Running title:

Fitness, adiposity and cardiovascular risk

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The influence of cardiorespiratory fitness on clustered cardiovascular disease risk factors and the mediator role of BMI in youth: The UP&DOWN study

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ABSTRACT

Background – The combined effect of cardiorespiratory fitness (CRF) and body mass index (BMI) on cardiovascular disease (CVD) risk in young people remains to be fully determined. We examined the individual and combined associations of CRF and BMI with clustered CVD risk factors, and the mediator role of BMI in the association between CRF and clustered CVD risk factors in children and adolescents.

Research Design and Methods - 237 children (111 girls) and 260 adolescents (120 girls) were included in this cross-sectional study. Height and weight were assessed and BMI was calculated. A CVD risk factor index (CVDRF-I) was computed from: waist circumference, systolic blood pressure, triglycerides, high-density lipoprotein cholesterol and glucose. CRF was assessed using the 20-m shuttle run test. Regression analysis, analysis of covariance and mediation analysis (Baron and Kenny procedures) were used to test the independent and combined effect of CRF and BMI on CVDRF-I, and to test mediation hypothesis, respectively.

Results - CRF was negatively associated with CVDRF-I (all $p < 0.05$); however, after adjusting for BMI the associations were no longer significant in children and adolescents of both sex groups. Contrary, the association between BMI and CVDRF-I was independent of CRF (all $p < 0.001$). The effect of CRF on CVDRF-I was mediated by BMI. The percentage of the total effect of CRF on CVDRF-I mediated by BMI for boys and girls children and boys and girls adolescents were 79.5%, 100%, 81.2% and 55.7%, respectively.

Conclusions - BMI is an independent predictor of CVDRF-I and a mediator of the association between CRF and CVDRF-I in children and adolescents. These results help to clarify the associations between CRF, weight status and cardiovascular health,

suggesting that future CVD health would benefit from maintaining an optimal weight status.

Keywords: body composition, physical fitness, cardiovascular diseases, child, adolescent.

ABBREVIATIONS

BMI: body mass index

CRF: cardiorespiratory fitness

CVD: cardiovascular disease

CVDRF-I: cardiovascular disease risk factor index

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

HDL-c: high-density lipoprotein cholesterol

NW: normalweight including thinness grades 1, 2 and 3

OW: overweight including obesity

INTRODUCTION

Future cardiovascular profile could be determined by lifestyles in childhood and adolescence since precursors of cardiovascular disease (CVD) are already present at early ages (1). Interestingly, they have been found to track from childhood and adolescence to adulthood (2,3). Given that there is no consensus on the establishment of cut-off points for individual CVD risk factors in youth, clustered CVD risk factors seems to provide a good measure of CVD risk. Clustering of CVD risk factors has shown a greater stability over time and might compensate for day-to-day fluctuations in single CVD risk factors (4). Preventing programmes conducted at early ages have the potential of reducing the prevalence of CVD and its economical impact on society. Research works aimed at identifying CVD's predictors have been conducted (5); however, little evidence exists on how these variables interact with each other in children and adolescents

For instance, high cardiorespiratory fitness (CRF) levels in childhood and adolescence has been associated with a healthier cardiovascular profile (6) and it is considered a strong predictor of CVD risk factors later in life (5,7). Moreover, the cardiovascular health at these ages and in future life is importantly affected by weight status during childhood and adolescence (8,9). Given that CRF affects weight status, they may lay on the same causal pathway, making it difficult to determine the extent to which each variable affects CVD risk. In this sense, while some evidence suggest an independent association of CRF and weight status with CVD risk factors (4,10,11), other studies have found an attenuation in the association between CRF and CVD risk factors when weight status was included in the analyses (12). In addition, a recent study by Nyström et al (13) concluded that the metabolic profile is negatively affected to a greater extent by obesity rather than by low CRF levels, in a sample of children aged 8-11 years. Interestingly, CRF proved to attenuate the deleterious effect of obesity.

Multivariate analyses (e.g. multiple linear regression, logistic regression or analysis of covariance) have been used in previous studies to control for potential confounders. Alternatively, mediation analysis goes a step further since it allows to determine the percentage of the total effect (i.e. the influence of an independent variable on an outcome variable) that is carried by a mediator (14). Using these procedures, only one study (15) has observed that weight status, assessed by body mass index (BMI), mediates the association between CRF and CVD risk factors in children aged 8-11 years. Furthermore, to our knowledge, the extent to which BMI acts as a mediator in this association in adolescents has not been analysed through mediation analyses.

Consequently, the aim of this study is twofold: (i) to test the individual and combined associations of CRF and BMI with clustered CVD risk factors; and (ii) to analyse if the association between CRF and clustered CVD risk factors is mediated by BMI in children and adolescents. We hypothesised that (i) CRF and BMI are independently associated with clustered CVD risk factors; (ii) those with higher BMI levels present the worst CVD profiles, with CRF levels attenuating these effects; and (iii) BMI mediates the association between CRF and clustered CVD risk factors.

METHODS

Study design and population

Baseline data of the UP&DOWN study (16) collected from September 2011 to June 2012 was analysed in this cross sectional study. A total convenience sample of 2225 children and adolescents aged 6-12.9 and 13-17.9 years, respectively, participated in the UP&DOWN study. According to the Spanish Institute of National Statistics, the UP&DOWN sample size represents the 50% and 5% of the total population of school children and adolescents, respectively. The main exclusion criterion was the presence of physical disability or any health problem which might influence the performance in the fitness tests. From the total sample, 514 participants were randomly selected for blood sampling. Only those participants with complete data on anthropometry, biochemistry and CRF field test (i.e. 237 children [111 girls] and 260 adolescents [120 girls]) were included in the present study. Further information concerning participant selection criteria for the UP&DOWN study can be found elsewhere (16).

Participants' parents were informed about the purposes of the study and written informed consents were provided. The study protocol was accepted by The Ethics Committee of the *Hospital Puerta del Hierro* (Madrid, Spain), the Bioethics Committee of the National Research Council (Madrid, Spain) and the Committee for Research Involving Human Subjects of the University of Cádiz (Cádiz, Spain).

Measurements

Anthropometric data

Weight and height were measured with an electronic scale (Type SECA 861; range, 0.05 to 130 Kg; precision, 0.05 kg) and a telescopic stature-measuring instrument (Type SECA

225; range, 60 to 200 cm; precision, 1 mm) respectively. BMI was calculated as weight/height squared (kg/m^2). Waist circumference was measured at the level of the narrowest part of the torso, using a non-elastic tape (SECA 200; range, 0 to 150 cm; precision, 1 mm). Two non-consecutive measurements were carried out and the average was recorded. Anthropometric measurements were performed according to Lohman's anthropometric standardization reference manual (17).

Systolic blood pressure

Systolic blood pressure was measured with a validated digital automatic blood pressure monitor (OMRON M6, OMRON HEALTH CARE Co., Ltd., Kyoto, Japan) according to the standardized and valid International Protocol of the European Society of Hypertension (18). Two measurements were taken 1-2 minutes apart. If the first two readings differed in >5 mmHg, an additional measurement was taken and the farthest value was removed. The average value of the two measurements was selected.

Blood sampling

After an overnight fast, 13.5 ml of blood were extracted from the cubital vein of each participant. 3.5 ml of the blood sample were collected in EDTA and analysed to acquire haemogram data. The remaining blood was collected in dried gel and sodium citrate and centrifuged to remove both serum and plasma. Finally, it was frozen at -80°C for future analyses. Enzymatic colorimetric methods (Olympus AU2700 Analyser; Olympus UK Ltd, Watford, UK) were used to analyse serum lipid triglycerides, high-density lipoprotein cholesterol (HDL-c) and fasting plasma glucose.

Clustered cardiovascular disease 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. waist circumference, systolic blood pressure, triglycerides, HDL-c and glucose) by population (children and adolescents) and sex (boys and girls). This index has been previously used by the International Diabetes Federation to assess cardiovascular health in children and adolescents (19). The standardized value for HDL-c was multiplied by (-1) since higher HDL-c levels represent lower CVD risk. Given the close relationship ($r=0.638-0.950$, all $p<0.01$ for the different sex and age groups) between BMI and waist circumference, an additional CVD risk factor index was computed excluding waist circumference (i.e. CVDRF-II).

Cardiorespiratory fitness

The 20-m shuttle run test was used to assess CRF (20). Participants were asked to run between two lines 20 meters apart at a pace marked by a pre-recorded sound signal. The initial speed was 8.5 km/h, with 0.5 km/h increments each stage. The test finished when the participant could not reach the line twice in a row. The last half-stage attained was recorded. This test has shown to be valid and reliable in children and adolescents (21,22).

Data analyses

Descriptive statistics are presented as mean $\pm$ standard deviation. Due to significant interactions between sex (boys and girls) and age (children and adolescents) groups 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. Sex differences were examined by one-way analysis of variance.

Linear regression models controlling for age (model 1) were used to assess the individual association of CRF 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 (23,24) were used to categorize participants as normal weight “NW” (including thinness grades 1, 2 and 3 and normal weight) or overweight “OW” (including overweight and 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 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 (14). 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.

All the aforementioned analyses were repeated using CVDRF-II as the dependent variable (see supplementary files).

Statistical analyses were conducted using SPSS version 21.0. for Windows (IBM, Armonk, NY), with significance set at $p < 0.05$.

RESULTS

Participant's characteristics are depicted in **Table 1**. In children, boys reached higher scores in the 20-m shuttle run test than girls ($p<0.001$) and girls had higher levels of triglycerides than boys ($p=0.037$). In adolescents, boys were significantly heavier and taller than girls ($p=0.005$ and $p<0.001$, respectively) and presented higher levels of waist circumference, systolic blood pressure (both $p<0.001$) and glucose ($p=0.029$). Besides, boys performed better in the 20-m shuttle run test compared to girls ($p<0.001$).

Associations between CRF and CVDRF-I are presented in **Table 2**. CRF was significantly associated with CVDRF-I in boys ($\beta=-0.437$; $p<0.001$) and girls ($\beta=-0.305$; $p=0.001$) children, and in boys ($\beta=-0.404$; $p<0.001$) and girls ($\beta=-0.191$; $p=0.048$) 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 3**. BMI showed a positive association with CVDRF-I in boys and girls children ($\beta=0.688$ and $\beta=0.709$, respectively) and in boys and girls 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 significant in all sex and age groups (all $p<0.001$). The same pattern was observed when CVDRF-II was used in the analyses (**Table S2** in supplementary content).

Combined effects of CRF and BMI on CVDRF-I are displayed in **Fig. 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 boys and girls and the OW+High CRF group in girls (all $p<0.05$). 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 girl children remained the same. However, significant differences only persisted between NW+High CRF and OW+Low CRF groups in boys children and boys adolescents ($p < 0.05$) (**Fig. S1** in supplementary content).

Results of mediation analyses are depicted in **Fig. 2**. Regression equations 1, 2 and 3 were significant in boys (all $p < 0.001$) and girls (all $p < 0.01$) children, and in boys (all $p < 0.001$) and girls (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 boys ($z = -5.287$, $p < 0.001$) and 100% for girls ($z = -4.437$, $p < 0.001$) children, and 81.2% for boys ($z = -4.772$, $p < 0.001$) and 55.7% for girls ($z = -2.231$, $p < 0.05$) adolescents. When CVDRF-II was entered as the dependent variable (**Fig. S2** in supplementary content), results of the different regression equations were similar in boys and girls children and boys adolescents. In contrast, the Sobel test for girls 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 the percentage of body fat (estimated through triceps and subscapular skinfolds thickness and using the equations proposed by Slaughter et al (26)) instead of BMI, and the results did not materially changed (data not shown).

DISCUSSION

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 (27) . However, some of these studies did not account for weight status in this association (28,29). 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 adiposity on CVD risk factors have been previously reported (4,10). Nevertheless, other part of the evidence suggest that the association between CRF and CVD risk factors does not remain significant when adiposity is taken into account (30).

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 (10) used the 9-

minute run/walking test. Although the accuracy of field tests are questioned against laboratory tests, the 20-m shuttle run test has shown to be valid and reliable (21,22). 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 (31), 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 (10) only included total cholesterol and blood pressure, individually, as outcomes. Alternatively, several studies have employed a clustered of individual CVD risk factors (4,10,12,15,29,32); 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 (19).

Weight status in youth is an essential determinant of cardiovascular health at this stage of life (8,33), and it predicts CVD risk factors levels in adulthood (34,35). BMI (8,34,35) and percentage of body fat (33) are the most frequent body composition measures in these studies. We found that BMI and percentage of body fat 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 (36) observed significant associations between adiposity (defined by waist circumference) and several cardiometabolic risk factors such as systolic blood pressure, 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 these evidence comes from studies conducted in adult population, and the information about the Fat but Fit paradox in children and adolescents is still sparse (37). Nonetheless, and despite differences in the methodology, regarding the cardiovascular risk factors and the statistical procedures used, our results coincide with those reported recently by Nyström et al (13), 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 (13) 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 (15). 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 boys and girls children and adolescents, respectively, were mediated by BMI. Since mediation analysis assumes that the independent variable causes the mediator (14), BMI may be an intermediate step in the causal pathway between CRF and CVD risk factors. Previously, CRF has been negatively associated with adiposity in youth (38) and it is considered a predictor of adiposity in later years (5,7,39). The physiological mechanisms by which CRF affects adiposity 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) (40). On the other hand, adipose tissue produces several adipokines involved in inflammation (41). 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 (41). Together with our results, this evidence indicates that increments in CRF through aerobic exercise programs will ameliorate the level of adiposity 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 (42), 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.

Limitations and strengths

The current study has some limitations. Due to the cross-sectional design, directionality of associations could not be correctly determined. The use of BMI as an index of adiposity has been extensively questioned due to its lack of discriminative capacity between fat mass and lean body mass. Nevertheless, BMI is considered a powerful tool to identify youth with high individual and clustered CVD risk factor levels (43). In fact, BMI could not be a good marker of adiposity but it has proved to be a strong predictor of CVD mortality (44). In addition, analyses were performed using body fat percentage, instead

of BMI, and the results persisted unchanged. Furthermore, median split is not the best method for CRF categorizations. 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 for CRF categorizations in adults (42). The rationale behind this is that the risk of CVD is markedly higher in the first quintile (<20th percentile) compared with the other quintiles (42). This approach has also been used in paediatrics (13) using previously created sex and age specific percentiles (31). However, in the current study this categorization method resulted in highly unbalanced groups. No data regarding family history of CVD is available in the UP&DOWN study. This variable could affect, to some extent, the associations observed in the current study since it has an impact on the risk of CVD mortality. However, it has been previously observed that family history of CVD, among other variables (i.e. hypertension, current smoking, diabetes, obesity, and ‘being unfit’), is the least associated with the risk of CVD mortality, with ‘being unfit’ and obesity occupying the first and second places in that ranking, respectively (45). Finally, the generalisability of these results should be considered cautiously since we could not determine the influence of ethnicity and country’s economic development on these associations, given that only urban, Caucasian Spanish children and adolescent participated. Otherwise, this study presents some strengths. The use of clustered CVD risk factors has been suggested as a better indicator of cardiovascular health, compared to individual CVD risk factors (2). Furthermore, the statistical analyses conducted allow determining the percentage of the total effect that is carried by the mediator variable.

Conclusion

BMI seems to be an independent predictor of CVDRF-I and a mediator in the association between CRF and CVDRF-I in children and adolescents of both sexes. These results extend the current knowledge regarding the associations between fitness and weight

status with CVD risk, and suggest that attaining/maintaining an optimal weight status should be considered the main objective of health promoting programmes at both, childhood and adolescence. Furthermore, changes in weight status could be considered an indicative of the effectiveness of exercise programmes, with few positive effects on the CVD profile expected from those programmes that do not positively affect weight status in children and adolescents. Future longitudinal studies with heterogeneous and representative samples in terms of ethnicity and country's economic development are claimed to corroborate these results.

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Data availability: Datasets used in the current study are no publicly accessible, but are available from the corresponding author on reasonable request.

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Table 1. Participant's characteristics by sex and age groups.

Variable	Children			Adolescents		
	Boys (n=126)	Girls (n=111)	<i>p</i>	Boys (n=140)	Girls (n=120)	<i>p</i>
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/cm ²)	18.2 (2.8)	18.1 (3.6)	0.799	21.0 (3.6)	21.1 (3.4)	0.942
Waist circumference (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 2. Associations between cardiorespiratory fitness and the cardiovascular disease risk factor index (CVDRF-I) in children and adolescents.

	Children							
	Boys				Girls			
	B	95% CI	β	<i>p</i>	B	95% CI	β	<i>p</i>
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							
	Boys				Girls			
	B	95% CI	β	<i>p</i>	B	95% CI	β	<i>p</i>
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 3. Associations between body mass index and the cardiovascular disease risk factor index (CVDRF-I) in children and adolescents.

	Children							
	Boys				Girls			
	B	95% CI	β	<i>p</i>	B	95% CI	β	<i>p</i>
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							
	Boys				Girls			
	B	95% CI	β	<i>p</i>	B	95% CI	β	<i>p</i>
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 LEGENDS

Fig. 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.

Fig. 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.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.