Muscle fitness cut points for early detection cardiovascular risk in children and adolescents.

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Short title: Muscle fitness cutoffs for cardiovascular risk in youth

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ABSTRACT

Objectives: To study (1) the cross-sectional and longitudinal (2-year follow-up) association between muscle fitness and CVD risk factors in youth; (2) whether there are muscle fitness cut points associated with CVD risk (cross-sectional); and (3) whether the health-related muscle fitness cut points identified at baseline are associated with CVD risk 2-years later.

Study design: 237 children (110 girls) aged 6-10 years and 274 adolescents (131 girls) aged 12-16 years with complete data were included in the study (10.3% drop out). The handgrip strength and the standing long jump tests were used to assess muscle fitness. CVD risk score was computed with: sum of two skinfolds, systolic blood pressure, insulin, glucose, triglycerides, and total cholesterol/high density lipoprotein cholesterol.

Results: Muscle fitness at baseline was inversely associated with single CVD risk factors and CVD risk score at baseline and 2-year follow-up (all $P < 0.05$). Receiver operating characteristics curve analyses showed a significant discriminating accuracy of handgrip strength in identifying CVD risk in children and adolescents (boys: ≥ 0.367 and ≥ 0.473 ; girls: ≥ 0.306 and ≥ 0.423 kg/kg body mass, respectively, all $P < 0.001$). Similarly, the standing long jump cut points for children and adolescents were ≥ 104.5 and ≥ 140.5 in boys, and ≥ 81.5 and ≥ 120.5 cm in girls, respectively (all $P < 0.05$). These cut points were associated with CVD risk two years later (all $P < 0.01$).

Conclusions: Muscle fitness is associated with present and future cardiovascular health in youth, and is independent of cardiorespiratory fitness. It should be monitored to identify youth at risk who could benefit from intervention programmes.

List of abbreviations:

CVD: cardiovascular disease

SBP: systolic blood pressure

TG: Triglycerides

TC: total cholesterol

HDL: high-density lipoprotein cholesterol

HOMA-IR: homeostasis model assessment for insulin resistance

SD: standard deviation

ROC: receiver-operating characteristic

AUC: area under the curve

ANCOVA: analysis of the covariance

INTRODUCTION

Physical fitness is nowadays considered a marker of cardiovascular health in children and adolescents (1, 2). Together with cardiorespiratory fitness, upper and lower body muscle fitness has been positively associated with a healthier cardiovascular profile during childhood and adolescence (1, 3) and also later in life (2, 4-6). Interestingly, the relationships of cardiorespiratory fitness (7, 8) and muscle fitness (9, 10) with cardiovascular risk, seems to be mediated by obesity.

Recently, a meta-analysis evaluated the relationship between cardiorespiratory fitness and CVD risk in children and adolescents from multiple countries. This study provided pooled cardiorespiratory fitness cut points in children and adolescents that could serve as standards for international comparisons and to identify the target population for primary CVD prevention (11). Given that cardiorespiratory and muscle fitness are independently associated with CVD risk factors (3), it would be desirable to determine generalized health-related muscle fitness (i.e. upper and lower body muscle fitness) cut points associated with a healthier cardiovascular profile in children and adolescents. One cross-sectional study determined health-related handgrip strength cut points for cardiovascular risk in Colombian children and adolescents, with low-middle socioeconomic status (12). It would be helpful to establish health-related muscle fitness cut points in a sample that includes children and adolescents across socioeconomic status.

Therefore, we studied (1) the cross-sectional association between muscle fitness and CVD risk factors in children (6-10 years old) and adolescents (12-16 years old), as well as the association between muscle fitness in children and adolescents and CVD risk factors two years later (longitudinal); (2) whether there are muscle fitness cut points associated with CVD risk in children and adolescents (cross-sectional); and (3) whether the health-related muscle fitness cut points identified at the ages of 6-10 and 12-16 years are associated with CVD risk two years later (longitudinal, 8-12 and 14-18 years old, respectively).

METHODS

The children and adolescents selected for this study were enrolled in the UP&DOWN study (13). Briefly, the UP&DOWN study was a 2-year follow-up study designed to assess the impact of physical activity and sedentary behaviour over time on health indicators. It also aimed to identify the psycho-environmental and genetic determinants of physical activity in a sample of Spanish children (Cádiz) and adolescents (Madrid). A total convenience sample of 2,225 healthy participants aged 6 to 18 years participated in the UP&DOWN study, from 23 primary school (Cádiz) and 22 secondary schools (Madrid). According to the database of the Spanish Institute of National Statistics, our sample size represented the 50% (n=1188) and 5% (n=1037) of the total population size of school children and adolescents, respectively; with a 3% percent error for both sample sizes. Blood sampling was randomly performed in one-fourth of the recruited children and adolescents (514; 244 girls). We collected baseline data from September 2011 to June 2012 on a subsample of 237 children (110 girls) aged 6-10 years and 274 adolescents (131 girls) aged 12-16 years, with complete data on muscle fitness, body composition, systolic blood pressure, and blood sampling. A total of 213 children (98 girls) aged 8-12 years and 245 adolescents (118 girls) aged 14-18 years completed the data in the follow-up study (10.1% and 10.6% drop out, respectively), which was performed from September 2013 to June 2014. The main exclusion criterion was to not to have physical disability or health problems, which might limit levels of physical activity. The study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the Hospital Puerta de Hierro (Madrid, Spain), the Bioethics Committee of the National Research Council (Madrid, Spain), and the Committee for Research Involving Human Subjects at University of Cádiz (Cádiz, Spain). Parents and school supervisors were informed by letter about the nature and purpose of the study, and written informed consent was provided. Sexual maturation was self-reported using adapted drawings of the five stages according to Tanner & Whitehouse (14). We used breast development in girls and genital development in boys.

We assessed triceps and subscapular skinfold thickness on the non-dominant side of the body with a Holtain calliper (range, 0 to 40 mm; precision, 0.2 mm) according to Lohman's anthropometric standardization reference manual (15). We carried out the measurements twice, but not consecutively, and the mean value of both measurements was used for analyses.

We measured the systolic blood pressure (SBP) with a validated digital automatic blood pressure monitor (OMRON M6, OMRON HEALTH CARE Co., Ltd., Kyoto, Japan) according to the International Protocol of the European Society of Hypertension (16). The participants sat quietly on a chair for 5 minutes before the measurements were conducted on the left arm in an extended position. We took two measures 1-2 minutes apart. We performed an additional measurement if the first two readings differed in >5 mmHg, and the farthest value was removed. Average values (mmHg) were calculated.

We obtained a fasting blood sample from the cubital vein at the schools early in the morning (13). Ten mL of blood were drawn from each subject (dry gel for serum) and centrifuged; serum removed was frozen at -80° C to be analysed later. We analysed serum lipid triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and glucose by enzymatic colorimetric methods (Olympus AU2700 Analyzer; Olympus UK Ltd, Watford, UK). TC/HDL-C ratio was calculated. We analysed insulin with the Bio-Plex® system and Luminex® xMAP technology (Bio-Rad Laboratories, Inc., USA). Furthermore, we calculated insulin resistance through the homeostasis model assessment for insulin resistance (HOMA-IR) score as: $[\text{insulin (mIU/mL)} \times \text{glucose (mmol/L)}] / 22.5$ (17).

Muscle fitness was assessed based on the handgrip strength (upper body isometric muscle fitness) and the standing long jump (lower body explosive muscle fitness) tests, according to the ALPHA health-related fitness test battery protocol (18, 19). We used a hand dynamometer with an adjustable grip (TKK 5101 Grip D; Takey, Tokyo, Japan) for the handgrip strength test (20). The grip-span of the dynamometer was adjusted according to the hand size for determining the

maximum handgrip strength using the equations specifically developed for children (21) and adolescents (22). We performed the test twice, and we recorded the maximum score for each hand in kilograms. The average of the maximum score achieved by left and right hands was used in the analysis. Since heavier children and adolescents have higher levels of handgrip strength (23-25), we normalized by body mass expressing the handgrip strength score divided by body mass. This allowed us to be more accurate when comparing individuals with different body sizes and to focus on muscle quality rather than muscle quantity.

The standing long jump test was performed from a starting position behind a line, standing with the feet approximately shoulder width apart. We performed the test twice and recorded the best score in centimetres. Trained personnel performed all the tests.

We computed a CVD risk score by sex and age, resulting in 8 different scores (boys and girls, age groups: 6-10, 12-16 years old). The variables included in the CVD risk score were: sum of two skinfolds; SBP, HOMA-IR, TG and TC/HDL-C ratio (26). These variables were standardized as follows: $\text{standardize value} = (\text{value} - \text{mean}) / \text{standard deviation}$. The CVD risk score was calculated as the mean of the 5 standardized scores separately for boys and girls and age groups. Those children with values greater than 1.0 standard deviation (SD) in the continuous composite risk score variable were defined as being at risk (27, 28).

Descriptive data are shown as mean and SD unless otherwise indicated. The Student's *t*-test was used to test differences in characteristics of study sample by sex at baseline and follow-up, except for the Tanner stage, which was analysed by χ^2 test.

We performed multiple linear regression analyses to examine the cross-sectional association between muscle fitness and CVD risk factors in children (6-10-year old) and adolescents (12-16-year old), and the association between muscle fitness in children and adolescents and CVD risk factors two years later (longitudinal). We entered single CVD risk factors and CVD risk score at baseline (6-10 and 12-16-year-olds) and follow-up (8-12 and 14-18-year old) as dependent

variables, handgrip strength test/body mass and standing long jump test at baseline as independent variables, and age as covariate, in separate models. Additionally, we controlled longitudinal analyses for the corresponding baseline value.

In order to determine whether there are muscle fitness cut points associated with CVD risk in children (6-10 years old) and adolescents (12-16 years old) (cross-sectional), we established the muscle fitness cut points (i.e. handgrip strength test/body mass and standing long jump test) at baseline associated with CVD risk score (CVD risk score >1.0 SD) by using the receiver operating characteristic (ROC) curve (29). We calculated the area under the curve (AUC), 95% confidence interval (CI), sensitivity, specificity, Youden Index, positive and negative predictive value and likelihood ratio of a positive and negative test results. Binary logistic regression was also used to study the relationship between the muscle fitness cut points (high vs. low) and CVD risk score (≤ 1 SD vs. >1 SD).

To study whether the health-related muscle fitness cut points identified in 6-10 and 12-16-year-olds are associated with CVD risk two years later (longitudinal, 8-12 and 14-18-year-olds, respectively) we conducted binary logistic regression analysis using muscle fitness cut points (high vs. low) at baseline and CVD risk score (≤ 1 SD vs. >1 SD) at follow-up, adjusting for age at follow-up. We also assessed differences on CVD risk score at follow-up by muscle fitness levels at baseline (high vs. low) by analysis of covariance (ANCOVA). We entered CVD risk score at follow-up as a dependent variable, muscle fitness tests (high vs. low) as an independent variable in separate models, and CVD risk score at baseline, sex, and age at follow-up as a covariate. We also analysed the association between muscle fitness at baseline and muscle fitness at follow-up by means of linear regression analysis.

The statistical analyses were performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp), and the level of significance was set at $P < 0.05$.

RESULTS

The characteristics of the study sample are shown in **Table 1** (online). 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 TG in children, both boys and girls, and TC/HDL-C ratio in children boys and adolescent boys and girls. In contrast, HOMA-IR significantly decreased from baseline to follow-up in adolescent boys and girls. Levels of handgrip strength and standing long jump significantly increased (all $P\leq 0.005$), except for the handgrip strength in children boys and the standing long jump in adolescent girls.

Levels of handgrip strength 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 children boys and adolescent boys and girls; and TC/HDL-C ratio at follow-up in adolescent boys (**Table 2**).

Levels of standing long jump performance were inversely associated with the sum of two skinfolds (β from -0.346 to -0.698; all $P<0.001$) and CVD risk score (β from -0.216 to -0.467; all $P<0.05$) at baseline and follow-up in children and adolescents in both sexes, with SBP at baseline in children in both sexes ($\beta=-0.118$; $P=0.007$ in boys and $\beta=-0.230$; $P=0.020$ in girls), TC/HDL-C ratio at baseline in children and adolescents in both sexes (β from -0.221 to -0.239; all $P<0.05$) and at follow-up in children in both sexes ($\beta=-0.289$; $P=0.005$ in boys and $\beta=-0.386$; $P=0.001$ in girls), TG at follow-up in children and adolescents in both sexes (β from -0.202 to -0.299; all $P<0.05$), and HOMA-IR at baseline in children boys ($\beta=-0.279$; $P=0.048$) and at follow-up in adolescent boys and girls ($\beta=-0.239$; $P=0.040$ in boys and $\beta=-0.271$; $P=0.017$ in girls) (**Table 3**).

The handgrip strength cut points associated with CVD risk were 0.367 and 0.306 kg/kg body mass in children boys and girls, respectively, and 0.473 and 0.423 kg/kg body mass in adolescents boys and girls, respectively (**Figure 1**). The standing long jump cut points associated with CVD risk were 104.5 and 81.5 cm in children boys and girls, respectively, and 140.5 and 120.5 cm in

adolescent boys and girls, respectively. Children and adolescents, both boy and girls, with levels of handgrip strength ≥ 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 favourable CVD risk score at baseline than their peers with lower levels of handgrip strength (**Table 4** online). Likewise, children and adolescents, both boys and girls, 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 favourable CVD risk score at baseline than their peers with lower levels of standing long jump. The AUC, sensitivity, specificity (**Figure 1**), and Youden Index, positive and negative predictive value and likelihood ratio of a positive and negative test results (**Table 5** online) are the diagnostic variables that have allowed determining the appropriated health-related muscle fitness cut points. Similarly, logistic regression analysis showed that children and adolescents, both boys and girls, with handgrip strength levels at or above the determined cut points were at greater odds for a favourable CVD risk score at follow-up compared with those with relative handgrip strength levels below the cut points (all $P < 0.05$) (**Table 4** online). Children and adolescents with levels of standing long jump at or above the determined cut points at baseline were at greater odds for a favourable CVD risk score at follow-up compared with those with standing long jump levels below the cut points (all $P < 0.05$).

Figure 2 (online) shows a positive association between levels of handgrip strength and standing long jump at baseline and follow-up in both boys and girls and in both age groups (R^2 ranging from 0.49 to 0.63; all $P < 0.001$). Children and adolescents, both boys and girls, with high levels of handgrip strength or standing long jump at baseline had lower CVD risk score (all $P < 0.01$ and all $P < 0.001$, respectively) at follow-up (**Figure 3**). We repeated all analyses after adjusting for pubertal development instead of age, and the results did not change (data not shown). Similarly, we repeated all analyses after further adjusting for cardiorespiratory fitness, assessed by the 20-metre shuttle run test (18) and the results persisted (data not shown).

DISCUSSION

The findings of the present study indicate that (1) poor levels of muscle fitness 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; (2) we identified a muscle fitness cut points associated with a more favourable cardiovascular health profile in children and adolescents, which seems to be more discriminative for boys than for girls; and (3) the health-related muscle fitness cut points identified are associated with CVD risk score two years later. The observed consistent cross-sectional and longitudinal associations between muscle fitness and cardiovascular health in children and adolescents are independent of cardiorespiratory fitness, which reinforces the importance of muscle fitness 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 (18, 19). This study included, besides the handgrip strength test, the standing long jump test to assess skeletal muscle fitness. Pate et al. (30) suggested that a USA survey of health-related physical fitness in youth should include both, upper and lower body muscle fitness. Furthermore, they recommended determining health-related muscle fitness cut points for children and adolescents.

Our findings confirm the observed strong evidence of an inverse association between muscle fitness and CVD risk(3). The results of the present study also concur with those from prospective studies showing that children and adolescents with higher levels of muscle fitness have a more favourable cardiovascular profile later in life (2, 3). Longitudinal studies also showed that muscle fitness was associated with risk of CVD mortality (6, 31). It is important to highlight that muscle fitness is associated with CVD risk regardless of cardiorespiratory fitness and other confounders (32, 33) in children and adolescents. Our results confirmed the findings reported in previous prospective studies (5, 6), suggesting that there is both a combined and additive effect of muscle fitness on CVD risk. On the other hand, we observed that children and adolescents with low muscle fitness levels

are very likely to have low muscle fitness levels 2 years later. It seems reasonable to include muscle fitness tests in monitoring to identify children and adolescents with poor cardiovascular health levels. Educational and public health policies are needed to promote healthy lifestyles in order to increase muscle fitness to prevent CVD risk. In this sense, global physical activity guidelines for children and adolescents should emphasise participation in high-intensity physical activities and include a recommendation to perform muscle physical activities at least 3 days per week (34).

The present study shows that handgrip strength cut points associated with a reduced CVD risk in children and adolescents, both boys and girls, 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 (35). These results are similar to those reported by Ramirez-Velez et al. (12) in Colombian children and adolescents aged 9-17 years. We also identified standing long jump cut points associated with a healthier cardiovascular profile in children (104.5 cm in boys and 81.5 cm in girls) and adolescents (140.5 cm in boys and 120.5 cm in girls). These health-related muscle fitness cut points presented high accuracy ($AUC > 0.7$), except in the standing long jump for adolescent boys and girls 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 muscle fitness should start in early childhood.

Limitations of the present study include the lack of consensus in children and adolescents regarding the definition of cardiovascular health. We included a clustered CVD risk score (z-score) because this type of outcome may be a better indicator of health in apparently healthy children and adolescents as compared with individual risk factors. Moreover, adiposity was assessed by anthropometry (skinfolds). The major drawback with the skinfold technique lies in the extensive expertise one must have in taking readings accurately and with consistency. However, trained professionals measured skinfolds using standard methods of data collection, and the skinfold

assessment has been suggested to be a valid tool to evaluate adiposity in children (36). Finally, muscle fitness was assessed by the handgrip strength and the standing long jump test, which may not represent overall muscle strength. Nevertheless, the standing long jump test is strongly associated with both lower and upper body muscle fitness, and has been considered a good marker of overall muscle fitness (37).

Muscle fitness should be considered in monitoring children at high risk of poor cardiovascular health. Public health as well as school-based strategies, and intervention programmes to increase physical activity levels in order to meet health-related muscle fitness levels could be helpful.

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CONFLICT OF INTEREST

The authors have no conflicts of interest applicable to this article to disclose

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FIGURE LEGENDS

Figure 1. Receiver operating characteristic curve summarising the potential of muscle fitness to identify cardiovascular health status in children and adolescents, both boys and girls. AUC indicates the area under the curve.

Figure 2. Scatterplot of muscle fitness levels at baseline (6-10 and 12-16-year old) and at 2-year follow-up (8-12 and 14-16-year-olds, respectively) in children and adolescents, both boys and girls.

Figure 3. Association between muscle fitness levels at baseline (6-10 and 12-16-year-olds) with cardiovascular risk score at 2-year follow-up (8-12 and 14-16-year-olds, respectively) for children (**A** and **B** for the relative handgrip strength and the standing long jump tests, respectively) and adolescents (**C** and **D** for the relative handgrip strength and the standing long jump tests, respectively), both boys and girls. Analysis adjusted for CVD risk score at baselines, sex, and age at 2-year follow-up.

Table 1. Baseline and 2-year follow-up characteristics of study sample by sex.

	Children						Adolescents					
	Boys			Girls			Boys			Girls		
	Baseline (n=127)	2-year follow-up (n=115)	P*	Baseline (n=110)	2-year follow-up (n=98)	P*	Baseline (n=143)	2-year follow-up (n=127)	P*	Baseline (n=131)	2-year follow-up (n=118)	P*
Pubertal development, n (%)			<0.001			<0.001			<0.001			<0.001
1	62 (48.8)	6 (5.2)		72 (65.2)	34 (33.3)		-	-		1 (0.8)	-	
2	56 (44.1)	73 (63.5)		28 (27.7)	27 (26.5)		16 (11.2)	-		11 (8.4)	-	
3	9 (7.1)	33 (28.7)		8 (7.3)	30 (29.4)		46 (32.2)	7 (5.5)		61 (46.6)	26 (20.0)	
4	-	3 (2.6)		2 (1.8)	11 (10.8)		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)	7.6 (1.5)	10.1 (1.5)	<0.001	7.5 (1.6)	10.1 (1.6)	<0.001	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)	21.7 (9.7)	26.7 (13.6)	<0.001	25.0 (12.8)	28.1 (14.9)	<0.001	23.7 (13.7)	25.3 (10.5)	<0.001	30.3 (11.9)	30.5 (13.4)	0.457
Systolic blood pressure (mmHg)	101.8 (11.0)	105.0 (9.3)	0.001	99.4 (11.6)	104.8 (10.8)	<0.001	112.9 (15.6)	114.7 (13.2)	0.071	106.8 (10.5)	104.6 (10.8)	0.002
HOMA-IR	0.8 (0.8)	1.1 (1.3)	0.021	1.2 (1.4)	1.1 (0.9)	0.630	2.8 (2.2)	0.9 (1.6)	<0.001	3.2 (2.7)	0.6 (0.5)	<0.001
Triglycerides (mg/dL)	41.1 (20.5)	40.8 (19.1)	0.906	46.3 (17.9)	48.3 (27.4)	0.248	54.38 (36.4)	67.8 (31.1)	<0.001	57.8 (29.6)	63.7 (24.2)	<0.001
TC/HDL-C ratio	3.0 (0.6)	3.1 (0.6)	0.223	3.2 (0.7)	3.0 (0.7)	0.022	2.8 (0.5)	2.9 (0.6)	0.245	2.8 (0.6)	2.7 (0.6)	0.162
Handgrip strength/body mass	0.41 (0.1)	0.41(0.1)	0.921	0.39 (0.1)	0.40 (0.1)	0.005	0.50 (0.1)	0.56 (0.1)	<0.001	0.43 (0.1)	0.46 (0.1)	<0.001
Standing long jump (cm)	114.8 (21.1)	129.8(25.3)	<0.001	106.5 (22.9)	122.9 (24.1)	<0.001	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.

*P: significant differences between baseline and 2-year follow-up are highlighted in bold.

Table 2. Association of the relative handgrip strength 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 2-year follow-up (8-10 and 14-16-year-olds, respectively).

	Children											
	Boys					Girls						
	B	95% CI			β	P	B	95% CI			β	P
<i>Baseline</i>												
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	-0.4084	;	-1.785	-0.424	< 0.001	-3.810	-5.146	;	-2.475	-0.484	< 0.001
<i>2-year follow-up*</i>												
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											
	Boys						Girls					
	B	95% CI			β	P	B	95% CI			β	P
<i>Baseline</i>												
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
<i>2-year follow-up*</i>										
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 2-year follow-up, cardiovascular risk factors and cardiovascular risk scores were adjusted for levels at baseline.

Table 3. 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 2-year follow-up (8-10 and 14-16-year-olds, respectively).

	Children									
	Boys					Girls				
	B	95% CI		β	P	B	95% CI		β	P
<i>Baseline</i>										
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
<i>2-year follow-up*</i>										
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									
	Boys					Girls				
	B	95% CI		β	P	B	95% CI		β	P
<i>Baseline</i>										
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
<i>2-year follow-up*</i>										
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 2-year follow-up, cardiovascular risk factors and cardiovascular risk scores were adjusted for levels at baseline.

Table 4. 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 the baseline and 2-year follow-up.

	Children											
	Boys					Girls						
	β	95% CI			OR	P	β	95% CI			OR	P
<i>Baseline</i>												
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
<i>2-year follow-up*</i>												
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											
	Boys					Girls						
	β	95% CI			OR	P	β	95% CI			OR	P
<i>Baseline</i>												
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
<i>2-year follow-up*</i>												
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 2-year follow-up, cardiovascular risk scores were adjusted for levels at baseline.

Table 5. Diagnostic variables for muscle fitness tests to determine elevated cardiovascular disease risk score.

	Children									
	Boys					Girls				
	Youden Index	PPV	NPV	LR+	LR+	Youden Index	PPV	NPV	LR+	LR-
Handgrip strength/body mass	0.42	5.0	75.0	2.10	0.31	0.61	3.4	75.7	2.90	0.10
Standing long jump	0.53	37.5	97.6	3.92	0.35	0.47	43.8	90.3	2.07	0.17
	Adolescents									
	Boys					Girls				
	Youden Index	PPV	NPV	LR+	LR-	Youden Index	PPV	NPV	LR+	LR-
Handgrip strength/body mass	0.49	5.4	65.5	2.39	0.24	0.44	3.4	75.7	2.14	0.29
Standing long jump	0.42	37.5	90.2	2.31	0.38	0.47	38.0	88.1	2.19	0.23

PPV indicates positive predictive value; NPV, negative predictive value; LR+, likelihood ratio of a positive test results; LR-, likelihood ratio of a negative test results.

Figure

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