- standardized body mass index values according to WHO growth reference standards; %BF: percentage of body fat; CRF: cardiorespiratory fitness; FMI: fat mass index; β: standardized regression coefficients.

Cross-sectional analyses were controlled for age, educational centre, socioeconomic status, mother's education level, and moderate-to-vigorous physical activity at baseline (model 1). Longitudinal analyses were adjusted for age, educational centre, socioeconomic status, and mother's education level at follow-up, and moderate-to-vigorous physical activity at baseline (model 1). Model 2 consisted in model 1 plus the corresponding body fatness index level at baseline.

Table 15. Linear regression models examining the associations between body fatness indices at baseline (exposures) and cardiorespiratory fitness at baseline and at two-year follow-up (outcomes).

		Children									
		Males					Females				
		r	R^2	β	95% CI	p	r	R^2	β	95% CI	p
Cross-sectional analysis Baseline											
BMI-CRF		0.634	0.402	-0.430	-0.898 ; -0.527	<0.001	0.617	0.380	-0.438	-0.699 ; -0.419	<0.001
ZBMI-CRF		0.629	0.396	-0.422	-3.126 ; -1.809	<0.001	0.612	0.375	-0.432	-2.514 ; -1.490	<0.001
ZWHO-CRF		0.615	0.379	-0.397	-2.316 ; -1.280	<0.001	0.631	0.398	-0.455	-2.210 ; -1.366	<0.001
%BF-CRF		0.657	0.432	-0.460	-0.289 ; -0.178	<0.001	0.656	0.430	-0.503	-0.327 ; -0.212	<0.001
FMI-CRF		0.638	0.407	-0.423	-0.864 ; -0.505	<0.001	0.634	0.402	-0.472	-0.989 ; -0.616	<0.001
Longitudinal analysis Follow-up											
BMI-CRF	Model 1	0.615	0.378	-0.479	-1.154 ; -0.710	<0.001	0.598	0.357	-0.423	-0.898 ; -0.524	<0.001
	Model 2	0.800	0.640	-0.180	-0.542 ; -0.158	<0.001	0.765	0.585	-0.168	-0.452 ; -0.112	0.001
ZBMI-CRF	Model 1	0.609	0.371	-0.472	-4.024 ; -2.448	<0.001	0.596	0.355	-0.421	-3.253 ; -1.891	<0.001
	Model 2	0.800	0.639	-0.175	-1.876 ; -0.526	0.001	0.766	0.586	-0.171	-1.660 ; -0.433	0.001
ZWHO-CRF	Model 1	0.585	0.342	-0.437	-2.952 ; -1.703	<0.001	0.601	0.361	-0.425	-2.760 ; -1.622	<0.001
	Model 2	0.794	0.631	-0.153	-1.337 ; -0.294	0.002	0.764	0.583	-0.162	-1.360 ; -0.309	0.002
%BF-CRF	Model 1	0.631	0.398	-0.493	-0.362 ; -0.227	<0.001	0.616	0.379	-0.455	-0.396 ; -0.241	<0.001
	Model 2	0.797	0.634	-0.179	-0.168 ; -0.046	0.001	0.763	0.583	-0.166	-0.191 ; -0.042	0.002
FMI-CRF	Model 1	0.618	0.382	-0.470	-1.109 ; -0.678	<0.001	0.609	0.371	-0.445	-1.243 ; -0.745	<0.001
	Model 2	0.797	0.635	-0.178	-0.527 ; -0.150	<0.001	0.765	0.585	-0.174	-0.622 ; -0.156	0.001
		Adolescents									
		Males					Females				
		r	R^2	β	95% CI	p	r	R^2	β	95% CI	p
Cross-sectional analysis Baseline											

BMI-CRF		0.610	0.373	-0.479	-1.230 ; -0.850	<0.001	0.505	0.255	-0.225	-0.651 ; -0.252	<0.001
ZBMI-CRF		0.622	0.387	-0.470	-4.328 ; -3.003	<0.001	0.516	0.267	-0.246	-2.217 ; -0.963	<0.001
ZWHO-CRF		0.597	0.356	-0.453	-3.842 ; -2.599	<0.001	0.494	0.244	-0.194	-1.912 ; -0.619	<0.001
%BF-CRF		0.638	0.407	-0.523	-0.432 ; -0.310	<0.001	0.531	0.282	-0.297	-0.357 ; -0.181	<0.001
FMI-CRF		0.631	0.398	-0.508	-1.382 ; -0.984	<0.001	0.525	0.275	-0.285	-1.038 ; -0.509	<0.001
Longitudinal analysis Follow-up											
BMI-CRF	Model 1	0.483	0.233	-0.325	-1.086 ; -0.587	<0.001	0.590	0.348	-0.228	-0.812 ; -0.342	<0.001
	Model 2	0.680	0.463	-0.040	-0.345 ; 0.137	0.397	0.808	0.653	-0.092	-0.409 ; -0.056	0.010
ZBMI-CRF	Model 1	0.482	0.232	-0.326	-3.873 ; -2.126	<0.001	0.598	0.357	-0.243	-2.730 ; -1.248	<0.001
	Model 2	0.676	0.457	-0.044	-1.261 ; 0.444	0.347	0.809	0.654	-0.096	-1.346 ; -0.219	0.007
ZWHO-CRF	Model 1	0.467	0.218	-0.295	-3.289 ; -1.671	<0.001	0.584	0.341	-0.207	-2.460 ; -0.945	<0.001
	Model 2	0.680	0.462	-0.018	-0.921 ; 0.613	0.694	0.808	0.653	-0.090	-1.303 ; -0.179	0.010
%BF-CRF	Model 1	0.504	0.254	-0.365	-0.385 ; -0.225	<0.001	0.597	0.357	-0.266	-0.410 ; -0.199	<0.001
	Model 2	0.679	0.461	-0.055	-0.128 ; 0.036	0.269	0.803	0.646	-0.085	-0.180 ; -0.015	0.020
FMI-CRF	Model 1	0.504	0.254	-0.361	-1.256 ; -0.734	<0.001	0.595	0.355	-0.261	-1.213 ; -0.579	<0.001
	Model 2	0.680	0.462	-0.064	-0.440 ; 0.086	0.186	0.804	0.646	-0.089	-0.551 ; -0.059	0.015

BMI: body mass index; ZBMI: sex- and age- standardized body mass index values; ZWHO: sex- and age- standardized body mass index values according to WHO growth reference standards; %BF: percentage of body fat; CRF: cardiorespiratory fitness; FMI: fat mass index; β: standardized regression coefficients.

Cross-sectional analyses were controlled for age, educational centre, socioeconomic status, mother's education level, and moderate-to-vigorous physical activity at baseline (model 1). Longitudinal analyses were adjusted for age, educational centre, socioeconomic status, and mother's education level at follow-up, and moderate-to-vigorous physical activity at baseline (model 1). Model 2 consisted in model 1 plus cardiorespiratory fitness at baseline.

The associations between CRF changes (exposure) and body fatness indices at follow-up (outcomes) are depicted in table 16. CRF changes were associated with all body fatness indices in children of both sexes and female adolescents, and with %BF and FMI in male adolescents after adjusting by sociodemographic and lifestyle confounders (model 1) (β ranging from -0.120 to -0.280; all P<0.05). When body fatness indices levels at baseline were included in the model (model 2), CRF changes were associated with all fatness indices at follow-up in children of both sexes and in male adolescents, and with %BF and FMI in female adolescents (β ranging from -0.062 to -0.173; all P<0.05).

Table 17 shows the association between fatness changes (exposures) and CRF at follow-up (outcome). Changes in each fatness index, except for ZWHO in male children, BMI, %BF and FMI in female children, and %BF and FMI in male adolescents were associated with CRF at follow-up (model 1) (β ranging from -0.162 to -0.360; all P<0.01). After including baseline CRF levels in the model (model 2), there were significant associations between changes in each body fatness index in male children and adolescents, in BMI, ZWHO, %BF and FMI in female children, and in FMI in female adolescents with CRF at follow-up (β ranging from -0.079 to -0.260; all P<0.05).

The same analyses were performed adjusting by pubertal development instead of age, sedentary time instead of moderate-to-vigorous PA, the KIDMED index assessing the adherence to the Mediterranean diet and the FTO gene polymorphisms, and the results did not materially change (data not shown). Finally, supplementary tables showing the associations between changes in CRF and fatness with changes in fatness (table S7 in supplementary content) and CRF levels (table S8 in supplementary content), respectively, are displayed in the 'Supplementary content' section. Tables S9 and S10, respectively, in the 'Supplementary content' section, show the probability of being OW at baseline and at follow-up according to CRF values at baseline, and the probability of having an unhealthy CRF at baseline and at follow-up according to baseline BMI values.

Table 16. Linear regression models examining the associations between cardiorespiratory fitness changes (exposure) and fatness indices at two-year follow-up (outcomes).

		Children														
		Males							Females							
		r	R^2	β	95% CI			p	r	R^2	β	95% CI			p
Longitudinal analysis Follow-up															
CRF-BMI	Model 1	0.479	0.229	-0.267	-0.362	;	-0.140	**<0.001**	0.269	0.073	-0.192	-0.320	;	-0.056	**0.006**
	Model 2	0.945	0.892	-0.131	-0.166	;	-0.081	**<0.001**	0.938	0.880	-0.065	-0.112	;	-0.016	**0.009**
CRF-ZBMI	Model 1	0.486	0.237	-0.280	-0.091	;	-0.037	**<0.001**	0.266	0.071	-0.196	-0.076	;	-0.014	**0.005**
	Model 2	0.939	0.882	-0.149	-0.045	;	-0.023	**<0.001**	0.934	0.873	-0.063	-0.026	;	-0.003	**0.015**
CRF-ZWHO	Model 1	0.477	0.228	-0.249	-0.112	;	-0.040	**<0.001**	0.238	0.057	-0.176	-0.091	;	-0.012	**0.011**
	Model 2	0.921	0.849	-0.132	-0.055	;	-0.024	**<0.001**	0.928	0.861	-0.062	-0.034	;	-0.003	**0.022**
CRF-%BF	Model 1	0.439	0.193	-0.252	-1.096	;	-0.385	**<0.001**	0.324	0.105	-0.177	-0.750	;	-0.105	**0.010**
	Model 2	0.821	0.674	-0.173	-0.735	;	-0.278	**<0.001**	0.806	0.650	-0.102	-0.450	;	-0.043	**0.018**
CRF-FMI	Model 1	0.453	0.206	-0.264	-0.337	;	-0.126	**<0.001**	0.319	0.102	-0.180	-0.251	;	-0.037	**0.009**
	Model 2	0.857	0.734	-0.166	-0.208	;	-0.084	**<0.001**	0.860	0.740	-0.082	-0.123	;	-0.007	**0.028**

		Adolescents														
		Males							Females							
		r	R^2	β	95% CI			p	r	R^2	β	95% CI			p
Longitudinal analysis Follow-up															
CRF-BMI	Model 1	0.251	0.063	-0.022	-0.060	;	0.041	0.714	0.196	0.038	-0.135	-0.157	;	-0.011	**0.024**
	Model 2	0.865	0.748	-0.109	-0.074	;	-0.021	**<0.001**	0.902	0.814	-0.046	-0.061	;	0.003	0.079
CRF-ZBMI	Model 1	0.125	0.016	-0.030	-0.019	;	0.011	0.617	0.174	0.030	-0.125	-0.046	;	-0.002	**0.035**
	Model 2	0.875	0.766	-0.119	-0.022	;	-0.008	**<0.001**	0.869	0.755	-0.039	-0.018	;	0.004	0.198
CRF-ZWHO	Model 1	0.148	0.022	0.034	-0.011	;	0.021	0.574	0.200	0.040	-0.126	-0.046	;	-0.002	**0.036**

	Model 2	0.885	0.784	-0.076	-0.018	;	-0.003	**0.009**	0.879	0.772	-0.036	-0.018	;	0.004	0.216
CRF-%BF	Model 1	0.218	0.047	-0.121	-0.314	;	-0.005	**0.042**	0.223	0.050	-0.131	-0.353	;	-0.022	**0.027**
	Model 2	0.850	0.723	-0.220	-0.376	;	-0.207	**<0.001**	0.843	0.711	-0.077	-0.202	;	-0.018	**0.020**
CRF-FMI	Model 1	0.206	0.043	-0.120	-0.098	;	-0.001	**0.045**	0.207	0.043	-0.136	-0.126	;	-0.010	**0.022**
	Model 2	0.856	0.732	-0.208	-0.113	;	-0.060	**<0.001**	0.872	0.761	-0.070	-0.065	;	-0.006	**0.019**

BMI: body mass index; ZBMI: sex- and age- standardized body mass index values; ZWHO: sex- and age- standardized body mass index values according to WHO growth reference standards; %BF: percentage of body fat; CRF: cardiorespiratory fitness; FMI: fat mass index; β: standardized regression coefficients.

Analyses were adjusted for age, educational centre, socioeconomic status, and mother's education level at follow-up, and moderate-to-vigorous physical activity at baseline (model 1). Model 2 consisted in model 1 plus the corresponding body fatness index level at baseline.

Table 17. Linear regression models examining the associations between fatness changes (exposures) and cardiorespiratory fitness at two-year follow-up (outcome).

		Children													
		Males						Females							
		r	R^2	β	95% CI			p	r	R^2	β	95% CI			p
Longitudinal analysis Follow-up															
BMI-CRF	Model 1	0.546	0.298	-0.347	-2.135	;	-1.076	**<0.001**	0.487	0.237	-0.236	-1.434	;	-0.444	**<0.001**
	Model 2	0.824	0.679	-0.255	-1.541	;	-0.815	**<0.001**	0.764	0.584	-0.151	-0.971	;	-0.231	**0.002**
ZBMI-CRF	Model 1	0.460	0.212	-0.167	-5.096	;	-0.835	**0.007**	0.435	0.190	-0.062	-3.059	;	1.073	0.344
	Model 2	0.813	0.661	-0.209	-5.111	;	-2.306	**<0.001**	0.755	0.570	-0.083	-2.829	;	0.191	0.087
ZWHO-CRF	Model 1	0.439	0.193	-0.112	-2.874	;	0.126	0.072	0.445	0.198	-0.112	-3.000	;	0.188	0.084
	Model 2	0.802	0.643	-0.171	-3.108	;	-1.099	**<0.001**	0.757	0.573	-0.102	-2.441	;	-0.110	**0.032**
%BF-CRF	Model 1	0.526	0.276	-0.303	-0.367	;	-0.165	**<0.001**	0.470	0.221	-0.178	-0.292	;	-0.052	**0.005**
	Model 2	0.801	0.642	-0.198	-0.246	;	-0.101	**<0.001**	0.758	0.574	-0.116	-0.202	;	-0.023	**0.014**
FMI-CRF	Model 1	0.559	0.312	-0.360	-1.516	;	-0.792	**<0.001**	0.487	0.238	-0.224	-1.172	;	-0.336	**<0.001**
	Model 2	0.807	0.651	-0.226	-0.989	;	-0.459	**<0.001**	0.760	0.577	-0.129	-0.753	;	-0.121	**0.007**
		Adolescents													
		Males						Females							
		r	R^2	β	95% CI			p	r	R^2	β	95% CI			p
Longitudinal analysis Follow-up															
BMI-CRF	Model 1	0.370	0.137	-0.047	-0.719	;	0.259	0.356	0.548	0.300	-0.015	-0.604	;	0.448	0.769
	Model 2	0.692	0.479	-0.133	-1.039	;	-0.269	**0.001**	0.805	0.648	-0.049	-0.636	;	0.113	0.170
ZBMI-CRF	Model 1	0.363	0.132	-0.057	-2.817	;	0.781	0.266	0.550	0.302	0.049	-0.710	;	2.263	0.305
	Model 2	0.691	0.477	-0.148	-4.063	;	-1.233	**<0.001**	0.804	0.646	-0.016	-1.315	;	0.818	0.647

ZWHO-CRF	Model 1	0.359	0.129	0.007	-1.627 ; 1.883	0.886	0.537	0.288	0.020	-1.227 ; 1.890	0.676
	Model 2	0.682	0.465	-0.097	-3.085 ; -0.287	**0.018**	0.801	0.642	-0.021	-1.447 ; 0.773	0.551
%BF-CRF	Model 1	0.392	0.153	-0.162	-0.371 ; -0.082	**0.002**	0.539	0.291	-0.023	-0.226 ; 0.139	0.641
	Model 2	0.720	0.519	-0.260	-0.474 ; -0.253	**<0.001**	0.802	0.644	-0.066	-0.257 ; 0.003	0.055
FMI-CRF	Model 1	0.394	0.155	-0.164	-1.250 ; -0.294	**0.002**	0.542	0.294	-0.066	-0.977 ; 0.181	0.177
	Model 2	0.718	0.516	-0.247	-1.531 ; -0.799	**<0.001**	0.803	0.645	-0.079	-0.891 ; -0.069	**0.022**

BMI: body mass index; ZBMI: sex- and age- standardized body mass index values; ZWHO: sex- and age- standardized body mass index values according to WHO growth reference standards; %BF: percentage of body fat; CRF: cardiorespiratory fitness; FMI: fat mass index; β: standardized regression coefficients.

Analyses were adjusted for age, educational centre, socioeconomic status, and mother's education level at follow-up, and moderate-to-vigorous physical activity at baseline (model 1). Model 2 consisted in model 1 plus cardiorespiratory fitness at baseline.

Table S7. Linear regression models examining the associations between cardiorespiratory fitness changes (exposure) and fatness changes (outcomes).

	Children													
	Males							Females						
	r	R^2	β	95% CI			p	r	R^2	β	95% CI			p
Longitudinal analysis Follow-up														
CRF-BMI	0.440	0.194	-0.370	-0.169	;	-0.086	**<0.001**	0.355	0.126	-0.192	-0.118	;	-0.022	**0.004**
CRF-ZBMI	0.382	0.146	-0.333	-0.041	;	-0.019	**<0.001**	0.356	0.127	-0.117	-0.023	;	0.001	0.078
CRF-ZOMS	0.340	0.116	-0.272	-0.051	;	-0.019	**<0.001**	0.323	0.104	-0.142	-0.032	;	-0.001	**0.036**
CRF-%BF	0.332	0.110	-0.278	-0.721	;	-0.267	**<0.001**	0.286	0.082	-0.154	-0.434	;	-0.028	**0.026**
CRF-FMI	0.360	0.129	-0.304	-0.209	;	-0.087	**<0.001**	0.309	0.096	-0.161	-0.127	;	-0.011	**0.019**

	Adolescents													
	Males							Females						
	r	R^2	β	95% CI			p	r	R^2	β	95% CI			p
Longitudinal analysis Follow-up														
CRF-BMI	0.232	0.054	-0.234	-0.083	;	-0.028	**<0.001**	0.318	0.101	-0.088	-0.058	;	0.007	0.124
CRF-ZBMI	0.254	0.064	-0.254	-0.024	;	-0.009	**<0.001**	0.136	0.018	-0.049	-0.016	;	0.007	0.412
CRF-ZOMS	0.231	0.053	-0.186	-0.020	;	-0.005	**0.002**	0.131	0.017	-0.049	-0.016	;	0.007	0.417
CRF-%BF	0.454	0.206	-0.384	-0.409	;	-0.230	**<0.001**	0.200	0.040	-0.127	-0.194	;	-0.008	**0.034**
CRF-FMI	0.409	0.167	-0.377	-0.120	;	-0.066	**<0.001**	0.222	0.049	-0.138	-0.064	;	-0.005	**0.020**

BMI: body mass index; ZBMI: sex- and age- standardized body mass index values; ZWHO: sex- and age- standardized body mass index values according to WHO growth reference standards; %BF: percentage of body fat; CRF: cardiorespiratory fitness; FMI: fat mass index; β: standardized regression coefficients.

Analyses were adjusted for age, educational centre, socioeconomic status, and mother's education level at follow-up, and moderate-to-vigorous physical activity at baseline. Statistically significant values are highlighted in bold.

Table S8. Linear regression models examining the associations between fatness changes (exposures) and cardiorespiratory fitness changes (outcome).

	Children									
	Males					Females				
	r	R^2	β	95% CI	p	r	R^2	β	95% CI	p
Longitudinal analysis Follow-up										
BMI-CRF	0.406	0.164	-0.384	-1.476 ; -0.751	**<0.001**	0.309	0.096	-0.198	-0.912 ; -0.172	**0.004**
ZBMI-CRF	0.368	0.136	-0.337	-5.161 ; -2.358	**<0.001**	0.272	0.074	-0.125	-2.879 ; 0.155	0.078
ZOMS-CRF	0.314	0.098	-0.278	-3.157 ; -1.154	**<0.001**	0.282	0.080	-0.146	-2.430 ; -0.085	**0.036**
%BF-CRF	0.321	0.103	-0.280	-0.230 ; -0.085	**<0.001**	0.291	0.084	-0.153	-0.192 ; -0.012	**0.026**
FMI-CRF	0.344	0.119	-0.308	-0.897 ; -0.371	**<0.001**	0.295	0.087	-0.163	-0.694 ; -0.063	**0.019**
	Adolescents									
	Males					Females				
	r	R^2	β	95% CI	p	r	R^2	β	95% CI	p
Longitudinal analysis Follow-up										
BMI-CRF	0.494	0.244	-0.187	-1.177 ; -0.393	**<0.001**	0.359	0.129	-0.085	-0.677 ; 0.083	0.124
ZBMI-CRF	0.501	0.251	-0.203	-4.575 ; -1.701	**<0.001**	0.353	0.124	-0.044	-1.528 ; 0.628	0.412
ZOMS-CRF	0.482	0.233	-0.151	-3.670 ; -0.836	**0.002**	0.362	0.131	-0.043	-1.589 ; 0.661	0.417
%BF-CRF	0.557	0.311	-0.334	-0.513 ; -0.289	**<0.001**	0.369	0.136	-0.114	-0.275 ; -0.011	**0.034**
FMI-CRF	0.551	0.303	-0.315	-1.651 ; -0.905	**<0.001**	0.372	0.139	-0.125	-0.913 ; -0.078	**0.020**

BMI: body mass index; ZBMI: sex- and age- standardized body mass index values; ZWHO: sex- and age- standardized body mass index values according to WHO growth reference standards; %BF: percentage of body fat; CRF: cardiorespiratory fitness; FMI: fat mass index; β: standardized regression coefficients.

Analyses were adjusted for age, educational centre, socioeconomic status, and mother's education level at follow-up, and moderate-to-vigorous physical activity at baseline. Statistically significant values are highlighted in bold.

Table S9. Binary logistic regression analyses testing the odds ratio of being overweight/obese at baseline and at two-years follow-up according to cardiorespiratory fitness groups (healthy or unhealthy) at baseline.

		Children							
		Males				Females			
		OR	95% CI			OR	95% CI		
Outcome: to be overweight/obese at baseline									
Unhealthy CRF		**4.602**	**1.976**	**;**	**10.721**	**7.916**	**3.539**	**;**	**17.708**
Healthy CRF	Ref. group	1.0		-		1.0		-	
Outcome: to be overweight/obese at follow-up									
Unhealthy CRF	model 1	**3.394**	**1.656**	**;**	**6.955**	**6.196**	**2.908**	**;**	**13.206**
	model 2	1.246	0.533	;	2.917	1.260	0.376	;	4.219
Healthy CRF	Ref. group	1.0		-		1.0		-	
		Adolescents							
		Males				Females			
		OR	95% CI			OR	95% CI		
Outcome: to be overweight/obese at baseline									
Unhealthy CRF		**3.531**	**1.863**	**;**	**6.691**	2.078	0.769	;	5.614
Healthy CRF	Ref. group	1.0		-		1.0		-	
Outcome: to be overweight/obese at follow-up									
Unhealthy CRF	model 1	**3.225**	**1.564**	**;**	**6.650**	1.386	0.545	;	3.525
	model 2	0.612	0.222	;	1.688	0.324	0.072	;	1.451
Healthy CRF	Ref. group	1.0		-		1.0		-	

CRF: cardiorespiratory fitness.

Cross-sectional analyses were controlled for age, educational centre, socioeconomic status, mother's education level, and moderate-to-vigorous physical activity at baseline (model 1). Longitudinal analyses were adjusted for age, educational centre, socioeconomic status, and mother's education level at follow-up, and moderate-to-vigorous physical activity at baseline (model 1). Model 2 consisted in model 1 plus body mass index levels at baseline. Statistically significant values are highlighted in bold.

Table S10. Binary logistic regression analyses testing the odds ratio of having unhealthy cardiorespiratory fitness levels at baseline and at two-years follow-up according to body mass index groups (normal weight and overweight/obese) at baseline.

		Children							
		Males				Females			
		OR	95% CI			OR	95% CI		
Outcome: to have unhealthy CRF levels at baseline									
Unhealthy CRF		**4.501**	**1.950**	**;**	**10.389**	**7.915**	**3.513**	**;**	**17.835**
Healthy CRF	Ref. group	1.0		-		1.0		-	
Outcome: to have unhealthy CRF levels at follow-up									
Unhealthy CRF	model 1	**3.599**	**1.553**	**;**	**8.341**	**6.217**	**2.712**	**;**	**14.250**
	model 2	0.976	0.355	;	2.686	2.318	0.917	;	5.861
Healthy CRF	Ref. group	1.0		-		1.0		-	
		Adolescents							
		Males				Females			
		OR	95% CI			OR	95% CI		
Outcome: to have unhealthy CRF levels at baseline									
Unhealthy CRF		**3.622**	**1.912**	**;**	**6.860**	2.074	0.764	;	5.630
Healthy CRF	Ref. group	1.0		-		1.0		-	
Outcome: to have unhealthy CRF levels at follow-up									
Unhealthy CRF	model 1	**2.654**	**1.556**	**;**	**4.525**	3.043	0.680	;	13.619
	model 2	1.417	0.770	;	2.607	1.294	0.218	;	7.680
Healthy CRF	Ref. group	1.0		-		1.0		-	

CRF: cardiorespiratory fitness

Cross-sectional analyses were controlled for age, educational centre, socioeconomic status, mother's educational level, and moderate to vigorous physical activity at baseline (model 1). Longitudinal analyses were adjusted for age, educational centre, socioeconomic status, and mother's education level at follow-up, and moderate-to-vigorous physical activity at baseline (model 1). Model 2 consisted in model 1 plus cardiorespiratory fitness at baseline.

Study VI (part I): The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study

The characteristics of the study sample are depicted in **table 18**. Overall, CRF, NC, SBP, DBP and MAP increased from baseline to follow-up in all sex and age groups (all $p<0.05$) except SBP, DBP and MAP in female adolescent which were significantly lower at follow-up (all $p<0.001$).

Results from cross-lagged panel analyses in male and female children and adolescents are depicted in **figure 11**.

Auto-associations (i.e. longitudinal associations to assess stability) were significant in all sex and age groups. Baseline CRF was associated with CRF two years later (β ranging from 0.667 to 0.765; all $p<0.001$) and NC at baseline with NC two years later (β ranging from 0.659 to 0.876; all $p<0.001$).

Synchronous associations (i.e. cross-sectional bidirectional associations) between CRF and NC were significant in both directions in all sex and age groups. CRF at time one and two were associated with NC at time one and two (β ranging from -0.105 to -0.370; all $p<0.05$), respectively. Similarly, NC at time one and two were associated with CRF at time one and two (β ranging from -0.115 to -0.437; all $p<0.05$), respectively.

Cross-lagged regressions (i.e. longitudinal bidirectional associations) between CRF and NC were only significant for male (β=-0.187; $p<0.001$) and female (β=-0.096; p=0.013) children when NC was introduced as the exposure and CRF as the outcome, independently of baseline CRF levels. These associations were not significant in adolescents and when CRF was considered the exposure.

Table 18. Participants' characteristics by sex and age groups at baseline and two-year follow-up.

	Children					
	Males (n=563)			Females (n=507)		
Variables	Baseline	Follow-up	p	Baseline	Follow-up	p
Age, yr	8.10 (1.58)	10.14 (1.57)	-	8.22 (1.57)	10.27 (1.57)	-
Cardiorespiratory fitness, stages	3.14 (1.71)	4.23 (1.96)	**<0.001**	2.29 (1.27)	3.18 (1.62)	**<0.001**
Neck circumference, cm	27.52 (2.11)	28.78 (2.41)	**<0.001**	26.78 (2.38)	28.12 (2.62)	**<0.001**
Systolic blood pressure, mmHg	103.37 (10.87)	106.18 (10.16)	**<0.001**	101.65 (11.50)	105.89 (12.45)	**<0.001**
Diastolic blood pressure, mmHg	65.42 (8.38)	66.12 (7.59)	**0.042**	66.86 (9.86)	67.54 (8.98)	0.105
MAP	78.07 (8.54)	79.47 (7.71)	**<0.001**	78.45 (9.74)	80.32 (9.51)	**<0.001**

	Adolescents					
	Males (n=379)			Females (n=337)		
Variables	Baseline	Follow-up	p	Baseline	Follow-up	p
Age, yr	13.77 (1.50)	15.75 (1.50)	-	13.76 (1.45)	15.73 (1.44)	-
Cardiorespiratory fitness, stages	6.50 (2.41)	7.74 (2.24)	**<0.001**	4.14 (1.61)	4.49 (1.70)	**<0.001**
Neck circumference, cm	32.42 (2.84)	34.33 (3.57)	**<0.001**	30.23 (1.71)	30.60 (1.57)	**<0.001**
Systolic blood pressure, mmHg	109.40 (13.61)	112.95 (11.89)	**<0.001**	105.99 (10.25)	102.79 (9.56)	**<0.001**
Diastolic blood pressure, mmHg	65.12 (7.13)	66.11 (7.60)	**0.01**	67.36 (6.90)	65.82 (6.99)	**<0.001**
MAP	79.88 (8.33)	81.72 (8.04)	**<0.001**	80.23 (7.17)	78.14 (7.00)	**<0.001**

Values are presented as means (standard deviation).

Statistically significant differences between baseline and follow-up values are highlighted in bold.

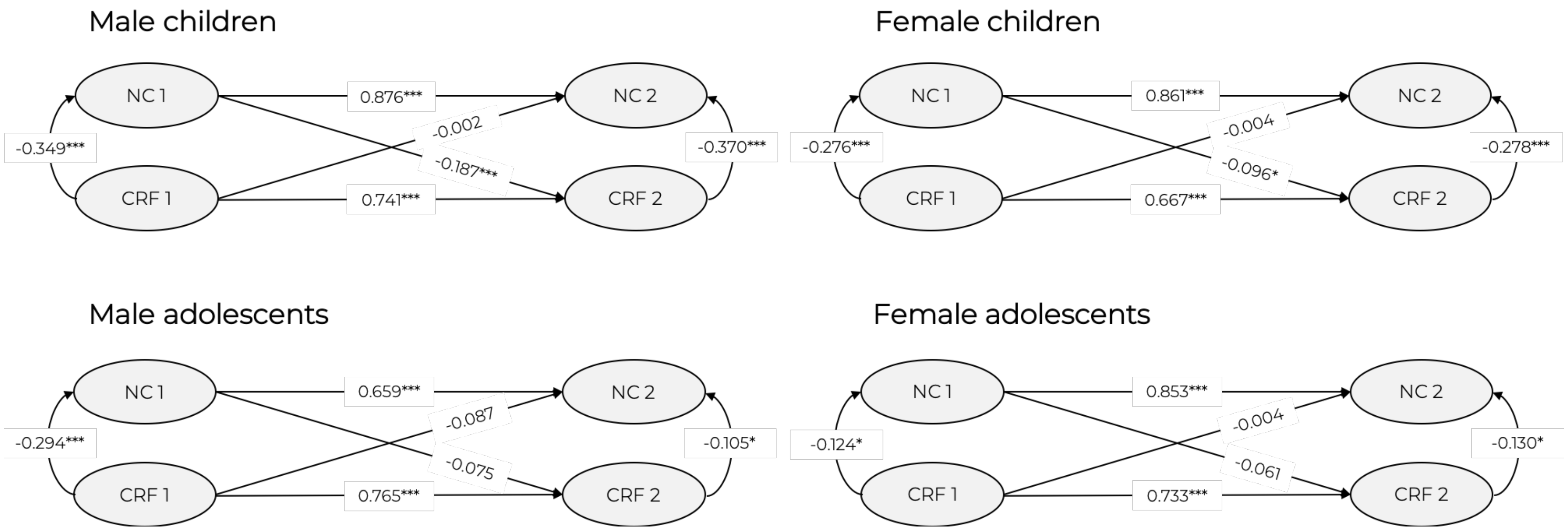

Figure 11. Cross-lagged panels assessing the stability and the bidirectional associations between CRF and NC in male and female children and adolescents. Results are presented as standardized regression coefficients (β). CRF 1: cardiorespiratory fitness (stages) at baseline; CRF 2: cardiorespiratory fitness at follow-up; NC 1: neck circumference at baseline; NC 2: neck circumference at follow-up. *: p<0.05; ***: p<0.001.

Study VII (part I): Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis. The UP&DOWN study

Characteristics of the study sample are depicted in table 19. All the studied measures increased from baseline to two-year follow-up, including MF tests, NC, and BP measures in all sex and age groups (all $p<0.05$), except DBP in female children ($p=0.105$), and in female adolescents, whose SBP, DBP, and MAP values decreased from baseline to follow-up (all $p<0.001$).

Results from cross-lagged panel analyses in male and female children and adolescents are depicted in figure 12.

Auto-associations (i.e. longitudinal associations to assess stability) in MF and NC over two-years was significant in all sex and age groups. MF at baseline was significantly associated with MF two years later (β ranging from 0.794 to 0.915; all $p<0.001$), and NC at baseline was associated with NC two years later (β ranging from 0.659 to 0.876; all $p<0.001$).

Synchronous associations (i.e. cross-sectional bidirectional associations) were observed at both time points. MF at time one and two were associated with NC at time one and two, respectively, in all sex and age groups (β ranging from -0.177 to -0.402; all $p<0.001$), except in male adolescents at time two. Besides, NC at time one and two were associated with MF at time 1 and 2, respectively, in all sex and age groups (β ranging from -0.174 to -0.462; all $p<0.01$), except in male adolescents at time two.

Cross-lagged regressions (i.e. longitudinal bidirectional associations) were only significant when NC was considered the exposure and MF the outcome in male and female children and adolescents (β ranging from -0.075 to -0.142; all $p<0.05$) after adjusting by MF at baseline.

Table 19. Participants' characteristics by sex and age groups at baseline and two-year follow-up.

Children						
	Males (n=563)			Females (n=507)		
Variables	Baseline	follow-up	p	Baseline	follow-up	p
Age, yr	8.10 (1.58)	10.14 (1.57)	-	8.22 (1.57)	10.27 (1.57)	-
Handgrip/weight (kg/kg)	0.40 (0.09)	0.41 (0.08)	**0.001**	0.38 (0.08)	0.40 (0.08)	**<0.001**
Standing long jump (cm)	115.91 (21.20)	130.62 (22.58)	**<0.001**	107.32 (21.04)	121.92 (21.78)	**<0.001**
Neck circumference, cm	27.52 (2.11)	28.78 (2.41)	**<0.001**	26.78 (2.38)	28.12 (2.62)	**<0.001**
Systolic blood pressure, mmHg	103.37 (10.87)	106.18 (10.16)	**<0.001**	101.65 (11.50)	105.89 (12.45)	**<0.001**
Diastolic blood pressure, mmHg	65.42 (8.38)	66.12 (7.59)	**0.042**	66.86 (9.86)	67.54 (8.98)	0.105
MAP	78.07 (8.54)	79.47 (7.71)	**<0.001**	78.45 (9.74)	80.32 (9.51)	**<0.001**

Adolescents						
	Males (n=379)			Females (n=337)		
Variables	Baseline	follow-up	p	Baseline	follow-up	p
Age, yr	13.77 (1.50)	15.75 (1.50)	-	13.76 (1.45)	15.73 (1.44)	-
Handgrip/weight (kg/kg)	0.49 (0.10)	0.57 (0.10)	**<0.001**	0.45 (0.08)	0.47 (0.08)	**<0.001**
Standing long jump (cm)	170.64 (33.11)	191.91 (31.23)	**<0.001**	142.36 (21.37)	147.23 (21.87)	**<0.001**
Neck circumference, cm	32.42 (2.84)	34.33 (3.57)	**<0.001**	30.23 (1.71)	30.60 (1.57)	**<0.001**
Systolic blood pressure, mmHg	109.40 (13.61)	112.95 (11.89)	**<0.001**	105.99 (10.25)	102.79 (9.56)	**<0.001**
Diastolic blood pressure, mmHg	65.12 (7.13)	66.11 (7.60)	**0.010**	67.36 (6.90)	65.82 (6.99)	**<0.001**
MAP	79.88 (8.33)	81.72 (8.03)	**<0.001**	80.23 (7.17)	78.14 (7.00)	**<0.001**

Values are presented as means (standard deviation).

Statistically significant differences between baseline and follow-up values are highlighted in bold.

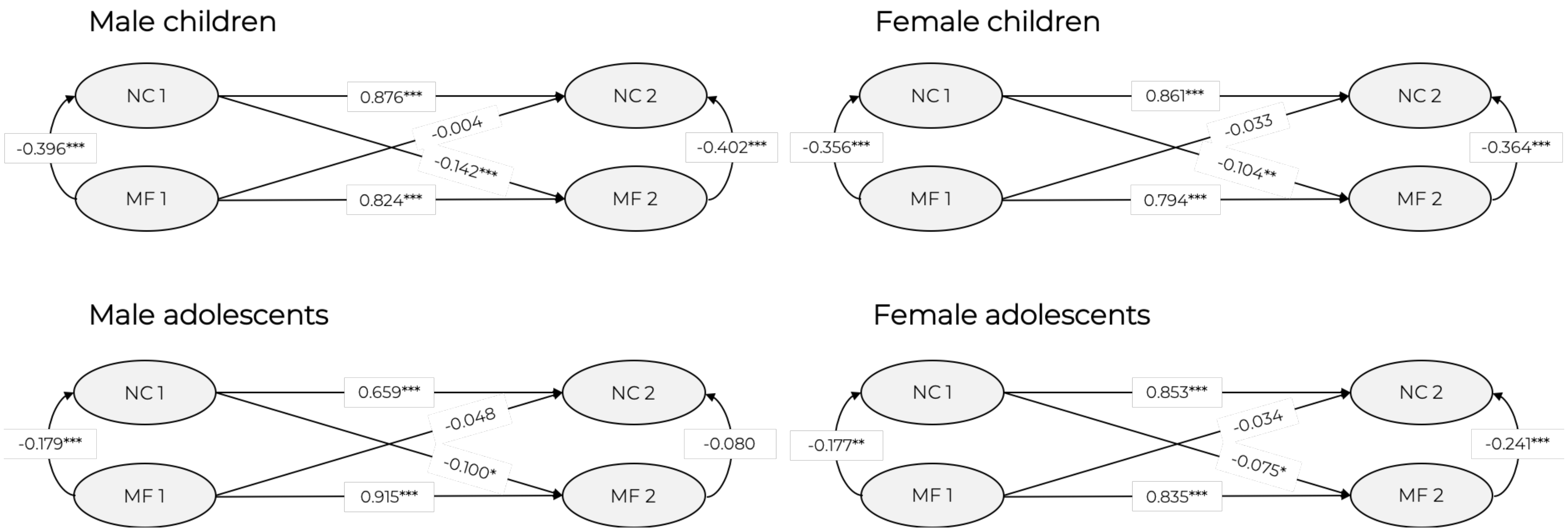

Figure 12. Cross-lagged panels assessing the stability and the bidirectional associations between MF and NC in male and female children and adolescents. Results are presented as standardized regression coefficients (β). MF 1: muscular fitness (global muscular fitness including handgrip/weight and standing long jump tests) at baseline; MF 2: muscular fitness at follow-up; NC 1: neck circumference at baseline; NC 2: neck circumference at follow-up. *: p<0.05; **: p<0.01; ***: p<0.001.

Chapter IV: Independent effect of fitness and fatness on BP

Study VI (part II): The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study

The independent effects of CRF and NC on BP measures is shown in **figure 13**. CRF at baseline was associated with DBP and MAP at baseline in female adolescents (β=-0.195 and β=-0.142; $p<0.05$). Longitudinal analyses adjusted by NC showed no significant associations between CRF and any BP variable in any sex and age group.

NC at baseline was associated with SBP, DBP, and MAP at baseline in all sex and age groups (β ranging from 0.218 to 0.728; all $p<0.001$), except for DBP in female adolescents. Longitudinal analyses showed significant associations between NC and SBP in children of both sexes (β=0.191 in males and β=0.266 in females; both $p<0.001$), DBP in male and female children and male adolescents (β=0.255, β=0.316 and β=0.192, respectively; all $p<0.01$), and MAP in male and female children and male adolescents (β=0.238, β=0.311 and β=0.169, respectively; all $p<0.05$). All these associations were independent of baseline CRF.

No substantial changes were observed when the aforementioned analyses were adjusted for pubertal status instead of age.

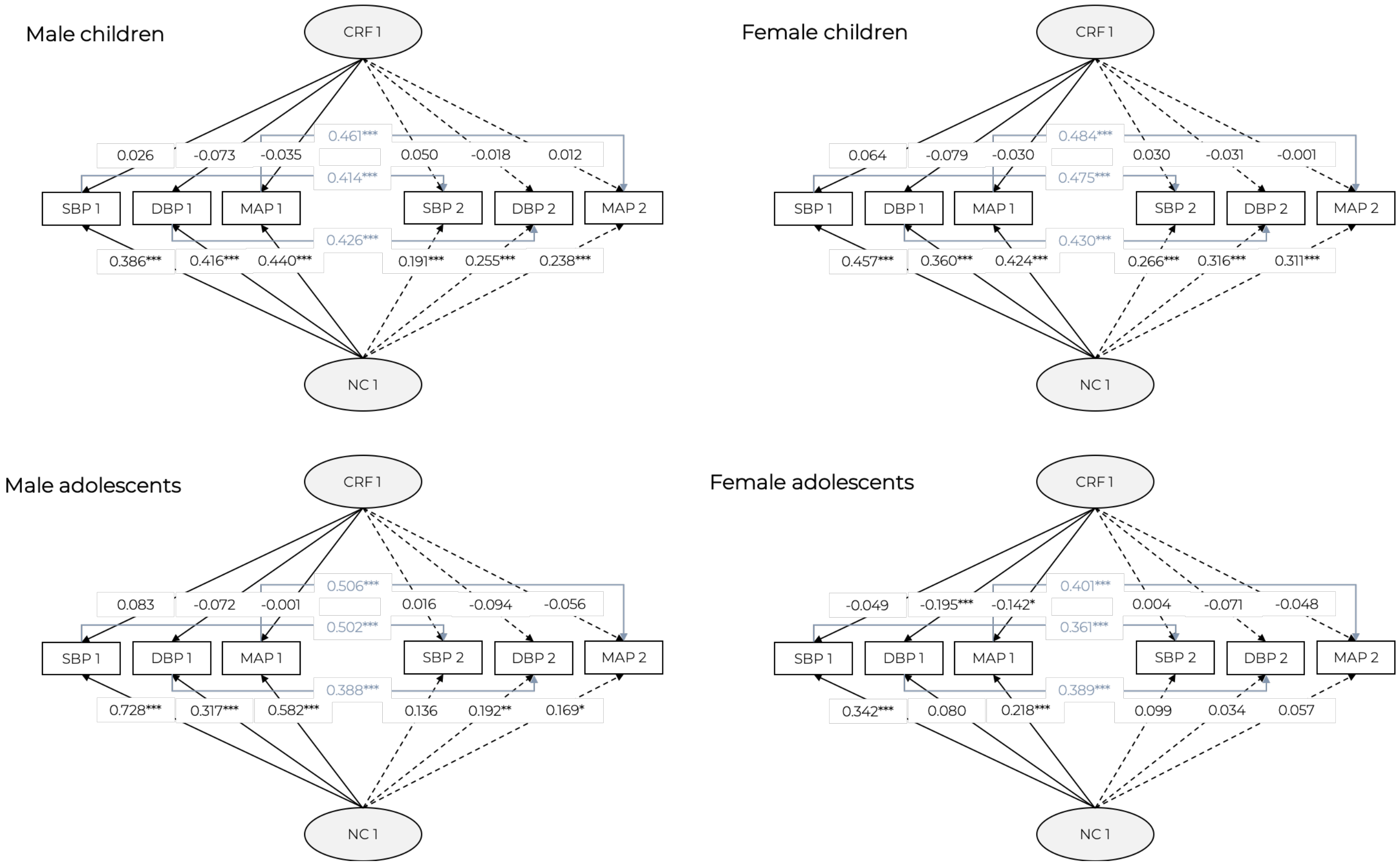

Figure 13. Independent associations between CRF and NC with BP measures at baseline (time 1) and follow-up (time 2). Results are presented as standardized regression coefficients (β). Cross-sectional analyses were adjusted by age and by the complementary exposure at baseline (i.e. CRF or NC depending on the fixed exposure). Longitudinal analyses were adjusted by age, the complementary exposure, and the outcome (i.e. the corresponding BP measure) at baseline. CRF: cardiorespiratory fitness (stages); DBP: diastolic blood pressure; MAP: mean arterial pressure; NC: neck circumference; SBP: systolic blood pressure; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Study VII (part II): Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis. The UP&DOWN study

The independent effects of MF and NC on BP measures is depicted in **figure 14**. MF at baseline was associated with DBP at baseline in female children (β=-0.110; p=0.027). Longitudinally, MF was positively associated with SBP in male children (β=0.107; p=0.025), and negatively associated with DBP (β=-0.139; p=0.009) and MAP (β=-0.123; p=0.020) in female adolescents, independently of NC and the BP values at baseline. On the other hand, NC at baseline was associated with SBP, DBP, and MAP at baseline in all sex and age groups (β ranging from 0.221 to 0.705; all p<0.001), except for DBP in female adolescents. NC was longitudinally associated with SBP in male (β=-0.219; p<0.001) and female (β=-0.273; p<0.001) children, DBP in male and female children and male adolescents (β=0.269, β=0.313, β=0.217; all p≤0.001), and MAP in male and female children and male adolescents (β=0.258, β=0.313, β=0.187; all p<0.01), after accounting for MF and BP values at baseline.

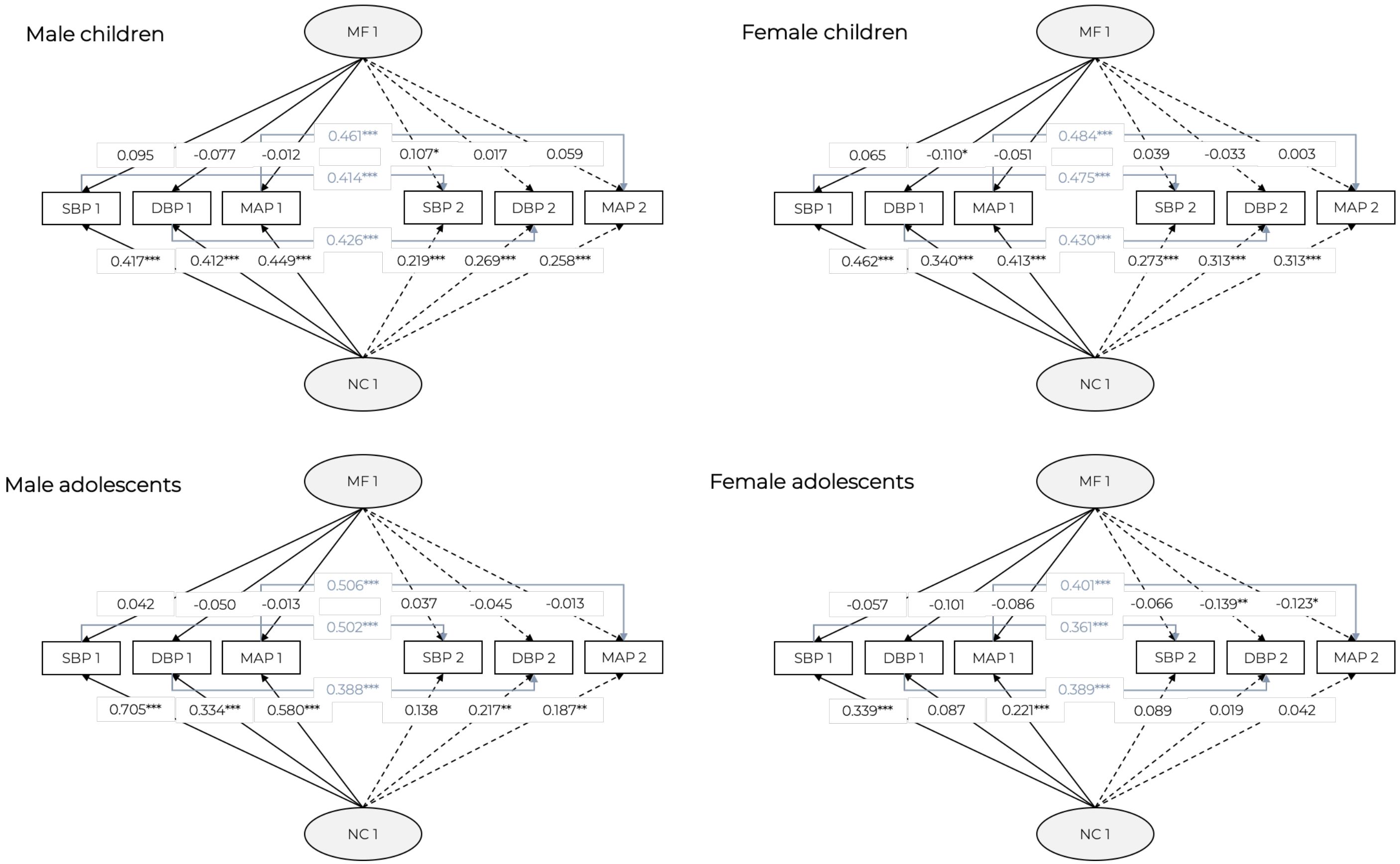

Figure 14. Independent associations between MF and NC with BP measures at baseline (time 1) and follow-up (time 2). Results are presented as standardized regression coefficients (β). Cross-sectional analyses were adjusted by age and by the complementary exposure at baseline (i.e. MF or NC depending on the fixed exposure). Longitudinal analyses were adjusted by age, the complementary exposure, and the outcome (i.e. the corresponding BP measure) at baseline. DBP: diastolic blood pressure; MAP: mean arterial pressure; MF: muscular fitness (global muscular fitness including handgrip/weight and standing long jump tests) NC: neck circumference; SBP: systolic blood pressure; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Study VIII: Fitness, fatness and their association with future blood pressure in youth: The UP&DOWN longitudinal study

The participants' characteristics by age groups are presented in table 20. Upper- and lower-body MF, WC, and BMI increased from baseline to follow-up in both children and adolescents (all $p<0.001$). CRF (VO_2max) decreased over the 2 years in children and adolescents (both $p<0.001$). All BP variables (i.e. SBP, DBP, and MAP) were higher at follow-up than at baseline in children (all $p<0.05$), but not in adolescents.

The longitudinal associations between MF, CRF and WC at baseline, and BP variables at follow-up are depicted in table 21. In simple models (model 1), WC predicted SBP and MAP in both children and adolescents (standardized β ranging from 0.093 to 0.178; all $p<0.05$). Upper-body MF (β=-0.100), global MF (β=-0.097), CRF (β=-0.104) and WC (β=0.235) predicted DBP in children, whereas lower-body MF (β=-0.100), global MF (β=-0.083), CRF (β=-0.113), and WC (β=0.118) predicted DBP in adolescents (all $p<0.05$). In joint models, including mutual adjustment for all explanatory variables (model 2), WC was independently associated with each BP variable in children (β ranging from 0.137 to 0.260; all $p<0.01$) and adolescents (β ranging from 0.094 to 0.127; all $p<0.05$). CRF was only independently associated with DBP in adolescents (β=-0.096; p=0.034).

The associations between changes in MF, CRF and WC over the 2 years, and BP variables at follow-up are shown in table 22. In simple models (model 1), WC changes were associated with SBP (β=0.101), DBP (β=0.144), and MAP (β=0.136) in children (all $p<0.01$). In adolescents, upper-body MF (β=-0.116 and β=-0.098; both $p<0.01$) and WC (β=0.099 and β=0.066; both $p<0.05$) changes were associated with SBP and MAP, respectively. In joint models (model 2), only WC changes were independently associated with SBP, DBP and MAP in children (β ranging from 0.121 to 0.142; all $p<0.01$). In adolescents, changes in upper MF (β=-0.092; p=0.001) and in WC (β ranging from 0.080 to 0.098; $p<0.05$) were independently associated with SBP. Upper-body MF changes were also associated with future MAP (β=-0.082; p=0.031).

Table 20. Participants' characteristics by age groups.

Variables	Children (n=1089)			Adolescents (n=787)		
	Baseline	Follow-up	p	Baseline	Follow-up	P
Sex, n (%)						
males	572 (52.5)	-	-	409 (52)	-	-
females	517 (47.5)	-	-	378 (48)	-	-
Age, yr	7.66 (1.56)	9.71 (1.59)	-	13.30 (1.53)	15.28 (1.52)	-
Handgrip strength, kg	11.99 (3.74)	15.70 (4.63)	**<0.001**	25.17 (7.18)	31.39 (7.83)	**<0.001**
Handgrip strength/weight, kg/kg	0.39 (0.08)	0.41 (0.08)	**<0.001**	0.47 (0.09)	0.52 (0.10)	**<0.001**
Standing long jump, cm	111.92 (21.59)	126.42 (22.71)	**<0.001**	156.95 (31.07)	165.98 (43.37)	**<0.001**
20-m shuttle run test, stages	2.73 (1.57)	3.73 (1.88)	**<0.001**	5.39 (2.38)	6.22 (2.58)	**<0.001**
VO_2max, $ml \cdot kg^{-1} \cdot min^{-1}$	46.67 (3.72)	45.37 (4.56)	**<0.001**	43.61 (6.04)	42.65 (7.54)	**<0.001**
Waist circumference, cm	59.11 (8.27)	62.92 (9.23)	**<0.001**	68.09 (7.86)	70.15 (8.46)	**<0.001**
Height, cm	129.49 (10.86)	141.80 (11.15)	**<0.001**	159.78 (9.50)	166.92 (8.48)	**<0.001**
Weight, kg	31.07 (9.67)	39.71 (12.42)	**<0.001**	53.92 (12.05)	60.53 (11.76)	**<0.001**
Body mass index, kg/m^2	18.14 (3.30)	19.36 (3.98)	**<0.001**	20.94 (3.38)	21.63 (3.32)	**<0.001**
Systolic blood pressure, mmHg	102.52 (11.14)	105.99 (11.24)	**<0.001**	108.15 (12.52)	108.04 (12.00)	0.802
Diastolic blood pressure, mmHg	66.10 (9.11)	66.72 (8.29)	**0.014**	66.42 (7.45)	66.03 (7.36)	0.152
Mean arterial pressure	78.22 (9.10)	79.81 (8.59)	**<0.001**	80.33 (8.10)	80.03 (7.85)	0.278

Values are presented as mean (standard deviation) or frequency (percentage) when stated.

Statistically significant differences between baseline and follow-up values are highlighted in bold.

Table 21. Longitudinal associations between muscular fitness, cardiorespiratory fitness, and waist circumference with blood pressure.

	Children											
	Systolic blood pressure				Diastolic blood pressure				Mean arterial pressure			
	Adjusted R^2	β	SE	p	Adjusted R^2	β	SE	p	Adjusted R^2	β	SE	p
Model 1												
Upper MF	0.264	-0.056	0.450	0.146	0.221	-0.100	0.476	**0.014**	0.285	-0.071	0.452	0.064
Lower MF	0.261	-0.004	0.002	0.917	0.217	-0.075	0.002	0.068	0.282	-0.038	0.002	0.335
Global MF	0.263	-0.039	0.021	0.302	0.221	-0.097	0.023	**0.013**	0.284	-0.064	0.021	0.088
CRF	0.262	-0.020	0.010	0.618	0.222	-0.104	0.010	**0.016**	0.284	-0.060	0.010	0.145
WC	0.272	0.113	0.005	**0.005**	0.258	0.235	0.005	**<0.001**	0.306	0.178	0.005	**<0.001**
Model 2												
Upper MF	0.272	-0.001	0.618	0.988	0.257	0.054	0.625	0.304	0.306	0.038	0.604	0.452
CRF	0.272	0.050	0.012	0.325	0.257	-0.015	0.013	0.775	0.306	0.016	0.012	0.741
WC	0.272	0.137	0.006	**0.009**	0.257	0.260	0.006	**<0.001**	0.306	0.208	0.006	**<0.001**
Lower MF	0.273	0.040	0.002	0.401	0.256	0.015	0.002	0.745	0.306	0.029	0.002	0.526
CRF	0.273	0.033	0.012	0.523	0.256	-0.001	0.013	0.986	0.306	0.018	0.012	0.719
WC	0.273	0.146	0.005	**0.002**	0.256	0.239	0.005	**<0.001**	0.306	0.197	0.005	**<0.001**
Global MF	0.272	0.017	0.030	0.741	0.256	0.040	0.030	0.450	0.306	0.036	0.029	0.485
CRF	0.272	0.042	0.013	0.432	0.256	-0.013	0.013	0.804	0.306	0.014	0.013	0.791
WC	0.272	0.144	0.006	**0.005**	0.256	0.251	0.006	**<0.001**	0.306	0.205	0.006	**<0.001**
	Adolescents											
	Systolic blood pressure				Diastolic blood pressure				Mean arterial pressure			

	Adjusted R^2	β	SE	p	Adjusted R^2	β	SE	p	Adjusted R^2	β	SE	p
Model 1												
Upper MF	0.178	0.008	0.386	0.825	0.147	-0.055	0.389	0.134	0.202	-0.028	0.378	0.437
Lower MF	0.179	-0.033	0.001	0.432	0.150	-0.100	0.001	**0.018**	0.205	-0.077	0.001	0.060
Global MF	0.179	-0.022	0.019	0.501	0.151	-0.083	0.019	**0.012**	0.205	-0.060	0.018	0.061
CRF	0.183	-0.013	0.007	0.743	0.156	-0.113	0.007	**0.005**	0.207	-0.075	0.006	0.057
WC	0.185	0.093	0.005	**0.013**	0.157	0.118	0.005	**0.001**	0.213	0.112	0.004	**0.002**
Model 2												
Upper MF	0.190	0.064	0.477	0.155	0.164	0.041	0.480	0.366	0.216	0.056	0.465	0.207
CRF	0.190	-0.005	0.007	0.912	0.164	-0.096	0.007	**0.034**	0.216	-0.063	0.007	0.148
WC	0.190	0.127	0.005	**0.004**	0.164	0.114	0.005	**0.007**	0.216	0.126	0.005	**0.003**
Lower MF	0.188	-0.024	0.002	0.632	0.163	-0.031	0.002	0.533	0.215	-0.033	0.002	0.498
CRF	0.188	0.029	0.008	0.542	0.163	-0.067	0.008	0.169	0.215	-0.028	0.008	0.554
WC	0.188	0.099	0.005	**0.013**	0.163	0.096	0.005	**0.014**	0.215	0.101	0.005	**0.008**
Global MF	0.188	0.018	0.023	0.664	0.163	-0.010	0.024	0.807	0.214	0.001	0.023	0.989
CRF	0.188	0.010	0.007	0.822	0.163	-0.077	0.007	0.093	0.214	-0.044	0.007	0.326
WC	0.188	0.108	0.005	**0.012**	0.163	0.094	0.005	**0.025**	0.214	0.104	0.005	**0.012**

β: standardized regression coefficient; CRF: cardiorespiratory fitness; MF: muscular fitness; SE: standard error; WC: waist circumference.

Model 1: adjusted for sex, age, socioeconomic status, and the corresponding outcome at baseline; Model 2: model 1 + muscular fitness variables alternatively, cardiorespiratory fitness, and waist circumference at baseline.

Table 22. Longitudinal associations between muscular fitness, cardiorespiratory fitness, and waist circumference changes with blood pressure.

	Children											
	Systolic blood pressure				Diastolic blood pressure				Mean arterial pressure			
	Adjusted R^2	β	SE	p	Adjusted R^2	β	SE	p	Adjusted R^2	β	SE	p
Model 1												
Upper MF change	0.262	0.024	0.720	0.518	0.217	-0.074	0.747	0.058	0.282	-0.039	0.714	0.293
Lower MF change	0.262	0.026	0.002	0.493	0.212	0.003	0.002	0.945	0.280	0.014	0.002	0.706
Global MF change	0.262	0.018	0.034	0.634	0.214	-0.041	0.035	0.289	0.281	-0.021	0.034	0.575
CRF change	0.266	0.015	0.013	0.628	0.218	-0.058	0.013	0.133	0.283	-0.030	0.012	0.410
WC change	0.271	0.101	0.009	**0.007**	0.233	0.144	0.010	**<0.001**	0.299	0.136	0.009	**<0.001**
Model 2												
Upper MF change	0.277	0.048	0.774	0.237	0.233	-0.029	0.804	0.484	0.298	-0.001	0.767	0.975
CRF change	0.277	0.045	0.013	0.257	0.233	-0.013	0.014	0.740	0.298	0.011	0.013	0.777
WC change	0.277	0.129	0.010	**0.002**	0.233	0.128	0.011	**0.003**	0.298	0.137	0.010	**0.001**
Lower MF change	0.276	0.042	0.002	0.271	0.233	0.029	0.002	0.457	0.299	0.038	0.002	0.310
CRF change	0.276	0.044	0.013	0.268	0.233	-0.021	0.014	0.598	0.299	0.005	0.013	0.900
WC change	0.276	0.121	0.010	**0.002**	0.233	0.141	0.010	**0.001**	0.299	0.142	0.010	**<0.001**
Global MF change	0.276	0.044	0.036	0.270	0.232	0.003	0.038	0.944	0.298	0.018	0.036	0.642
CRF change	0.276	0.043	0.013	0.276	0.232	-0.017	0.014	0.670	0.298	0.008	0.013	0.840
WC change	0.276	0.126	0.010	**0.002**	0.232	0.138	0.011	**0.001**	0.298	0.142	0.010	**<0.001**
	Adolescents											
	Systolic blood pressure				Diastolic blood pressure				Mean arterial pressure			

	Adjusted R^2	β	SE	p	Adjusted R^2	β	SE	p	Adjusted R^2	β	SE	p
Model 1												
Upper MF change	0.189	-0.116	0.501	**0.001**	0.148	-0.066	0.512	0.072	0.209	-0.098	0.494	**0.006**
Lower MF change	0.180	0.041	0.001	0.233	0.145	0.027	0.001	0.450	0.203	0.041	0.001	0.231
Global MF change	0.179	-0.017	0.025	0.601	0.144	0.008	0.026	0.803	0.201	-0.003	0.025	0.931
CRF change	0.190	0.037	0.008	0.327	0.145	-0.020	0.008	0.602	0.205	0.008	0.008	0.830
WC change	0.188	0.099	0.006	**0.003**	0.147	0.026	0.006	0.436	0.207	0.066	0.006	**0.043**
Model 2												
Upper MF change	0.203	-0.092	0.540	**0.017**	0.147	-0.059	0.552	0.135	0.213	-0.082	0.001	**0.031**
CRF change	0.203	0.073	0.008	0.060	0.147	-0.003	0.008	0.935	0.213	0.037	0.007	0.344
WC change	0.203	0.080	0.006	**0.021**	0.147	0.013	0.006	0.725	0.213	0.048	0.021	0.164
Lower MF change	0.199	0.061	0.002	0.128	0.145	0.015	0.002	0.722	0.209	0.037	0.002	0.349
CRF change	0.199	0.041	0.008	0.308	0.145	-0.018	0.008	0.656	0.209	0.012	0.008	0.769
WC change	0.199	0.098	0.006	**0.004**	0.145	0.023	0.006	0.524	0.209	0.063	0.006	0.066
Global MF change	0.196	-0.004	0.032	0.917	0.145	0.009	0.032	0.806	0.208	0.002	0.031	0.948
CRF change	0.196	0.057	0.008	0.148	0.145	-0.016	0.008	0.684	0.208	0.021	0.008	0.596
WC change	0.196	0.094	0.006	**0.007**	0.145	0.023	0.006	0.514	0.208	0.061	0.006	0.076

β: standardized regression coefficient; CRF: cardiorespiratory fitness; MF: muscular fitness; SE: standard error; WC: waist circumference.

Model 1: adjusted for sex, age, and socioeconomic status, and the corresponding outcome at baseline; Model 2: model 1 + changes in muscular fitness variables alternatively, cardiorespiratory fitness, and waist circumference.

The associations between changes in MF, CRF and WC, and changes in BP variables are presented in table S11 (supplementary content). In simple models (model 1), changes in WC were the only variable associated with changes in SBP (β=0.110), DBP (β=0.132) and MAP (β=0.138) in children. In adolescents, changes in upper-body MF were associated with changes in SBP (β=-0.098; p=0.014) and changes in WC were associated with changes in SBP (β=0.121) and MAP (β=0.072) (both $p<0.05$). In joints models (model 2), WC changes was the only variable independently associated with changes in SBP, DBP and MAP in children (β ranging from 0.111 to 0.145; all $p<0.05$) and with SBP in adolescents (β ranging from 0.103 to 0.117; all $p<0.01$).

Differences in BP variables at follow-up according to WC groups at baseline are depicted in figure S6 (supplementary content). Those children and adolescents who exhibited high WC values, had higher levels of SBP, DBP, and MAP 2 years later, regardless of their MF, CRF levels, and additional covariates, including BP at baseline (all $p<0.05$).

Results remained similar when BMI was used instead of WC (data not shown).

Table S11. Associations between changes in muscular fitness, cardiorespiratory fitness, and waist circumference with changes in blood pressure variables.

	Children											
	Systolic blood pressure change				Diastolic blood pressure change				Mean arterial pressure change			
	Adjusted R^2	β	SE	p	Adjusted R^2	β	SE	p	Adjusted R^2	β	SE	p
Model 1												
Upper MF change	0.008	0.027	0.822	0.533	0.003	-0.083	0.866	0.059	0.003	-0.047	0.807	0.287
Lower MF change	0.008	0.026	0.002	0.542	-0.004	0.002	0.003	0.964	0.001	0.015	0.002	0.733
Global MF change	0.008	0.019	0.039	0.654	-0.001	-0.051	0.041	0.240	0.002	-0.028	0.038	0.522
CRF change	0.009	0.016	0.014	0.720	-0.001	-0.053	0.015	0.223	0.002	-0.030	0.014	0.495
WC change	0.020	0.110	0.011	**0.012**	0.013	0.132	0.011	**0.003**	0.020	0.138	0.011	**0.001**
Model 2												
Upper MF change	0.021	0.051	0.883	0.280	0.011	-0.045	0.933	0.342	0.015	-0.011	0.868	0.819
CRF change	0.021	0.048	0.015	0.299	0.011	-0.011	0.016	0.811	0.015	0.013	0.015	0.770
WC change	0.021	0.140	0.012	**0.004**	0.011	0.111	0.013	**0.021**	0.015	0.137	0.012	**0.004**
Lower MF change	0.021	0.046	0.002	0.298	0.010	0.028	0.003	0.533	0.017	0.041	0.002	0.354
CRF change	0.021	0.046	0.015	0.313	0.010	-0.021	0.016	0.654	0.017	0.006	0.015	0.902
WC change	0.021	0.131	0.011	**0.005**	0.010	0.128	0.012	**0.006**	0.017	0.145	0.011	**0.002**
Global MF change	0.021	0.048	0.041	0.293	0.009	-0.012	0.044	0.798	0.015	0.011	0.040	0.804
CRF change	0.021	0.046	0.015	0.322	0.009	-0.014	0.016	0.755	0.015	0.010	0.015	0.825
WC change	0.021	0.138	0.012	**0.004**	0.009	0.121	0.012	**0.011**	0.015	0.143	0.012	**0.003**

	Adolescents											
	Systolic blood pressure change				Diastolic blood pressure change				Mean arterial pressure change			
	Adjusted R^2	β	SE	p	Adjusted R^2	β	SE	p	Adjusted R^2	β	SE	p
Model 1												
Upper MF change	0.003	-0.098	0.596	0.014	-0.004	-0.042	0.617	0.295	0.000	-0.077	0.582	0.051
Lower MF change	-0.004	0.036	0.001	0.341	0.000	0.073	0.001	0.056	-0.001	0.069	0.001	0.073
Global MF change	-0.004	-0.026	0.030	0.472	-0.004	0.025	0.031	0.485	-0.005	0.003	0.029	0.943

CRF change	-0.003	0.044	0.009	0.301	-0.005	-0.009	0.009	0.829	-0.005	0.015	0.009	0.722
WC change	0.009	0.121	0.007	**0.001**	-0.005	0.013	0.007	0.716	0.000	0.072	0.007	**0.047**
Model 2												
Upper MF change	0.008	-0.057	0.634	0.179	-0.007	-0.042	0.654	0.333	-0.001	-0.056	0.618	0.193
CRF change	0.008	0.076	0.009	0.079	-0.007	0.003	0.010	0.940	-0.001	0.040	0.009	0.360
WC change	0.008	0.103	0.007	**0.008**	-0.007	0.007	0.008	0.852	-0.001	0.058	0.007	0.132
Lower MF change	0.012	0.097	0.002	0.030	-0.007	0.046	0.003	0.302	0.000	0.074	0.002	0.101
CRF change	0.012	0.041	0.009	0.354	-0.007	-0.016	0.010	0.711	0.000	0.011	0.009	0.811
WC change	0.012	0.117	0.007	**0.002**	-0.007	0.016	0.008	0.678	0.000	0.071	0.007	0.065
Global MF change	0.006	0.014	0.037	0.710	-0.008	0.014	0.038	0.712	-0.003	0.014	0.036	0.714
CRF change	0.006	0.062	0.009	0.153	-0.008	-0.008	0.010	0.858	-0.003	0.026	0.009	0.550
WC change	0.006	0.115	0.007	**0.003**	-0.008	0.016	0.008	0.675	-0.003	0.070	0.007	0.073

β: standardized regression coefficient; CRF: cardiorespiratory fitness; MF: muscular fitness; SE: standard error; WC: waist circumference.
Model 1: adjusted for sex, age, and socioeconomic status at baseline; Model 2: model 1 + changes in muscular fitness variables alternatively, cardiorespiratory fitness and waist circumference.

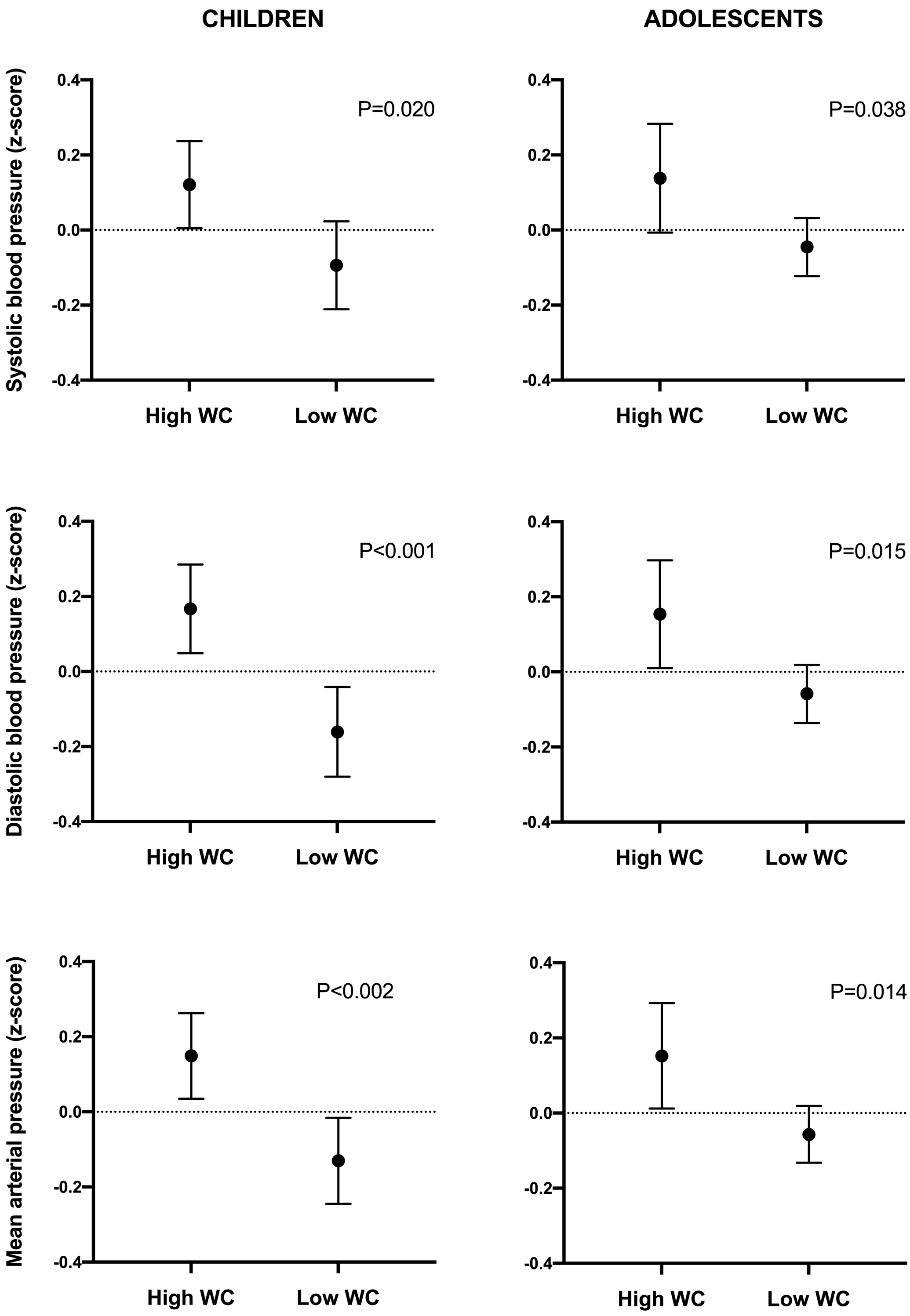

Figure S6. Differences in blood pressure variables at follow-up according to waist circumference groups at baseline. Dots and error bars indicate estimated mean and 95% CI, respectively. Analyses were adjusted by sex, age, socioeconomic status, global muscular fitness, cardiorespiratory fitness and the outcome value at baseline.

DISCUSSION

DISCUSSION

The specific discussions for each study are shown below.

Chapter I: Mediation

Study I: The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of body mass index in youth: The UP&DOWN Study

In the present study we aimed to examine the individual and combined associations of CRF and BMI with clustered CVD risk factors in children and adolescents, and to determine if the association between CRF and clustered CVD risk factors is mediated by BMI. CRF was negatively associated with CVDRF-I in children and adolescents, and this association was dependent of BMI. On the other hand, BMI was positively associated with CVDRF-I, with independence of CRF levels. Overall, combined analyses indicated that those participants with a favourable weight status (NW) displayed lower CVDRF-I than their OW counterparts, regardless of CRF levels. Finally, BMI proved to play a mediator role in the association between CRF and CVDRF-I in children and adolescents of both sex groups.

Previous evidence has clearly demonstrated the importance of CRF levels on cardiovascular health in young people (1). However, some of these studies did not account for weight status in this association (2,3). Contrary to what we hypothesised, our results showed that the association between CRF (assessed by 20-m shuttle run test) and CVDRF-I became non-significant when we adjusted for BMI in both sex groups. By contrast, an independent effect of CRF and fatness on CVD risk factors have been previously reported (4,5). Nevertheless, other part of the evidence suggests that the association between CRF and CVD risk factors does not remain significant when fatness is taken into account (6).

The discordance between studies could be partly due to methodological sources of variation. For instance, while the 20-m shuttle run test was used in our study, Andersen et al (4) assessed CRF as the maximal power output attained in an incremental test performed on a cycle ergometer. On the other hand, Bergmann et al. (5) used the 9-minute run/walking test. Although the accuracy of field tests is questioned against laboratory tests, the 20-m shuttle run test has shown to be valid and reliable (7,8). Additionally, worldwide reference values for this test has been proposed by a recent meta-analysis, which includes more than a million children and adolescents from 50 countries (9), making this test one of the most useful fitness screening tools. Another source of variation that should be mentioned is the use of different CVD risk factors and the way in which they are used in the analyses (i.e. individually or clustered). In this sense, Bergmann et al. (5) only included TC and BP, individually, as outcomes. Alternatively, several studies have employed a clustered of individual CVD risk factors (2–5,10,11); nonetheless, it is important to highlight that none of these studies used exactly the same cluster to define CVD risk. The rationale of using the risk factors included in the current study, lies in the need of a consensus in the definition of cardiovascular risk in children and adolescents to make the results between studies comparable. For that reason, we used the variables proposed by the International Diabetes Federation to define cardiometabolic risk in children and adolescents (12).

Weight status in youth is an essential determinant of cardiovascular health at this stage of life (13,14), and it predicts CVD risk factors levels in adulthood (15,16). BMI (13,15,16) and %BF (14) are the most frequent body composition measures in these studies. We found that BMI and %BF were positively associated with CVDRF-I and these associations remained significant after the inclusion of CRF in the model. In a similar way, Demmer et al (17) observed significant associations between fatness (defined by WC) and several cardiometabolic risk factors such as SBP, triglycerides, low-density lipoprotein cholesterol, and C-reactive protein in adolescents, with no significant effects found for CRF on the aforementioned outcomes. This evidence reinforces the crucial role of weight status reported in the current study. Moreover, the association between BMI and CVD risk in our study was not affected by the exclusion of waist circumference from the index (CVDRF-II). In agreement, combined analyses in our study showed that having an optimal weight status exerts a greater CVD risk protection than having high levels of CRF. Our results do not support the so call ‘Fat but Fit paradox’ in relation with CVD risk. In this sense, it has been previously suggested that CRF has the potential to counteract the deleterious effect that weight status exerts on the CVD profile; however, most of this evidence comes from studies conducted in adult population, and the information about the Fat but Fit paradox in children and adolescents is still sparse (18). Nonetheless, and despite differences in the methodology, regarding the CVD risk factors and the statistical procedures used, our results coincide with those reported recently by Nyström et al (19), who found that weight status has a larger negative effect on the metabolic profile than low CRF. It is important to highlight that Nyström et al (19) used two different scores of metabolic syndrome and HOMA-IR, separately, as outcomes, what suggests that this trend in the results does not depend on specific cardiometabolic variables.

Previous mediation analyses performed in children aged 8-11 years showed that the association between CRF and CVD risk factors was mediated by BMI in both sexes (11). Our results are in accordance, showing that 79.5%, 100%, 81.2% and 55.7% of the total effect of CRF on CVDRF-I in male and female children and adolescents, respectively, were mediated by BMI. Since mediation analysis assumes that the independent variable causes the mediator (20), BMI may be an intermediate step in the causal pathway between CRF and CVD risk factors. Previously, CRF has been negatively associated with fatness in youth (21) and it is considered a predictor of fatness in later years (22–24). The physiological mechanisms by which CRF affects fatness may include the effect that aerobic exercise exerts on the expression and activity of carnitine palmitoyl transferase I (an enzyme that controls the fatty acids movement into the mitochondria), as well as the increase in β-Hydroxy acyl-CoA dehydrogenase protein expression (a key enzyme that regulates fat oxidation) (25). On the other hand, adipose tissue produces several adipokines involved in inflammation (26). Obesity-induced inflammation is well known for being responsible of several cardiometabolic disorders including insulin resistance; however, it is also responsible of fibrosis and necrosis which may produce important damages in tissues of several organs including the heart (26). Together with our results, this evidence indicates that increments in CRF through aerobic exercise programs will ameliorate the level of fatness and, ultimately the CVD risk profile. Since clustered CVD risk factors track into adulthood and given that the duration of obesity over the years is also a determinant of CVD risk (27), public health policies should start at early ages. Therefore, school-based interventions aimed at improving physical activity participation are warranted among youths who fail to meet health-related CRF levels and, specially, optimal weigh status levels. Further studies testing the mediator role of weight status in the prospective association between physical fitness components and CVD risk factors would be of great interest.

Study II: The role of adiposity in the association between muscular fitness and cardiovascular disease

We found a negative association between MF and CVDRF-I in children of both sexes and in male adolescents. However, this association was not independent of BMI. In contrast, no associations between MF and CVDRF-I were found in female adolescents. BMI was markedly and positively related to CVDRF-I in children and adolescents of both sexes, and this association remained unchanged after further adjustment for MF. In general, combined analyses showed that those participants with a favorable weight status presented lower CVDRF-I than their overweight counterparts, regardless of their MF levels. Mediation analyses indicated that BMI acts as a full mediator in the association between MF and CVDRF-I in children of both sexes and male adolescents.

Several studies have found a relationship between MF and CVD risk factors in children and adolescents of both sexes (2,28–31). Our results agree with this evidence. Intriguingly, none of the MF tests or the MF index was related to CVDRF-I in female adolescents. Gracia-Marco et al (32) observed that, unlike in males, lean mass was positively associated with clustered CVD risk factors in female adolescents after controlling for body fat, what suggests that lean mass could exert a protective role in males but not in females. The different associations observed in male and female adolescents in our study can be explained, at least partially, by methodologic aspects. For instance, males were better distributed across the different tanner stages than females, and the prevalence in the last tanner stage was higher for males compared to females (data not shown). Given that maturation at these ages is accompanied by increases in muscle strength in both males and females (33), the less scattered and the lower levels of MF in female adolescents seem reasonable. Consequently, the association between MF and CVDRF-I is less likely to occur in female adolescents.

Adiposity seems to be a key determinant of cardiovascular health in children and adolescents (34,35). We found a strong positive association between BMI and CVDRF-I in children and adolescents, regardless of sex and independent of MF. These results persisted when we used %BF instead of BMI in the analyses. We found that the negative association between MF and CVDRF-I became not significant when adiposity was taken into account, which is in line with previous findings (36–38). Accordingly, combined analyses showed that CVDRF-I were more influenced by weight status rather than by MF level. Using similar analyses, the authors of previous studies (2,31,38) have found that MF exerts a protective effect on the CVD risk profile within each weight status group, which means that MF could counteract, at least partially, the deleterious effect of obesity on CVD risk factors in youth. In our study, this protective role of MF in participants who were OW was only seen in male children. The lack of this protective effect of MF on CVDRF-I within weight status groups observed in female children and in male and female adolescents could be due to the unequal distribution of participants across the weight status-MF groups. For instance, only the 11.5% of the female children (n=13), 11.3% of male adolescents (n=16), and 8.6% of female adolescents (n=11) were categorized in the OW+high MF.

Finally, mediation analyses showed that the effect of MF on the CVD risk profile was fully mediated by BMI in male and female children and in adolescent boys. The same results have been observed previously in children aged 8-11 years of both sexes (39). These results suggest that cardiovascular health in youth is more influenced by weight status than by MF.

CRF has also been related to cardiovascular health in childhood and later in life (23,28). A previous study by Díez-Fernández et al (11) concluded that BMI mediated the association between CRF and cardiovascular risk in a study sample of 1158 children aged 8-11 years. Taken together, the results of the study by Díez-Fernández et al, and the results of the current study suggest that both CRF and MF are associated with cardiovascular risk in children, but these associations are mediated by BMI.

Study III: Cardiorespiratory fitness cut-off points for early detection of present and future cardiovascular risk in children: a 2-year follow-up study

The findings from the present study indicate that (1) poor CRF levels in 6-10 years-old were associated with higher CVD risk factors and CVD risk scores at these ages as well as two years later; (2) CRF levels of at least 39.0 ml/kg/min in males and at least 37.5 ml/kg/min in females were associated with a more favorable cardiovascular health profile in 6-10 years-old; (3) the health related CRF cut-off points proposed were associated with CVD risk two years later; and (4) maintaining high levels of CRF or shifting from low to high levels in a two-year period was associated with a healthier cardiovascular profile in 8-12 years old. These findings extend previous results found in older children, and provide CRF cut-off points for early detection of present and future cardiovascular risk in children.

Cross-sectional studies have reported a negative association between CRF and CVD risk factors in children and adolescents (28). Likewise, prospective studies have found that children and adolescents with higher levels of CRF have a more favorable cardiovascular profile (23,40–43), and a reduced risk of myocardial infarction (44), stroke (43), and mortality (45,46) later in adulthood. On the other hand, we found that children with low CRF levels are very likely to have low CRF levels two years later. Levels of CRF tend to track from childhood to adolescence (42,47) and adulthood (48,49). These findings, together with the fact that the origins of CVD begin in early childhood and the tracking of CVD risk factors from early childhood to adulthood (42,50), suggest the importance of including CRF tests in monitoring systems from early ages with the purpose of identifying children with potential CVD risk. Moreover, educational and public health policies should promote healthy lifestyles to increase cardiorespiratory levels to prevent CVD risk factors and improve cardiovascular health (7).

In the past decade, several studies have developed CRF cut-off points associated with CVD risk in children and adolescents (51–58). Recently, a meta-analysis systematically evaluated the relationship between CRF and CVD risk in children and adolescents from multiple countries and provided pooled CRF cut-off points in children and adolescents that could serve as standards for international comparisons and for broader adoption in research and clinical applications (59). The study found that CRF levels associated with lower CVD risk ranged from 41.8 to 47.0 ml/kg/min for males and from 34.6 to 39.5 ml/kg/min for females. However, it was pointed out that there is a lack of studies with a cut-off point calculated in children younger than 10 years. The present study found that CRF cut-off points associated with reduced CVD risk in 6-10 years-old children were 39.0 ml/kg/min for males and 37.0 ml/kg/min for females. These cut-off points are similar to those reported by Ruiz et al (59), although it was slightly lower in males. This might be due to the fact that CRF levels increase more with age in males than in females from 6-12 years-old (60). Children who did not meet the health-related CRF levels (39.0 ml/kg/min in males and 37.0 ml/kg/min in females) were 12 and 11 times, in males and females, respectively, more likely to present higher CVD risk compared with those who met the standards.

We found that CRF levels in childhood were associated with CVD risk two years later and that 6-10 years-old children who met the health related CRF levels had lower values of CVD risk factors (ie, HOMA-IR and TC/HDL-c levels) and CVD risk score when they were 8- 12 years-old. Moreover, males and females with CRF values below the cut-off points were 7 and 4 times, respectively, more likely to have a higher CVD risk score two years later compared with those who met the standards. The results, again, support the idea that the development of high levels of CRF should start in early childhood.

Finally, we examined whether changes with age (from 6-10 to 8-12 years-old) in CRF are associated with a healthier cardiovascular profile in 8-12 years-old children. Shah et al (46) analyzed the effect of changes in CRF levels on long-term prognosis and subclinical CVD from late adolescence to adulthood and found that each one-minute reduction in exercise test duration was associated with a 21% increase in all-cause mortality and a 20% increase in incident CVD. Dwyer et al (48) reported that a decline and a persistent low CRF level (lowest tertile) from childhood to adulthood were associated with a higher prevalence of obesity and HOMA-IR in adulthood. Similarly, we observed that children who maintained or increased their CRF with similar levels or above the cut-off points, had lower levels of CVD risk score than their counterparts who maintained or decreased their CRF levels below the cut-off points. These findings suggest that the promotion of high CRF levels should start at early childhood, and emphasize the development of strategies to motivate unfit children to increase their CRF.

Study IV: Muscle fitness cut points for early assessment of cardiovascular risk in children and adolescents

The findings of the present study indicate that poor levels of MF in children (6-10 years-old) and adolescents (12- 16 years-old) are associated with a higher CVD risk score at these ages as well as two years later. We identified MF cut-off points associated with a more favorable cardiovascular health profile in children and adolescents, which seems to be more discriminative for males than for females. The health-related MF cut-off points identified are associated with CVD risk score two years later. The observed consistent cross-sectional and longitudinal associations between MF and cardiovascular health in children and adolescents are independent of CRF, which reinforces the importance of MF as a marker of health at these ages.

The ALPHA study developed a valid, reliable, feasible, and safe health-related fitness test battery for children and adolescents (61,62). This study included, besides the handgrip strength test, the standing long jump test to assess skeletal MF. Pillsbury et al (63) suggested that a US survey of health-related physical fitness in youth should include both, upper- and lower-body MF. Furthermore, they recommended determining health-related MF cut-off points for children and adolescents.

Our findings confirm the observed strong evidence of an inverse association between MF and CVD risk (64). The results of the present study also concur with those from prospective studies showing that children and adolescents with higher levels of MF have a more favorable cardiovascular profile later in life (23,64). Longitudinal studies also showed that MF was associated with risk of CVD mortality (65,66). It is important to highlight that MF is associated with CVD risk regardless of CRF and other confounders (2,31) in children and adolescents. Our results confirmed the findings reported in previous prospective studies (29,65) suggesting that there is both a combined and additive effect of MF on

CVD risk. On the other hand, we observed that children and adolescents with low MF levels are very likely to have low MF levels two years later. It seems reasonable to include MF tests in monitoring to identify children and adolescents with poor cardiovascular health levels. Educational and public health policies are needed to promote healthy lifestyles to increase MF to prevent CVD risk. In this sense, global physical activity guidelines for children and adolescents should emphasize participation in high-intensity physical activities and include a recommendation to perform muscle physical activities at least three days per week (67).

The present study shows that handgrip strength cut-off points associated with a reduced CVD risk in children and adolescents, both males and females, were 0.367 kg/kg body mass, 0.306 kg/ kg body mass, 0.473 kg/kg body mass, and 0.423 kg/kg body mass, respectively (68). These results are similar to those reported by Ramirez-Velez et al (69) in Colombian children and adolescents aged 9-17 years-old. We also identified standing long jump cut-off points associated with a healthier cardiovascular profile in children (104.5 cm in males and 81.5 cm in females) and adolescents (140.5 cm in males and 120.5 cm in females). These health-related MF cut-off points presented high accuracy (AUC >0.7), except in the standing long jump for adolescent males and females which were moderate (0.665 and 0.676).

Finally, we observed that children and adolescents who met the health-related handgrip strength or standing long jump levels had lower values of CVD risk two years later, which supports the idea that the development of high levels of MF should start in early childhood.

Study V: Bidirectional associations between fitness and fatness in youth: A longitudinal study

The aim of this study was to analyze the bidirectional associations between CRF and body fatness in a two-year longitudinal study conducted in children and adolescents. Overall, a bidirectional association was observed in children and adolescents of both sexes, with CRF and body fatness indices levels being prospectively associated with body fatness indices and CRF, respectively, after accounting for a set of confounders including sociodemographic and lifestyle factors.

Previous bidirectional studies have been conducted analyzing directionality between health-related features such as %BF and physical activity (70); physical activity against motor skills, CRF, and %BF (71); and fatness (i.e. BMI, skinfolds, and hip circumference) and self-reported screen time (72). Nonetheless, to our knowledge, the existing evidence regarding the associations between CRF and fatness in young people has only been addressed by longitudinal studies unidirectionally. Regarding the prospective association between fatness (exposure) and CRF (outcome), Fairchild et al. (73) observed that OW children had significant lower CRF levels two years later compared to their NW counterparts. Similarly, the study by Zaqout et al. (74) showed that changes in CRF levels over a two-year period were associated with baseline BMI values. Conversely, attending to the opposite direction of this association, previous evidence suggests that CRF (exposure) is associated with future fatness indices (outcomes) in children and adolescents. He et al. (75) analyzed the associations between CRF and weight gain in children followed during an 18 month period, and observed that children with the lowest baseline CRF levels were those who gained more weight. Furthermore, those children who were categorized as "low CRF" (based on sex-specific median splits) at baseline were at higher a risk of becoming or remaining OW, independently of baseline BMI values. As far as we know, the current study is the first one addressing the prospective association between CRF and fatness bidirectionally (i.e. CRF predicting future fatness levels and fatness predicting future CRF levels), showing a prospective negative association between fatness and CRF levels, each one being introduced as exposure and outcome alternatively.

These associations were, in general, independent of a set of confounders including SES, mother's education level and baseline moderate-to-vigorous physical activity levels. Furthermore, the inclusion of sedentary time in the model instead of moderate-to-vigorous physical activity did not affect these results. Compared to the aforementioned bidirectional studies (70–72) which mainly included demographic variables in their models (i.e. sex, age, ethnicity), our adjustments strengthen considerably the current results. Interestingly, the association between CRF and fatness indices, especially in adolescents, weakened when baseline fatness indices values were included in the model.

Related to the latter, it is important to highlight the low levels of CRF attained by the adolescent subsample in our study. Thus, according to previously determined CRF cut-off points (59), 86.8 and 72.7% of the females and males adolescents, respectively, had CRF levels associated with higher CVD risk (i.e. CRF below the sex-specific cut-off points). In addition, mean CRF values expressed as VO_2max of our adolescent sample are below the 20th and the 30th percentiles of female and male adolescents, respectively, according to the normative 20-m shuttle run test values proposed by Tomkinson et al (9). In this regard, it has been widely demonstrated the large impact that high CRF levels exert on various health parameters, reducing the deleterious effect of fatness (18,76). This condition has been called "The Fat but Fit paradox" and, although evidence of this paradox in young populations is scarce (18), it could explain the prevalent role of fatness against CRF and the lack of prospective associations between CRF and fatness observed in our adolescent subsample, since a significant effect of CRF on health is only expected with higher levels of CRF. Thus, bidirectional associations between CRF and fatness in adolescents with higher CRF would be presumable.

To analyze the bidirectionality between these variables removing the influence of baseline exposures values, we examined whether changes in CRF and fatness (exposures) over the two-years period predicted future fatness and CRF levels (outcomes), respectively. The same analyses were performed but including changes in fatness and changes in CRF also as outcomes (supplemental tables S7, and S8). Overall, these confirmatory analyses showed that changes in CRF and changes in fatness indices were significantly associated with future fatness indices and CRF levels, respectively, independently of the baseline values of the corresponding outcomes. Likewise, bidirectional associations were also observed when changes in fatness indices and changes in CRF were considered the outcomes.

Supplementary analyses also showed that having a healthy CRF level at baseline reduces the likelihood of being OW two years later; however, this association weakened when baseline BMI values were considered (table S9). Although it concurs with previous findings (77,78), the role of CRF should not be misjudged, since changes in CRF seem to be an important predictor of future weight status (77). Similarly, we observed that the likelihood of having an unhealthy CRF at follow-up was markedly higher in those children and adolescents who were overweight/obese at baseline (table S10). Likewise, this association was attenuated when baseline CRF levels were taken into account. It also reinforces the importance of CRF, since it suggests a tracking in fitness levels, which means that those with higher CRF levels at baseline are more likely to have higher CRF in the future.

The influence of CRF on fatness could be explained by physiological aspects. For instance, aerobic exercise has been related to the expression and activity of carnitine palmitoyl transferase I, the so called CPT-I, which mediates the movement of fatty acids into the mitochondria to be oxidized (79). Furthermore, CRF is one of the major predictors of the maximal fat oxidation capacity (80). Interestingly, maximal fat oxidation capacity has also shown to influence several CVD risk factors (81–83), although these studies are generally focused on adults. On the other hand, no physiological aspects seem to explain the effect of body fatness on future CRF; however, it could be explained by behavioural aspects. In this sense, Ekelund et al. (84) observed that either high baseline body weight and BMI were associated with lower levels of moderate-to-vigorous and vigorous physical activity six years later. This notion is interesting since decreases in habitual physical activity levels over the years are accompanied by reductions in CRF (71).

It is important to highlight the existence of some important factors associated with pediatric obesity. First, a recent systematic review has proven the role of dietary patterns in the prediction of obesity at early ages (85). According to their results, those children and adolescents who adhered to obesogenic food patterns (i.e. including sugary drinks, processed foods, fast food, candies, and refined grains) were more likely to develop obesity. Interestingly, they concluded that additional confounders such as physical activity should be considered. In this sense, previous evidence has suggested that physical activity could have a higher impact on childhood obesity than dietary habits (86). Accordingly, when we accounted for the KIDMED index (87), the association between CRF and fatness remained significant (data not shown). Additionally, gene profile has also proven to impact obesity in young people (88). We included fat mass and obesity associated (FTO) rs9939609 polymorphism in our models, which has been previously linked to an increased risk of obesity among children and adolescents (89). However, the bidirectional associations between CRF and fatness did not materially change.

Taking all this evidence together, an important public health message has arisen: improvements in CRF levels could reduce the probability of becoming OW, no matter the CRF level a person has. This statement is reinforced by previous evidence (77), which showed that the risk of developing OW after six years of follow-up is decreased by 10% for every 1 ml/kg/min increase in CRF. Additionally, given the bidirectionality observed in this study, the attainment of optimal fatness levels at early ages should also be promoted in order to ensure higher future CRF levels.

Study VI (part I): The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study

The main findings of the present study indicate that: (i) There exists a bidirectional association between CRF and NC only cross-sectionally. Longitudinally, only NC seems to predict CRF levels in children; (ii) CRF at baseline was not associated with future BP values after adjusting by NC at baseline. In contrast, NC was associated with different BP measures in children of both sexes and male adolescents, independently of baseline CRF.

Several studies have analyzed the longitudinal associations between CRF and fatness in children and adolescents. Overall, decreases in CRF has been linked to an increased risk of overweight and obesity later in life (48,75,77,90). Similarly, high levels of fatness or its increases over the years seem to be associated with lower levels and declines in CRF (73,74,91). However, the previously mentioned studies normally used BMI, waist or hip circumferences, and body fat percentage as markers of fatness (24). Furthermore, to our knowledge the bidirectional association between CRF and fatness has only been addressed once within the same study-cohort in a recent study by our group (92). In this study, a bidirectional association was observed between CRF and several fatness indices including BMI, %BF, and FMI. Thus, no previous evidence exists on the bidirectional association between CRF and NC. The current results showed a bidirectional association between CRF and NC only in cross-sectional analyses, which occurred in children and adolescents of both sexes. Longitudinally, only NC was associated with CRF in children of both sexes, independently of baseline CRF. According to these results, it seems more likely that upper-body fat (assessed through NC) leads to impaired CRF than vice versa. The lack of longitudinal bidirectional associations between CRF and NC, in contrast with the results obtained in the aforementioned study (92), could be explained in two ways: (i) the use of a fat indicator at a specific location (i.e. upper body fat) rather than total body fat indices (such as BMI or body fat percentage), since it has been previously stated that distribution, rather than total fatness, may have a greater relevance on health indicators (93), and (ii) the result obtained in a body weight bearing fitness test (i.e. 20-m shuttle run test) is more likely to be associated with an indicator of total body fat rather than a measure of fat at a specific part of the body (i.e. NC).

Study VII (part I): Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis. The UP&DOWN study

The current results suggest that: (i) The cross-sectional bidirectional associations observed between MF and NC do not occur longitudinally, since baseline NC was associated with future MF, but not the other way around; (ii) Overall, future BP measures seems to be affected to a higher extent by baseline NC rather than MF, except in female adolescents, where MF seems to be a better predictor of BP.

To the authors' knowledge, no previous studies have analyzed the bidirectional association between MF and fatness features within the same study cohort. Nonetheless, different measures of MF have shown to be longitudinally and negatively associated with fatness indicators (64,94,95). Similarly, OW have a negative impact in the performance of children and adolescents in MF tests (96). Our results show a bidirectional association between MF and NC, as an indicator of upper-body fat (97,98), in cross-sectional analyses, while longitudinally only NC was associated with MF. These results differ from those obtained in previous studies where MF was associated with future overall and central fatness indicators (64,94,95); nonetheless, not all of these studies adjusted for the baseline values of fatness, what might reduce the reliability of the results. In this sense, MF was longitudinally associated to NC in our study when baseline NC was not considered (data not shown). This may imply that baseline fatness, which is potentially affected by MF, is a major predictor of future fatness. More evidence is needed to correctly understand the longitudinal association between MF and NC. Regarding the associations between NC and MF in our study, two possible mechanisms have been proposed to explain the deleterious effect of fatness on MF; First, OW children and adolescents may be more reticent to participate in weight-bearing activities due to the extra energy cost that these tasks entail for them, affecting their physical activity levels and their MF (99). A second explanation is that the excess body weight could directly affect the performance of OW children in those fitness test requiring propulsion or lifting their body weight (96,99). Interestingly, consistent evidence supports that OW children and adolescents perform better in those MF tests that do not require lifting their body weight, such as the handgrip test, suggesting that NW individuals have a higher MF relative to their body weight but a lower absolute MF (96,99,100). Since the SLJ is a weight-bearing test, we relativized the handgrip test by body weight to create a relative MF index (101). The use of this index implies the limitation that its results could be driven by muscular strength, weight status, or both. However, a recent meta-analysis of longitudinal studies stated that MF measured in both, absolute and relative to body weight terms are similarly and inversely associated with fatness later in life (94). Furthermore, the standing long jump test has been validated by comparing its results with one repetition maximum in the leg extension test (102). In this sense, both approaches (i.e. absolute and relative

MF scores) seem justified for the assessment of their impact on health indicators, but caution should be taken when comparing results from studies that use opposing approaches to avoid drawing erroneous conclusions.

Chapter IV: Independent effect of fitness and fatness on BP

Study VI (part II): The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study

The longitudinal evidence regarding the association between CRF and BP or the risk of hypertension has led to conflicting results. Some studies have reported important protective effects derived from CRF. Juraschek et al. (103) concluded that having higher CRF at baseline was associated with a lower probability of prevalent and incident hypertension. Similarly, Carnethon et al. (104) observed that those participants with low (<20th percentile) or moderate (<60th percentile) CRF had twice the risk of developing hypertension compared to their fittest counterparts (>60th percentile). Physical activity, and specifically aerobic exercise, are associated with different mechanisms that potentially lower BP values by decreasing cardiac output, peripheral vascular resistance, sympathetic nervous and renin-angiotensin systems activity, peripheral vascular resistance, insulin resistance, and inflammation, and is also associated with improved endothelial function (105,106). Nonetheless, these effects are generally attributed to physical activity and not to CRF, which is highly determined not only by lifestyles such as physical activity but also by genetics (107). In fact, a recent systematic review on the longitudinal association between CRF and several CVD risk factors did not find convincing evidence of the associations between CRF and BP values based on the heterogeneity in the results (24). In agreement, our results showed no longitudinal associations between CRF and SBP, DBP or MAP. Importantly, and based on the cross-sectional associations observed between CRF and NC, we accounted for baseline NC in this association.

On the other hand, pediatric obesity is positively associated with SBP and DBP in adulthood (108) and those who maintain the obese status from childhood have an increased risk of adult hypertension (109). Several mechanisms seem to explain the deleterious effect of excess fat on BP, including the increased sympathetic nervous, and renin-angiotensin-aldosterone systems activity, which lead to increased total blood volume, stroke volume, and cardiac output (105,110). Interestingly, the different types of fat deposit have intrinsic characteristics and, as previously discussed, it has been recently suggested that fat distribution is more relevant in the quantification of CVD risk in young people than total body fat (93). In this sense, upper-body fat may yield additional risk of future hypertension beyond total and visceral fat (111,112). To our knowledge, the association between NC, as a surrogate marker of upper body fat, and BP has only been studied cross-sectionally in the pediatric population. Overall, these studies have observed a significant and positive association between NC and SBP (98,111,113–117), DBP (98,111,113–117), pre-hypertension (115,117) and hypertension risk (111,117). Importantly, none of these studies accounted for the effect of CRF in the associations. The results of the current study showed significant and positive associations between NC at baseline and future SBP, DBP and MAP in children of both sexes, and DBP and MAP in male adolescents. No associations were observed in female adolescents. This might be due to the homogeneity in their NC and BP levels, being the sex and age group with the lowest SD in these variables. Thus, the homogeneity observed in the female adolescents included in the current study may hinder the correct interpretation of the studied associations in this group.

Taking all together, NC seems to have a direct effect on future BP whereas CRF, based on its cross-sectional associations with NC, may exert an indirect effect on BP measures by reducing NC values. In agreement, baseline CRF was associated with future DBP, which seems to be more consistently related to mortality in later life than SBP (118), when the effect of NC was not accounted (data not shown).

Study VII (part II): Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis. The UP&DOWN study

Studies analyzing the longitudinal effects of MF on BP measures show a high degree of heterogeneity in their results, leading to inconsistent conclusions (94). Importantly, some of the studies that found a longitudinal association between MF and BP observed an attenuation of these associations after considering baseline WC values (119,120). In agreement, MF was not longitudinally associated with BP measures in our study when baseline NC values were considered in children of both sexes and male adolescents. In this sense, it has been suggested that fatness may play a mediator role in the associations between MF and BP measures (120,121). Accordingly, although it did not occur longitudinally, MF was cross-sectionally associated with NC, and NC longitudinally associated with BP measures, suggesting an indirect pathway in which MF leads to reductions in NC, which finally affect BP levels. The effect of MF on fatness may be explained by the fact that higher MF levels is normally accompanied by higher fat-free mass, which is considered a key determinant of energy expenditure and the main determinant of resting energy expenditure (122). No previous evidence nor physiological mechanisms have been proposed to explain the specific association between MF and DBP and MAP observed in female adolescents in the current study. Thus, it seems more likely that this association is due to the greater homogeneity in NC values in this sex and age group, which may have increased the importance of MF against NC within the model.

On the other hand, we found that, overall, NC was positively associated with future BP values independently of their MF and their BP levels at baseline. A consistent body of evidence suggest a longitudinal association between pediatric obesity and adult SBP and DBP values as well as the risk of hypertension (108,109). Excess fatness seems to elevate BP values through different mechanisms including an increased sympathetic nervous, and renin-angiotensin-aldosterone systems activity, which lead to increased total blood volume, stroke volume, and cardiac output (105,110). Although it is well known that obesity is a major modifiable risk factor for hypertension development, it is considered a fast-growing epidemic, which makes further attention and support from health institutions a must (123). In this sense, not only overall fat but also its distribution plays an important role in the quantification of CVD risk, including hypertension (93). In fact, upper-body fat measured through NC seems to add important information on BP levels beyond that obtained through total and visceral adiposity screening (111). Accordingly, high NC values have been linked to greater SBP (98,111,113–117), DBP (98,111,113–117), pre-hypertension (115,117), and hypertension risk (111,117). Nonetheless, all this evidence comes from cross-sectional studies, with the longitudinal evidence between these variables yet to be determined. Furthermore, these studies did not include any physical fitness variable as predictors in their analyses so, to our knowledge, this is the first study analyzing the longitudinal and independent association between NC and BP measures. Furthermore, the evidence regarding the independence of MF and fatness indicators on CVD risk factors including BP is scarce, reinforcing the need of future longitudinal and intervention studies to clarify this issue (68).

Taking all this evidence together, NC rather than MF seems to be independently associated with future BP values. Nonetheless, and given the effects that MF exerts on future fatness (94), the inclusion of strengthening exercises in hypertension prevention programs should be promoted.

Study VIII: Fitness, fatness and their association with future blood pressure in youth: The UP&DOWN longitudinal study

We aimed to determine whether MF, CRF and WC were associated with an increase in BP over two years in a large sample of Spanish children and adolescents. Overall, our results showed that WC was positively associated with BP two years later and its changes over this period, independent of MF, CRF and additional confounders.

Convincing evidence support that adult CVD risk factors relate to physical fitness at a young age (24,94). However, conflicting results have been found for the prospective association between MF, CRF and BP features. While some studies support a significant prospective associations of MF (29,119,124) and CRF (125,126) with BP, other studies have failed to observe such associations in the childhood population (120,127–130). Noteworthy, several of these studies are hampered by the lack of adjustments for potential confounders, including fatness (127–129). Overall, in the current study MF and CRF were only significantly associated with future DBP while not adjusting for WC and the other measure of fitness. Previous studies also concurs with these results, suggesting that MF and CRF in young people are more closely associated with DBP rather than SBP (29,120). Importantly, high DBP is the predominant BP abnormality in young people, and its levels during young adulthood relates more strongly to future mortality compared to SBP (118). However, these associations did not persist after accounting for WC, suggesting that MF and CRF do not exert a direct effect on future BP values.

On the other hand, we found a significant and independent prospective association between WC and BP. These results are in accordance with previous evidence regarding the association between WC in adolescence with adult DBP and MAP values (128), and total body fat and BMI in children aged 7-11 years old with SBP two years later (adjusted by the baseline value of the outcome, CRF and several sociodemographic covariates) (130). Moreover, when we classified our participants according to previously determined WC cut-off points (131), those who displayed lower WC levels at baseline, had lower levels of SBP, DBP, and MAP at follow-up, independently of MF and CRF at baseline, compared to those with higher WC levels. A similar pattern was also observed by Eisenmann et al (128) for DBP and MAP using median split according to WC levels.

Several pathophysiological mechanisms, i.e. increased total blood volume, stroke volume, and cardiac output, partly due to increased sympathetic nervous system and renin-angiotensin-aldosterone system, seem to explain some of the deleterious effect of OW on hypertension and cardiovascular structure and function (105). Nevertheless, the protective effects of fitness should not be underestimated since it is likely that it exerts an indirect effect on future BP (i.e. fitness affecting fatness and, finally, fatness affecting BP). This hypothesis is supported by the results obtained by Eisenmann et al. (128) who observed that subjects with high CRF during adolescence showed lower levels of fatness as adults (BMI, WC, and body fat percentage), and those with high levels of WC during adolescence showed higher BP during adulthood. Similar conclusions could be drawn from the results obtained by Fraser et al (120) that used different measures of MF (i.e. handgrip strength, leg and shoulder extension and flexion) which were associated with future WC but not with SBP or DBP. In fact, in our sample, baseline CRF (β=-0.033; p=0.04) and global MF (β=-0.045; p=0.002) in children, and global MF in adolescents (β=-0.071; p=0.005) were associated with WC two years later, independently of baseline WC.

Our results are not in compliance with previous evidence that found significant prospective associations between MF (29,119,124) and CRF (125,126) with BP. These discordances may be due to several differences between studies with regard to the measurement and analytical procedures. A large heterogeneity in CRF test used in previous studies has been pointed out, from laboratory based (objectively measured CRF through maximal exercise test with direct assessment of VO_2max), to submaximal or field test estimating VO_2max (24). Likewise, different measures of MF have been used, including muscular endurance of trunk and upper-body muscles, muscular strength of upper (mainly handgrip test) and lower (mainly the standing long jump test) limbs, or global MF scores (94). In this respect, Grontved et al (29) used isometric tests to determine back extension and abdominal flexion strength. Last, although BMI is the most common feature for the classification of pediatric obesity, other fatness measures such as WC, %BF, waist-to-hip-ratio, or sum of skinfold thicknesses have also been adopted. In the current study, results did not materially change when WC was replaced by BMI (data not shown). According to the analytical procedures, a possible source of variation is the treatment of BP variables. Thus, and since SBP and DBP in pediatric population increase with age (given the age-related changes in body size) and differs by sex, the use of their age- and sex-standardized values (z-scores) instead of the direct measured value, has been previously encouraged (132). Finally, some studies, including the one from Mikkelsson et al (126), did not control for the baseline value of the outcome in the analyses, what has been suggested to be a key issue for the correct interpretation of the temporal sequence of the observed associations (94).

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LIMITATIONS AND STRENGTHS

LIMITATIONS AND STRENGTHS

The studies included in the current International Doctoral Thesis present some general and specific limitations that should be taken into consideration when interpreting their results. These limitations, along with the main strengths, are set out below.

General limitations

- The cross-sectional (studies I and II) and longitudinal (studies III to VIII) designs do not allow to properly determine the direction of the studied associations.
- The UP&DOWN study included only urban, Caucasian Spanish children and adolescents. The homogeneity in ethnicity and country economic development limits the generalizability of these results.
- CVD risk factors are affected by other factors that have not been systematically assessed, such as dietary patterns. The results could therefore be subjected to residual confounding.
- BP measures were only assessed twice on one occasion. Since BP presents fluctuations during the day, a simple measurement may not represent the usual BP value (1).
- No information regarding family history of CVD is available in the UP&DOWN study.

Specific limitations

- Studies I, II, and V: the use of BMI as an index of fatness has been extensively questioned due to its lack of discriminative capacity between fat and lean body mass. Nevertheless, BMI is considered a powerful tool to identify youth with high individual and clustered CVD risk factors levels (2). In fact, BMI could not be a good marker of total fatness, but it has proven to be a strong predictor of CVD mortality (3).
- Studies III, IV, and V: fatness was assessed using skinfolds. In the studies III and IV, the sum of subscapular and triceps skinfolds was used, and in study V, these skinfolds were used to estimate the %BF through the Slaughter equation (4), and also the FMI. The mayor drawback with the skinfold technique lies in the extensive expertise needed for taking readings accurately and with consistency. However, skinfolds

have been suggested to be a valid tool to assess fatness in youth (5), and trained professionals carried out these measurements.

- Studies I, and II: Median split is not the best method of categorization. It has been previously stated that defining unfit and fit groups as being below or above the 20th percentile, respectively, is a more accurate way of fitness categorization (6,7). Nonetheless, this way of categorization would have resulted in highly unbalanced groups.
- Studies III, IV, V, and VIII: Related to the latest limitation, dichotomization is generally not recommended since it results in a loss of power equivalent to a loss of sample size. This loss of power occurs mainly when the mean or the median are selected as the split points. However, rather than placing the split at a conveniently chosen point (as it could be the mean or median), previously validated cut-off points for fitness (8) and WC (9) were employed to that end in studies III, IV, V, and VIII.
- Studies III, V, and VIII: The use of a ratio-scaled value of CRF (i.e. absolute VO_2 divided by body weight) may hamper the correct interpretation of the association between fitness and fatness (10). In the case of the 20-m shuttle run test, participants have to carry their weight over a series of 20-m shuttle runs, which adversely affect the performance in the heavier participants (11). Nonetheless, we used an equation (12) to estimate VO_2max that was validated without correcting the test performance by any anthropometric measure. The results should be interpreted cautiously, since no direct measure of absolute VO_2max was available in our study.

General strengths

- The fitness tests used in the UP&DOWN study are part of a previously validated and commonly used fitness test battery for children and adolescents (13).
- The relatively large sample allowed to performed the analyses differentiating by sex and age groups.

Specific strengths

- Studies I, II, III, and IV: The use of clustered CVD risk factors has been suggested as a better indicator of cardiovascular health, compared to individual CVD risk factors.
- Studies III, IV, V, VI, VII, and VIII: Although the longitudinal design

does not allow to determine causation, it is a strength of these studies, since it provides novel evidence given that most evidence in this subject comes from cross-sectional studies.

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CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

CONCLUSIONS

The general and specific conclusions of this International Doctoral Thesis derived from studies performed in Spanish children and adolescents are depicted below.

General conclusions

The main conclusions of this International Doctoral Thesis are: (i) fitness seems to exert an indirect effect on clustered CVD risk factor levels, with BMI mediating these associations; (ii) CRF and MF cut-off points have been identified, which are associated with a more favorable cardiovascular health two years later; (iii) a bidirectional association seem to exist, cross-sectionally and longitudinally, between fitness and different fatness indicators. Nevertheless, the longitudinal associations between fitness (both CRF and MF) and fatness weakened when the baseline value of the corresponding fatness indicator was considered; and (iv) fatness indicators (i.e. NC and WC) but not fitness (i.e. CRF and MF) seems to be independently associated with future BP values.

Specific conclusions

The specific conclusions of the studies included in each chapter are depicted below:

Chapter I: Mediation

Study I: BMI seems to be an independent predictor of clustered CVD risk factors and a mediator in the association between CRF and clustered CVD risk factors.

Study II: The effect of MF on clustered CVD risk factor seems to be fully mediated by BMI.

Chapter II: Fitness cut-off points

Study III: CRF levels cut-off points associated with reduced CVD risk two years later in male and female children were ≥39.0 ml/kg/min and ≥37.5 ml/kg/min, respectively. Keeping low levels of CRF and its decline below the proposed CRF cut-off points in a two-year period are associated with higher CVD risk during childhood.

Study IV: Handgrip/weight cut-off points associated with reduced CVD risk two years later in male and female children and adolescents were ≥0.367, ≥0.306, ≥0.473, and ≥0.423 kg/kg, respectively. The corresponding standing long jump cut-off points were: ≥104.5, ≥81.5, ≥140.5, and ≥120.5 cm.

Chapter III: Bidirectionality

Study V: Bidirectional longitudinal associations seem to exist between CRF and fatness indicators in children and adolescents. Nonetheless, the longitudinal associations between CRF (exposure) and fatness (outcome) weakened when fatness indicators at baseline were considered.

Study VI (part I): A bidirectional association exists cross-sectionally between CRF and NC. Longitudinally, NC seems to affect CRF levels independently of baseline CRF in children, while CRF is not independently associated with future NC.

Study VII (part I): the bidirectional association between MF and NC was only observed in cross-sectional analyses. NC was longitudinally associated with MF, independently of baseline MF values, but MF was not independently associated with NC two years later.

Chapter IV: Independent effect of fitness and fatness on BP

Study VI (part II): Future SBP, DBP and MAP values seem to be affected to a higher extent by NC rather than CRF. Nonetheless, CRF might exert an indirect effect on future BP by reducing NC.

Study VII (part II): Overall, future BP values appear to be affected to a higher extent by NC than by MF. Nonetheless, the inclusion of strengthening exercises in strategies for hypertension prevention in the pediatric population seems to be justified by the potential indirect effect that MF exert on future BP by reducing fatness levels.

Study VIII: WC, but neither MF nor CRF, is independently associated with BP and its changes over two years.

FUTURE RESEARCH DIRECTIONS

- Performance in fitness tests is intimately linked to weight status. Thus, the understanding of the independent effect of fitness and fatness on health parameters is a tricky matter. Different studies have concluded that improvement in CRF (ml/kg/min) is associated with future CVD risk factors in children and adolescents. However, they did not include any adjustment by weight status. In this sense, CRF, expressed in ml/kg/min, could be improved by increasing aerobic capacity, but also by reducing weight. Well design intervention studies with direct measures of fitness and adjustments are needed for clarity.

- Although clustered CVD risk factors has been shown to be a better indicator of the cardiovascular profile of children and adolescents, it implies that each CVD risk factor included in the cluster is equally important for cardiovascular health. It seems logical to think that this is not the case, and that some variables have a greater weight in the prediction of CVD than others. Several studies have compared the association of specific risk factors with cumulative risk through factor analysis, but additional evidence is needed to fully understand how individual CVD risk factors affects independently cardiovascular health.
- Longitudinal studies with longer follow-up periods analyzing how fitness and fatness levels in childhood and adolescence affects cardiovascular health at adulthood are needed.

ANNEXES

ANNEX I: Short curriculum vitae

Personal information

Alejandro Pérez Pérez (signature on articles: Alejandro Perez-Bey)

Born: August the 6th of 1993. San Fernando, Cádiz, Spain

Contact: alejandro.perezperez@uca.es, +34 657588624

Education

2011-2015	Bachelor's degree in Sport Sciences. University of Cádiz, Spain.
2015-2016	Master's degree in Physical Activity and Health. University of Cádiz, Spain.
2016-2020	PhD Student in Health Sciences. University of Cádiz, Spain.

Previous and current positions

2016-2020	Predoctoral FPU fellow. Department of Physical Education, Faculty of Education Sciences, University of Cádiz, Spain.
2020-	Substitute teaching tutor university-level. Department of Physical Education, Faculty of Education Sciences, University of Cádiz, Spain.

Publications derived from the Doctoral Thesis

1. **Perez-Bey A**, Segura Jiménez V, Fernández-Santos JR, Esteban-Cornejo I, Gómez-Martínez S, Veiga OL, et al. The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of body mass index in youth: The UP&DOWN Study. Pediatr Diabetes. 2019;20(1):32-40.
2. **Perez-Bey A**, Segura-Jiménez V, Fernández-Santos JR, Esteban-Cornejo I, Gómez-Martínez S, Veiga OL, et al. The role of adiposity in the association between muscular fitness and cardiovascular disease. J Pediatr. 2018;199:178-185.e4.
3. Castro-Piñero J, **Perez-Bey A**, Segura-Jiménez V, Aparicio VA, Gómez-Martínez S, Izquierdo-Gómez R, et al. Cardiorespiratory fitness cutoff points for early detection of present and future cardiovascular risk in children: A 2-year follow-up study. Mayo Clin Proc. 2017;92(12):1753-62.

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5. **Perez-Bey A**, Ruiz JR, Ortega FB, Martinez-Gomez D, Mota J, Veiga OL, et al. Bidirectional associations between fitness and fatness in youth: A longitudinal study. Scand J Med Sci Sport. 2020;30(8):1483-96.
6. **Perez-Bey A**, Marín-Jiménez N, Jiménez-Iglesias J, Sánchez-Oliva D, Conde-Caveda J, Cabanas-Sánchez V, et al. The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study. *Submitted*
7. **Perez-Bey A**, Sánchez-Delgado A, Cruz-León C, Padilla-Moledo C, Izquierdo-Gómez R, Aadland E, et al. Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis: The UP&DOWN longitudinal study. *Submitted*
8. **Perez-Bey A**, Delgado-Alfonso A, Aadland E, Resaland GK, Martinez-Gomez D, Veiga OL, et al. Fitness, fatness and their associations with future blood pressure in youth: The UP&DOWN longitudinal study. *Under review (minor comments).*

Other publications

9. Delgado-Alfonso A, **Perez-Bey A**, Conde-Caveda J, Izquierdo-Gómez R, Esteban-Cornejo I, Gómez-Martínez S, et al. Independent and combined associations of physical fitness components with inflammatory biomarkers in children and adolescents. Pediatr Res. 2018;84(5):704–12.
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11. Cabanas-Sánchez V, Martínez-Gómez D, Esteban-Cornejo I, **Perez-Bey A**, Castro Piñero J, Veiga OL. Associations of total sedentary time, screen time and non-screen sedentary time with adiposity and physical fitness in youth: the mediating effect of physical activity. J Sports Sci. 2019;37(8):839–49.
12. Martinez-Tellez B, Sanchez-Delgado G, Alcantara JMA, Acosta FM, Amaro-Gahete FJ, Osuna-Prieto FJ, et al. Evidence of high 18F-fluorodeoxyglucose

uptake in the subcutaneous adipose tissue of the dorsocervical area in young adults. Exp Physiol. 2019;104(2):168–73.
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Research stays

2017 Faculty of Sport Sciences, University of Granada, Spain.
Prof: Jonatan Ruiz Ruiz

Duration: 3 months

2018 CIAFEL Research Center, Faculty of Sport, University of Porto, Portugal.
Prof: Jorge Mota
Duration: 3 months

2019 Faculty of Education, Arts and Sports, Western Norway University Of Applied Sciences
Prof: Geir Kåre Resaland
Duration: 3 months

Research Experience

- Health-related physical fitness in school children and adolescents, and adolescents with down syndrome and its relationship with health indicators: a 3-year longitudinal study. Funded by the Spanish Ministry of Economy, Industry, and Competitiveness. 450,000 €.
- Diagnosis of the pattern of commuting and physical activity of students, teaching and research academic staff, and administration personnel of the University of Cádiz. Funded by the University of Cádiz. 3,400 €.
- Combined effects of the Mediterranean diet and physical exercise on cardiovascular disease risk factors in university Students. Funded by the University of Cádiz. 2,000 €.
- Mediating effect of physical activity, physical fitness, and nutrition on the influence of the FTO and PPARGC1A on adiposity and fat oxidation capacity during exercise: The NutAF Study. Funded by the University of Cádiz. 1,600 €.
- Health-related physical fitness test battery design for the adult population: The ADULTFIT Study. Funded by the Spanish Ministry of Economy, Industry, and Competitiveness. 112,000 €.

Awards

2016 Award to the Best Academic Record in the Master degree.

2018 Study IV of the current Doctoral Thesis was included in the Expert's choice of the "Most exciting Research in the Field of Pediatric Exercise Science" published in the journal "Pediatric Exercise Science".

2019 Study IV of the current Doctoral Thesis received the "XXIII Research Award 2019" by the "Illustrious Official College of Graduates in Physical

Education and Physical Activity and Sport Sciences of Andalusia (COLEF)".

Other merits

- Author and co-author of 27 congress communications (both national and international).
- Organizing committee in 1 national congress and 1 international congress.
- Lecturer in the degree of Sport Sciences. Faculty of Education Sciences, University of Cádiz. A total of 200 hours (2017-present).
- Lecturer in the degree in Early Childhood Education. Faculty of Education Sciences, University of Cádiz. A total of 12.64 hours (2020)
- Lecturer in the Master degree in Compulsary Secondary Education and High-School Teaching, Vocational Training, and Language Teaching. Faculty of Education Sciences, University of Cádiz. A total of 8 hours (2020).

ANNEX II: Agreement of co-authors and contributions of the doctoral student

The following pages contain the approval of the co-authors for the presentation of the included studies as part of the current Doctoral Thesis, and their resignation to present these studies as part of another Doctoral Thesis at any other University.

Alejandro Pérez Pérez (signing as Alejandro Perez-Bey in publications) states his contribution in the included studies as follows:

- Studies I, II, V, VI, VII, and VIII: Alejandro Pérez Pérez contributed in the acquisition of the data, the creation of the databases, performed the data analyses, proceeded with the presentation of the results, and wrote the manuscript, being the first author and the corresponding author.
- Studies II and III: Alejandro Pérez Pérez contributed in the acquisition of the data, the creation of the databases, the elaboration of the manuscripts' figures, gave critical discussion of the manuscript, and contributed in the final manuscript as accepted.

UCA Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

D. **Alejandro Sánchez Delgado** con DNI o Pasaporte: 26250969B coautor de la publicación que se identifica a continuación:

- Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis: The UP&DOWN Study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de la citada publicación como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar la citada publicación como parte de otra tesis doctoral en cualquier otra universidad.

En Málaga, a 18 de Enero de 2021

Fdo.: ______________________________________

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

D. **Álvaro Delgado Alfonso** con DNI o Pasaporte: 45114004-X coautor de la publicación que se identifica a continuación:

- Fitness, fatness and their association with future blood pressure in youth: The UP&DOWN longitudinal study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de la citada publicación como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar la citada publicación como parte de otra tesis doctoral en cualquier otra universidad.

En Oslo a 20 de enero de 2021

Fdo.: ______________________________

UCA Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

Dña. **Ascensión Marcos Sánchez** con DNI o Pasaporte: 00265107D, coautora de las publicaciones que se identifican a continuación:

- The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of body mass index in youth: The UP&DOWN Study.
- The role of adiposity in the association between muscular fitness and cardiovascular disease.
- Cardiorespiratory fitness cutoff points for early detection of present and future cardiovascular risk in children: A 2-year follow-up study.
- Muscle fitness cut points for early assessment of cardiovascular risk in children and adolescents.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de las citadas publicaciones como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar las citadas publicaciones como parte de otra tesis doctoral en cualquier otra universidad.

En Madrid, a 29 de enero de 2021

Fdo.: Ascensión Marcos

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

Dña. **Carmen Padilla Moledo** con DNI o Pasaporte: ______31243940G________________ coautora de la publicación que se identifica a continuación:

- Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis: The UP&DOWN Study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de la citada publicación como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada “Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN”.

Y expresa su renuncia a presentar la citada publicación como parte de otra tesis doctoral en cualquier otra universidad.

En Cádiz , a 18 de enero de 2021

PADILLA MOLEDO MARIA DEL CARMEN - 31243940G

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Dña. **Carolina Vaney Cruz León** con DNI o Pasaporte: Y7499243S coautora de la publicación que se identifica a continuación:

- Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis: The UP&DOWN Study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de la citada publicación como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar la citada publicación como parte de otra tesis doctoral en cualquier otra universidad.

En Cádiz, a 18 de enero de 2021

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Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

D. **David Martínez Gómez** con DNI: 53411668X coautor de las publicaciones que se identifican a continuación:

- Bidirectional associations between fitness and fatness in youth: A longitudinal study.
- Fitness, fatness and their association with future blood pressure in youth: The UP&DOWN longitudinal study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de las citadas publicaciones como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar las citadas publicaciones como parte de otra tesis doctoral en cualquier otra universidad.

En Madrid , a 18 de Enero de 2021

David Martinez-Gomez

Digitally signed by David Martinez-Gomez
DN: cn=David Martinez-Gomez, o, ou, email=d.martinez@uam.es, c=US
Date: 2021.01.18 14:26:16 +01'00'

Fdo.: ______________________________

UCA Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

D. **David Sánchez Oliva** con DNI: 76129318-P coautor de la publicación que se identifica a continuación:

- The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de la citada publicación como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar la citada publicación como parte de otra tesis doctoral en cualquier otra universidad.

En Cáceres, a 18 de Enero de 2021

David Sánchez Oliva

Firmado digitalmente por David Sánchez Oliva
Fecha: 2021.01.18 15:35:34 +01'00'

Fdo.: David Sánchez Oliva

UCA | Universidad de Cádiz

Thesis by compendium of publications.
Document of agreement and resignation of co-authors.

Mr. **Eivind Aadland** with ~~ID~~ number or Passport: _12128149579_ co-author of the following publications:

- Fitness, fatness and their association with future blood pressure in youth: The UP&DOWN longitudinal study.
- Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis: The UP&DOWN Study.

In accordance with the **Article 23.4** of the **Regulation UCA CG06-2012, June 27, 2012, which regulates the ordinance of doctoral studies at the University of Cádiz (BOUCA No. 208).**

States his approval to the presentation of these publications as a part of the doctoral thesis written by Mr. Alejandro Pérez Pérez, entitled "The effects of physical fitness and fatness on cardiovascular disease risk factors in children and adolescents: the UP&DOWN longitudinal study".

And expresses his resignation to present said publications as a part of another doctoral thesis at any other University.

Sogndal, 19th of January, 2021

Signed: Eivind Aadland

UCA Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

D. **Francisco B Ortega Porcel** con DNI o Pasaporte: __75228381G__ coautor de las publicaciones que se identifican a continuación:

- The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of body mass index in youth: The UP&DOWN Study.
- Bidirectional associations between fitness and fatness in youth: A longitudinal study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de las citadas publicaciones como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar las citadas publicaciones como parte de otra tesis doctoral en cualquier otra universidad.

En Granada , a 18 de Enero de 2021

ORTEGA PORCEL FRANCISCO BARTOLOME - 75228381G

Firmado digitalmente por ORTEGA PORCEL FRANCISCO BARTOLOME - 75228381G
Fecha: 2021.01.18 13:46:26 +01'00'

Fdo.: ____

UCA Universidad de Cádiz

Thesis by compendium of publications.
Document of agreement and resignation of co-authors.

Mr. **Geir Kåre Resaland** with ID number or Passport: **34040169** co-author of the following publication:

- Fitness, fatness and their association with future blood pressure in youth: The UP&DOWN longitudinal study.

In accordance with the **Article 23.4** of the **Regulation UCA CG06-2012, June 27, 2012, which regulates the ordinance of doctoral studies at the University of Cádiz (BOUCA No. 208).**

States his approval to the presentation of this publication as a part of the doctoral thesis written by Mr. Alejandro Pérez Pérez, entitled "The effects of physical fitness and fatness on cardiovascular disease risk factors in children and adolescents: the UP&DOWN longitudinal study".

And expresses his resignation to present said publication as a part of another doctoral thesis at any other University.

In Sogndal, Norway of 19th January, **2021**

Signed: Geir K. Resaland

If there is any questions, please contact me.

Professor Geir K. Resaland
Leader, Center for physically active learning
Faculty of Education, Arts and Sports
Western Norway University of Applied Sciences, *www.hvl.no*
Campus Sogndal, P.O. Box 133, 6851 Sogndal, Norway
Phone: +47 57676097 | Cell: +47 41621333 Email: *geirkr@hvl.no*

UCA Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

Dña. **Irene Esteban Cornejo** con DNI o Pasaporte: 70257673X coautora de las publicaciones que se identifican a continuación:

- The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of body mass index in youth: The UP&DOWN Study.
- The role of adiposity in the association between muscular fitness and cardiovascular disease.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de las citadas publicaciones como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar las citadas publicaciones como parte de otra tesis doctoral en cualquier otra universidad.

En Granada, a 19 de Enero de 2021

Fdo.: ______________________________

UCA Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

D. **Jesús Gustavo Ponce González** con DNI o Pasaporte: 48904255-E coautor de la publicación que se identifican a continuación:

- Fitness, fatness and their association with future blood pressure in youth: The UP&DOWN longitudinal study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de la citada publicación como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar la citada publicación como parte de otra tesis doctoral en cualquier otra universidad.

En Puerto Real, a 18 de enero de 2021

Fdo.: ______________________________

Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

D. **Jonatan Ruiz Ruiz** con DNI o Pasaporte: 46779494R coautor de las publicaciones que se identifican a continuación:

- Cardiorespiratory fitness cutoff points for early detection of present and future cardiovascular risk in children: A 2-year follow-up study.
- Muscle fitness cut points for early assessment of cardiovascular risk in children and adolescents.
- Bidirectional associations between fitness and fatness in youth: A longitudinal study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de las citadas publicaciones como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar las citadas publicaciones como parte de otra tesis doctoral en cualquier otra universidad.

En Granada, a 18 de Enero de 2021

Firmado por RUIZ RUIZ JONATAN - 46779494R el día 18/01/2021 con un certificado emitido por AC FNMT Usuarios

Fdo.: Jonatan Ruiz Ruiz

Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

D. **Jorge del Rosario Fernández Santos** con DNI o Pasaporte: 75778284T coautor de las publicaciones que se identifican a continuación:

- The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of body mass index in youth: The UP&DOWN Study.
- The role of adiposity in the association between muscular fitness and cardiovascular disease.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de las citadas publicaciones como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar las citadas publicaciones como parte de otra tesis doctoral en cualquier otra universidad.

En Cádiz, a 27 de enero de 2021

Fdo.: FERNANDEZ SANTOS JORGE DEL ROSARIO - 75778284T

Firmado digitalmente por FERNANDEZ SANTOS JORGE DEL ROSARIO - 75778284T
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UCA Universidad de Cádiz

Thesis by compendium of publications.
Document of agreement and resignation of co-authors.

Mr. **Jorge Mota** with ID number or Passport: CB 364562 co-author of the following publication:

- Bidirectional associations between fitness and fatness in youth: A longitudinal study.

In accordance with the **Article 23.4** of the **Regulation UCA CG06-2012, June 27, 2012, which regulates the ordinance of doctoral studies at the University of Cádiz (BOUCA No. 208).**

States his approval to the presentation of this publication as a part of the doctoral thesis written by Mr. Alejandro Pérez Pérez, entitled "The effects of physical fitness and fatness on cardiovascular disease risk factors in children and adolescents: the UP&DOWN longitudinal study".

And expresses his resignation to present said publication as a part of another doctoral thesis at any other University.

In Porto, 18 of January, 2021

Signed: ____________________

UCA Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

D. **José Jiménez Iglesias** con DNI o Pasaporte: **76648742-T** coautor de la publicación que se identifica a continuación:

- The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de la citada publicación como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar la citada publicación como parte de otra tesis doctoral en cualquier otra universidad.

En Cádiz, a 02 de febrero de 2021

JIMENEZ IGLESIAS JOSE - 76648742 T

Firmado digitalmente por JIMENEZ IGLESIAS JOSE - 76648742T
Fecha: 2021.02.02 09:17:15 +01'00'

Fdo.: José Jiménez Iglesias

UCA Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

D. **José Castro Piñero** con DNI o Pasaporte 31656395-T, coautor de las publicaciones que se identifican a continuación:

- The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of body mass index in youth: The UP&DOWN Study.
- The role of adiposity in the association between muscular fitness and cardiovascular disease.
- Cardiorespiratory fitness cutoff points for early detection of present and future cardiovascular risk in children: A 2-year follow-up study.
- Muscle fitness cut points for early assessment of cardiovascular risk in children and adolescents.
- Bidirectional associations between fitness and fatness in youth: A longitudinal study.
- Fitness, fatness and their association with future blood pressure in youth: The UP&DOWN longitudinal study.
- The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study.
- Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis: The UP&DOWN Study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de las citadas publicaciones como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar las citadas publicaciones como parte de otra tesis doctoral en cualquier otra universidad.

En Cádiz, a 18 de enero de 2021

CASTRO PIÑERO JOSE - 31656395T

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Nombre de reconocimiento (DN): c=ES, serialNumber=IDCES-31656395T, givenName=JOSE, sn=CASTRO PIÑERO, cn=CASTRO PIÑERO JOSE - 31656395T
Fecha: 2021.01.18 14:56:35 +01'00'

Fdo.: José Castro Piñero

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

D. **Julio Conde Caveda** con DNI o Pasaporte: 00820007B coautor de la publicación que se identifica a continuación:

- The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de la citada publicación como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar la citada publicación como parte de otra tesis doctoral en cualquier otra universidad.

En Cádiz , a 18 de enero de 2021

Fdo.:Julio Conde Caveda

CONDE DE CAVEDA JULIO - 00820007B

Firmado digitalmente por CONDE DE CAVEDA JULIO - 00820007B
Nombre de reconocimiento (DN): c=ES, serialNumber=IDCES-00820007B, givenName=JULIO, sn=CONDE DE CAVEDA, cn=CONDE DE CAVEDA JULIO - 00820007B
Fecha: 2021.01.18 14:22:41 +01'00'

UCA Universidad de Cádiz

Thesis by compendium of publications.
Document of agreement and resignation of co-authors.

Mr. **Carl J. Lavie** with ID number or Passport: 575595507, co-author of the following publication:

- Bidirectional associations between fitness and fatness in youth: A longitudinal study.

In accordance with the **Article 23.4** of the **Regulation UCA CG06-2012, June 27, 2012, which regulates the ordinance of doctoral studies at the University of Cádiz (BOUCA No. 208).**

States his approval to the presentation of this publication as a part of the doctoral thesis written by Mr. Alejandro Pérez Pérez, entitled "The effects of physical fitness and fatness on cardiovascular disease risk factors in children and adolescents: the UP&DOWN longitudinal study".

And expresses his resignation to present said publication as a part of another doctoral thesis at any other University.

In ____, ____ of _____________, 20__

Signed: C Lavie, MD

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

Dña. **Magdalena Cuenca García** con DNI o Pasaporte: 74728962-F coautora de la publicación que se identifica a continuación:

- Muscle fitness cut points for early assessment of cardiovascular risk in children and adolescents.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de la citada publicación como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar la citada publicación como parte de otra tesis doctoral en cualquier otra universidad.

En Jerez de la Frontera, a 18 de enero de 2021

CUENCA GARCIA MARIA MAGDALENA - 74728962F

Firmado digitalmente por CUENCA GARCIA MARIA MAGDALENA - 74728962F
Fecha: 2021.01.18 16:53:18 +01'00'

Fdo.: ______________________________________

UCA Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

Dña. **Nuria Marín Jiménez** con DNI o Pasaporte: 77371723E, coautora de la publicación que se identifica a continuación:

- The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de la citada publicación como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar la citada publicación como parte de otra tesis doctoral en cualquier otra universidad.

En Puerto Real, a 18 de enero de 2021

Fdo.: Nuria Marín Jiménez

UCA Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

D. **Oscar Luis Veiga Núñez** con DNI o Pasaporte: 50. 080.855-B coautor de las publicaciones que se identifican a continuación:

- The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of body mass index in youth: The UP&DOWN Study.
- The role of adiposity in the association between muscular fitness and cardiovascular disease.
- Muscle fitness cut points for early assessment of cardiovascular risk in children and adolescents.
- Bidirectional associations between fitness and fatness in youth: A longitudinal study.
- Fitness, fatness and their association with future blood pressure in youth: The UP&DOWN longitudinal study.
- The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study.
- Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis: The UP&DOWN Study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**
Manifiesta su conformidad para la presentación de las citadas publicaciones como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar las citadas publicaciones como parte de otra tesis doctoral en cualquier otra universidad.

En Madrid , a 25 de Enero de 2021

Fdo.: Dr. Óscar L. Veiga Núñez

UCA Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

Dña. **Rocío Izquierdo Gómez** con DNI o Pasaporte: 49017224-S coautora de las publicaciones que se identifican a continuación:

- Cardiorespiratory fitness cutoff points for early detection of present and future cardiovascular risk in children: A 2-year follow-up study.
- Muscular fitness, neck circumference, and blood pressure: an insight into their relationship through cross-lagged panel analysis: The UP&DOWN Study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de las citadas publicaciones como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar las citadas publicaciones como parte de otra tesis doctoral en cualquier otra universidad.

En Cádiz, a 18 de Enero de 2021

Fdo.: Rocío Izquierdo Gómez

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

Dña. **Sonia Gómez Martínez** con DNI o Pasaporte: 70512966A coautora de las publicaciones que se identifican a continuación:

- The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of body mass index in youth: The UP&DOWN Study.
- The role of adiposity in the association between muscular fitness and cardiovascular disease.
- Cardiorespiratory fitness cutoff points for early detection of present and future cardiovascular risk in children: A 2-year follow-up study.
- Muscle fitness cut points for early assessment of cardiovascular risk in children and adolescents.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de las citadas publicaciones como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar las citadas publicaciones como parte de otra tesis doctoral en cualquier otra universidad.

En Madrid , a 19 de Enero de 2021

Firmado por GOMEZ MARTINEZ SONIA - DNI 70512966A
el día 19/01/2021 con un certificado emitido por
AC Administración Pública

Fdo.: ______________________________

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

Dña. **Verónica Cabanas Sánchez** con DNI o Pasaporte 12.401.878W, coautora de las publicaciones que se identifican a continuación:

- Muscle fitness cut points for early assessment of cardiovascular risk in children and adolescents.
- The influence of cardiorespiratory fitness and neck circumference on blood pressure in children and adolescents: The UP&DOWN longitudinal study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de las citadas publicaciones como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar las citadas publicaciones como parte de otra tesis doctoral en cualquier otra universidad.

En Madrid, a 21 de enero de 2021

Fdo.: ______________________________

UCA Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

D. **Víctor Segura Jiménez** con DNI o Pasaporte: 74934073G coautor de las publicaciones que se identifican a continuación:

- The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of body mass index in youth: The UP&DOWN Study.
- The role of adiposity in the association between muscular fitness and cardiovascular disease.
- Cardiorespiratory fitness cutoff points for early detection of present and future cardiovascular risk in children: A 2-year follow-up study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de las citadas publicaciones como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada “Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN”.

Y expresa su renuncia a presentar las citadas publicaciones como parte de otra tesis doctoral en cualquier otra universidad.

En Puerto Real , a 21 de Enero de 2021

Fdo.: Víctor Segura Jiménez

UCA Universidad de Cádiz

Tesis por compendio de publicaciones
Documento de conformidad y renuncia de coautores

Dña. **Virginia A. Aparicio García Molina** con DNI o Pasaporte: 53265170E coautora de la publicación que se identifica a continuación:

- Cardiorespiratory fitness cutoff points for early detection of present and future cardiovascular risk in children: A 2-year follow-up study.

de acuerdo con lo establecido en el **Artículo 23.4** del **Reglamento UCA/CG06/2012, de 27 de junio de 2012, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BUOCA nº 208).**

Manifiesta su conformidad para la presentación de la citada publicación como parte de la tesis doctoral de D. Alejandro Pérez Pérez, titulada "Efectos de la condición física y la adiposidad sobre indicadores de salud cardiovascular en niños y adolescentes: estudio longitudinal UP&DOWN".

Y expresa su renuncia a presentar la citada publicación como parte de otra tesis doctoral en cualquier otra universidad.

En Granada, a 18 de enero de 2021

Fdo.: ______________________________________

ANNEX III: List of studies and quality indexes of published studies

Study I:

Title: The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of body mass index in youth: The UP&DOWN Study

Year of publication: 2019

Journal: Pediatric Diabetes

Journal impact factor (JCR): 3.052

Quartile: Q1

Study II:

Title: The role of adiposity in the association between muscular fitness and cardiovascular disease

Year of publication: 2018

Journal: The Journal of Pediatrics

Journal impact factor (JCR): 3.739

Quartile: Q1 (1st decile)

Study III:

Title: Cardiorespiratory fitness cutoff points for early detection of present and future cardiovascular risk in children: A 2-year follow-up study

Year of publication: 2017

Journal: Mayo Clinic Proceedings

Journal impact factor (JCR): 7.199

Quartile: Q1 (1st decile)

Study IV:

Title: Muscle fitness cut points for early assessment of cardiovascular risk in children and adolescents

Year of publication: 2019

Journal: The Journal of Pediatrics

Journal impact factor (JCR): 3.700

Quartile: Q1 (1st decile)

Study V:

Title: Bidirectional associations between fitness and fatness in youth: A longitudinal study

Year of publication: 2020

Journal: Scandinavian Journal of Medicine & Science in Sports

Journal impact factor (JCR): 3.255

Quartile: Q1

*Studies VI and VII are currently submitted for their publication. Study VIII is under revision with minor comments in the Journal of Science and Medicine in Sport (Journal impact factor (JCR): 3.607, Q1).

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