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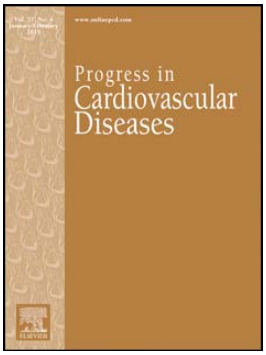

Body Composition Indices and Single and Clustered Cardiovascular Disease Risk Factors in Adolescents: Providing Clinical-Based Cut-Points

Luis Gracia-Marco, Luis A. Moreno, Jonatan R. Ruiz, Francisco B. Ortega, Augusto César Ferreira de Moraes, Frederic Gottrand, Romana Roccaldo, Ascensión Marcos, Sonia Gómez-Martínez, Jean Dallongeville, Anthony Kafatos, Denes Molnar, Gloria Bueno, Stefaan de Henauw, Kurt Widhalm, Jonathan C. Wells

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TITLE PAGE

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ABSTRACT

The aims of the present study in adolescents were 1) to examine how various body composition-screening tests relate to single and clustered cardiovascular disease (CVD) risk factors, 2) to examine how lean mass and body fatness (independently of each other) relate to clustered CVD risk factors and, 3) to calculate specific thresholds for body composition indices associated with an unhealthier clustered CVD risk. We measured 1089 European adolescents (46.7% boys, 12.5-17.49yr) in 2006-2007. CVD risk factors included: systolic blood pressure, maximum oxygen uptake, homeostasis model assessment, C-reactive protein (n=748), total cholesterol/high density lipoprotein cholesterol and triglycerides. Body composition indices included: height, body mass index (BMI), lean mass, the sum of four skinfolds, central/peripheral skinfolds, waist circumference (WC), waist-to-height ratio (WHtR) and waist-to-hip ratio (WHR). Most body composition indices are associated with single CVD risk factors. The sum of four skinfolds, WHtR, BMI, WC and lean mass are strong and positively associated with clustered CVD risk. Interestingly, lean mass is positively associated with clustered CVD risk independently of body fatness in girls. Moderate and highly accurate thresholds for the sum of four skinfolds, WHtR, BMI, WC and lean mass are associated with an unhealthier clustered CVD risk (all AUC > 0.773). In conclusion, our results support an association between most of the assessed body composition indices and single and clustered CVD risk factors. In addition, lean mass (independent of body fatness) is positively associated with clustered CVD risk in girls, which is a novel finding that helps to understand why an index such as BMI is a good index of CVD risk but a bad index of adiposity. Moderate to highly accurate thresholds for body composition indices associated with a healthier clustered CVD risk were found. Further studies with a longitudinal design are needed to confirm these findings.

Keywords: Adolescence, cardiovascular risk, C-reactive protein, fitness, fat mass, lean mass.

Abbreviations and definitions in alphabetical order:

AUC (area under the curve), BF (body fat), BMI (body mass index), BP (blood pressure), CV (cardiovascular), CVD (cardiovascular disease), CRP (C-reactive protein), HC (hip circumference), HDLc (high-density lipoprotein cholesterol), HELENA-CSS (Healthy Lifestyle in Europe by Nutrition in Adolescence cross-sectional study), HOMA-IR (homeostasis model assessment – insulin resistance) index, LV (left ventricular), ROC (receiver operating characteristic), SBP (systolic blood pressure), TC (total cholesterol), VO_{2max} (maximum oxygen consumption), WC (waist circumference), WHR (waist-to-hip ratio), WHtR (waist-to-height ratio)

INTRODUCTION:

Cardiovascular (CV) diseases (CVD) usually occur during adulthood although it might have its origin already in childhood or adolescence[1,2]. Some body composition indices seem to predict CVD risk and are recommended as possible screening tools in the absence of other CV risk measures.

From a methodological perspective, clustering of CVD risk factors seems to be a much stronger measure of CV health than single risk factors in children, as a subject with CVD risk may present high levels of several risk factors simultaneously [3]. There is controversy as to which body composition indices are the best when screening youths and adults for cardio metabolic risk, with some studies suggesting the use of body mass index (BMI) or body fat (BF) percentage (%) [4] while others suggesting waist circumference (WC) and skinfolds [5].

Most research to date has focused in finding an association between CVD risk factors and adiposity. However, little research has included lean mass (independent of BF) as a possible body composition index associated with single CVD risk factors [6] and no one has focused on understanding the association between lean mass and clustered CVD risk factors. Recently, several lines of evidence have also implicated chronic inflammation in CVD and, some inflammatory markers, such as C-reactive protein (CRP) have received much attention since elevated serum CRP concentrations have been associated with CVD risk [7,8]As a consequence, its use to predict CVD risk has been supported [9].

Therefore, the aims of the present study in adolescents are 1) to examine how various body composition-screening tests relate to single and clustered CVD risk factors, 2) to examine how lean mass and BF (independently of each other) relate to clustered CVD risk factors and, 3) to calculate specific thresholds for body composition indices associated with an unhealthier clustered CVD risk.

METHODS:

Study design and study sample

The current report is based on data derived from the Healthy Lifestyle in Europe by Nutrition in Adolescence cross-sectional study (HELENA-CSS). Participants were recruited at schools in 10 European cities: Stockholm (Sweden), Athens and Heraklion (Greece), Rome (Italy), Zaragoza (Spain), Pecs (Hungary), Ghent (Belgium), Lille (France), Dortmund (Germany) and Vienna (Austria). To ensure that the heterogeneity of social background of the population would be represented, schools were randomly selected after stratification by school zone or district. In cases where the selected schools refused to participate, a second list of substitute schools had already been drawn up. Up to three classes from two grades were selected per school. A class was considered eligible if the participation rate was at least 70%. Detailed descriptions of the HELENA sampling and recruitment approaches, standardization and harmonization processes, data collection, analysis strategies, quality control activities and inclusion criteria have been described in detail elsewhere [10]. An extended and detailed manual of operations was designed for and thoroughly read by every researcher involved in the field work before data collection started.

Data collection took place between October 2006 and December 2007 and the age range considered valid for the HELENA study was 12.5–17.49 years (n=3528). One-third of the school classes were randomly selected in each center for blood collection, resulting in a total of 1089 adolescents. However, valid data for CRP was only available in 748 adolescents. In order to make a better use of the data, sample sizes may vary depending on the outcome since the study samples did not differ in sex distribution, mean age and mean BMI from the whole HELENA sample (all $p>0.05$).

The study was approved by the Research Ethics Committees of each city involved (for most this was the country's ministry of health) and was performed following the ethical guidelines

of the Declaration of Helsinki, 1961 (revision of Edinburgh 2000) [11]. We obtained written informed consent from the parents of the adolescents and the adolescents themselves.

Blood pressure (BP)

Systolic BP (SBP) has been consistently used as an individual CVD risk factor in youths [12,13]. We measured SBP with an automatic oscillometric device (OMRON M6) which has been approved by the British Hypertension Society [14]. The procedures to measure SBP have already been published[15]. Briefly, measurements were taken twice (10 min apart) and the lowest SBP value was retained.

Cardiorespiratory fitness (CRF)

Physical fitness characteristics of the study sample, as well as all the procedures used to assess physical fitness in the HELENA study, have already been published [16]. CRF was assessed with the 20m shuttle run test (stage). A stage is the period of time in which the speed maintains constant. In this test, the initial speed is 8.5km·h^{-1}, which is increased by 0.5km·h^{-1}·min^{-1} (1 min equals one stage) [17]. The equation reported by Léger et al. [17] was used to estimate the maximum oxygen consumption (VO_{2max}, ml/kg/min). This test has been shown to be reliable in the HELENA adolescents (inter-trial difference -0.1 ± 1.5 stages in boys and 0.0 ± 1.1 stages in girls)[18]. We multiplied VO_{2max} by -1 to indicate higher CVD risk with increasing value and be consistent with the other individual CVD risk factors.

Biochemical measurements

The blood sampling procedure and sample logistics have been previously described [19]. We obtained venous blood samples after a 10-h overnight fast and we sent them to a central laboratory (the Analytical Laboratory at the University of Bonn's Institut für Ernährungs- und

Lebensmittelwissenschaften). We measured serum concentrations of triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), and glucose on the Dimension RxL clinical chemistry system (Dade Behring, Schwalbach, Germany) with enzymatic methods that used the manufacturer's reagents and instructions. The TC/HDLc ratio was calculated. We measured insulin by a solid-phase two-site chemiluminescent immunometric assay with an Immulite 2000 analyzer (DPC Biermann GmbH, Bad Nauheim, Germany). We calculated insulin resistance (IR) through the homeostasis model assessment (HOMA-IR) index as: [insulin (mLU/mL) X glucose (mmol/L)] / 22.5 [20]. In addition, we measured high sensitive C-reactive protein by immunoturbidimetry (Olympus AU2700 Analyzer, Olympus UK Ltd., Watford, UK).

CVD risk score

We computed a composite CVD risk score following the one used by Andersen et al. [12], but excluding the sum of skinfolds (as this is one of the predictor variables) and including CRP. Briefly, we summed the age-and sex- specific Z scores of the individual risk factors (SBP, VO_{2max}, HOMA-IR, CRP, TC/HDLc and triglycerides). We considered individuals with a CVD risk score higher than 1SD at risk and individuals with ≤1SD as having a healthier CVD risk score, as performed in previous studies [12,21].

Anthropometry and body composition

Harmonization and standardization of anthropometric measurements used to assess body composition were strictly controlled. Participants were barefoot and wearing underwear. Briefly, we measured body weight with an electronic scale (Type SECA 861; range, 0.05–130 kg; precision, 0.05 kg). We measured height in the Frankfurt plane with a telescopic height measuring instrument (Type SECA 225; range, 60–200 cm; precision, 1 mm). Then, we calculated BMI as body weight (kg) divided by the height (m) squared.

We measured a set of skinfold thickness (biceps, triceps, subscapular, suprailiac, thigh, and calf) in triplicate on the left side of the body with a Holtain caliper (Crymmych, UK, range, 0–40 mm; precision, 0.2 mm).

We measured (in triplicate) WC at the midpoint between the lowest rib cage and the top of the iliac crest and hip circumference (HC) around the widest portion of the buttocks with a non-elastic tape (Seca 200, MWS Ltd, Scalesmart) to the nearest 0.1 cm.

We computed the sum of four skinfolds (biceps, triceps, subscapular and suprailiac), central-peripheral skinfolds ratio (subscapular + suprailiac / biceps + triceps), waist-to-height ratio and (WHtR) and waist-to-hip ratio (WHR) from the original measurements.

Lean body mass (kg) was obtained using a tetra-polar bioelectrical impedance device (BIA 101 AKERN, Srl., Firenze, Italy) through the measurement of resistance and using the Bodygram Software V.1.41 (Akern S.r.l. Bioresearch, Pontassieve, Italy) for Windows. The following formulas were used to obtain lean mass from bioelectrical impedance analysis measurements[22]:

Lean mass (kg) for males = $-9.88 + 0.65$ stature^2/resistance $+ 0.26$ weight $+ 0.02$ resistance.

Lean mass (kg) for females = $-11.03 + 0.70$ stature^2/resistance $+ 0.17$ weight $+ 0.02$ resistance.

Statistical analysis

Descriptive data are shown as mean and standard deviation (SD) and were obtained by running analysis of variance (ANOVA) tests. We computed age- and sex-specific z-scores from our participants for all body composition indices included (height, BMI, lean mass, the sum of four skinfolds, central/peripheral skinfolds, WC, WHtR and WHR). We then divided the residual (actual minus predicted) value for each subject from the regression by its standard error to give a "studentized" residual. We performed linear regression models with single CVD risk factors (z-scores of SBP, VO_{2max}, HOMA-IR index, CRP, TC/HDLc and

triglycerides) as dependent variables, and body composition indices (z-scores) as independent variables. In addition, we also performed linear regression models with the composite CVD risk score as dependent variable and the body composition indices as independent variables.

It is known that BF and lean mass have a strong colinearity. Therefore, in order to analyze the association of the sum of four skinfolds (index of body fatness) independently of lean mass on CVD risk we performed a linear regression model of lean mass (independent variable) on CVD risk score (dependent variable) adjusting by the residuals of a previous regression of the sum of four skinfolds on lean mass. Similarly, to analyze the association of lean mass independently of the sum of four skinfolds on CVD risk we performed a linear regression model of the sum of four skinfolds (independent variable) on CVD risk score (dependent variable) adjusting by the residual of a previous regression of lean mass on the sum of four skinfolds [23].

Finally, we performed Receiver Operating Characteristic (ROC) analyses curves for those body composition indices most associated with clustered CVD risk in both sexes. ROC curve provides the whole spectrum of specificity/sensitivity values for all the possible cut-offs. The area under the curve (AUC) is determined from plotting sensitivity versus 1-specificity of a test as the threshold varies over its entire range. Taking into account the suggested cut-off points, the test can be non-informative/ test equal to chance (AUC=0.5); less accurate ($0.5<AUC\leq0.7$); moderately accurate ($0.7<AUC\leq0.9$); highly accurate ($0.9<AUC<1.0$); and perfect discriminatory tests (AUC=1.0) [24]. Cut-off points were selected for those scores optimizing the sensibility– specificity relationship.

The statistical analyses were performed using the SPSS IBM statistics (version 21.0 for WINDOWS, Chicago, IL, USA), and the statistical software package Stata (version 12.0, Stata Corp., college Station, TX, USA). Alpha error was set at 5%.

RESULTS:

Table 1 shows descriptive characteristics (mean ± SD) of the study sample. The ANOVA showed that there were no sex differences in age, BMI, WHtR ratio, HOMA-IR index score, TC/HDLc and CVD risk score; however, most variables differed by sex.

Body composition and single CVD risk factors

Table 2 shows that all body composition indices were positively associated with SBP (boys, adjusted R^2 from 0.024 to 0.139 and girls, adjusted R^2 from 0.008 to 0.11). In addition, all but height (in boys) were positively associated with VO_{2max} (boys, adjusted R^2 from 0.022 to 0.173 and girls, adjusted R^2 from 0.006 to 0.107). Note that VO_{2max} was multiplied by -1 to indicate higher CVD risk with increasing value. Moreover, all body composition indices except height were positively associated with HOMA-IR index (boys, adjusted R^2 from 0.014 to 0.181 and girls, adjusted R^2 from 0.034 to 0.102). Table 3 shows that all body composition indices except height were positively associated with TC/HDLc (boys, adjusted R^2 from 0.007 to 0.106 and girls, adjusted R^2 from 0.015 to 0.078). In addition, all but height (both sexes) and the sum of four skinfolds (in girls) were positively associated with triglycerides (boys, adjusted R^2 from 0.015 to 0.074 and girls, adjusted R^2 from 0.011 to 0.022). Finally, and all but central/peripheral skinfolds (both sexes) and height (in girls) were positively associated with CRP (boys, adjusted R^2 from 0.006 to 0.078 and girls, adjusted R^2 from 0.007 to 0.045).

Body composition and clustered CVD risk factors

Table 4 shows that the top five body composition indices positively associated with clustered CVD risk were: the sum of four skinfolds, WHtR, BMI, WC and lean mass (adjusted R^2 from 0.182 to 0.523 in boys; adjusted R^2 from 0.167 to 0.388 in girls).

Table 5 shows that lean mass in girls (adjusted R^2 = 0.569) was positively associated with clustered CVD risk independently of the sum of four skinfolds (used as an index of BF) while the sum of four skinfolds was positively associated with clustered CVD risk independently of lean mass (adjusted R^2 = 0.729 and 0.785, boys and girls respectively).

Body composition-related thresholds associated with an unhealthier clustered CVD risk

Table 6 shows z-scores and specific thresholds for BMI, the sum of four skinfolds, WC, WHtR, and lean mass associated with an unhealthier clustered CVD risk in boys and girls (ROC curve analyses). In boys, highly accurate thresholds associated with an unhealthier clustered CVD risk were found for the sum of four skinfolds (≥68.5 mm; AUC=0.973), WHtR (≥0.46 cm; AUC=0.96), BMI (≥24.2 kg/m^2; AUC=0.956) and WC (≥83.5 cm; AUC=0.951). In addition, this threshold was moderately accurate for lean mass (≥63.5 kg; AUC=0.822) (all p<0.001). In girls, moderately accurate thresholds associated with an unhealthier clustered CVD risk were found for the sum of four skinfolds (≥76 mm; AUC=0.819), WHtR (≥0.43 cm; AUC=0.854), BMI (≥24.3 kg/m^2; AUC=0.816), WC (≥80.9 cm; AUC=0.817) and lean mass (≥46.1 kg; AUC=0.773) (all p<0.001).

DISCUSSION:

The main findings of the present study indicate that: i) the majority of the assessed body composition indices are associated with single and clustered CVD risk in adolescents, ii) the sum of four skinfolds, BMI, WHtR, WC and lean mass are the strongest indices associated with clustered CVD risk, iii) lean mass, independently of the sum of four skinfolds (index of BF), is a significant contributing factor of clustered CVD risk in girls, iv) moderate to highly accurate specific thresholds for the sum of four skinfolds, BMI, WHtR, WC and lean mass are associated with an unhealthier clustered CVD risk in European adolescents.

Body composition and single CVD risk factors

In the present study, most body composition indices but specially BMI, WHtR, WC and the sum of four skinfolds are positively associated with the single CVD risk factors herein included. Controversial results have been published in this regard. Data in adolescent populations have shown that measurements of WC or fat mass are not stronger than BMI in identifying associations with CVD risk factors (i.e. SBP and diastolic BP, insulin, triglycerides fasting glucose, high low density lipoprotein cholesterol and low HDLc) [25]. In this line, changes in age-specific BMI percentile from childhood to adulthood and pediatric metabolic syndrome were found to be predictors of CVD [26]. Some studies have shown WC and WHR (but not BMI) to be linked to an increased risk of CVD mortality [27]; whereas others have shown a similar association using BMI, WC, WHR and WHtR [28]. In addition, both BMI and the sum of skinfold thicknesses have been shown to similarly identify children and adolescents at metabolic risk [29].

Body composition and clustered CVD risk

Our results show that the sum of four skinfolds, BMI, WHtR, WC and lean mass are the body composition indices more strongly associated with clustered CVD risk in boys and girls. The strength of the associations was quite similar among the body composition indices but it was higher in boys than girls. Our results agree with previous studies showing that measurements related to body circumferences (i.e. WC and WHR) were more related to clustered CVD risk than measurements related to body lengths (i.e. height). In this regard, height was not associated to clustered CVD risk in our study. WC, that reflects the amount of abdominal fat, has been shown as a good tool for the screening of metabolic syndrome in children [30]. In addition, elevated skinfold thickness and WC measures in childhood have been found as the

strongest predictors of metabolic syndrome in early adulthood [5], suggesting tracking of adiposity status [31].

It is known that there is collinearity between lean mass and BF and therefore, overweight and obese adolescents have more lean mass than their normoweight peers [32]. The literature has consistently omitted the possibility that lean mass may be a significant contributing factor to clustered CVD risk independently of BF. In our study, we examined the association between lean mass and BF with clustered CVD risk after accounting for each other. On one hand, our results showed that the sum of 4 skinfolds (as an index of BF) was positively associated to clustered CVD risk in both sexes after accounting for lean mass. These findings support the notion of keeping BF to low levels to reduce the risk of CVDs. On the other hand and interestingly, lean mass was positively associated with clustered CVD risk in girls after accounting for BF while no association was found in boys, suggesting that lean mass might have a more protective factor for clustered CVD risk in boys than in girls. This positive association between lean mass and clustered CVD risk helps to explain why an index such as BMI (which includes both fat and lean mass) has been widely related to CVD risk, in spite of the surrounding controversy about its use as an index of adiposity. A possible physiological approach to this positive association may be related to the fact that a higher lean mass leads to a higher circulating blood volume, increasing the left ventricular (LV) stroke volume and, as a consequence, cardiac output. Previous studies have shown that lean mass explains a much larger proportion of the variance of cardiac output (33% vs. 3%) and stroke volume (49% vs. 2%) than does fat mass [33], which relates to the fact that lean mass is a tissue with much higher metabolic demand than BF [34]. As a consequence of these changes, there may be LV alterations that may lead to LV hypertrophy [35], a condition whose risk is increased in individuals with an excess of body weight and women, which may also explain our sex differences. This finding is of importance to pediatric research since age-related increases in

body size during youth mainly reflect increases in lean mass rather than BF [36,37]. In this line, Brion et al. [6] found a positive association between blood pressure (a single CVD risk factor) and lean mass after accounting for BF. To the best of our knowledge, this is the first study that shows this association convincingly and using a large sample. However, future studies with a longitudinal design are needed to confirm our findings.

Body composition-related thresholds and unhealthier clustered CVD risk

BMI, WHtR and WC are all body composition indices that include lean mass and have been used worldwide in epidemiologic studies. In the present study, the sum of four skinfolds, BMI, WHtR, WC and lean mass showed strong and positive associations with clustered CVD risk (that includes inflammation). Moderate to highly accurate thresholds in boys (all AUCs > 0.822) and moderately accurate thresholds in girls (all AUCs > 0.773) associated with an unhealthier clustered CVD risk were found. Interestingly, our BMI threshold (24.2 and 24.3 kg/m^2, boys and girls respectively) is very similar to the one provided by Cole et al. for 15 yr old overweight boys and girls (23.3 and 23.9 kg/m^2) [38]. In our study, the mean age of the participants was 14.8 yr. Cole et al. reported BMI values for 15 yr old obese boys and girls of 28.3 and 29.1 kg/m^2 respectively, which are much higher than our BMI thresholds. This comparison suggests that slightly overweight adolescents already have an unhealthier clustered CVD risk. Neovius et al. [39] in their study with adolescents aged 17 years observed that the BMI, percentage body fat and WC weakly identified individual CVD risk factors (i.e. adverse insulin and lipid profile). However, the use of a cluster was more accurate (AUCs 0.76-0.91). Freedman et al. [40] in their study with children aged 5-17 years observed a similar prediction of clustered CVD risk between BMIz and WHtR (AUCs 0.85 and 0.86, respectively). Sardinha et al. [41] in their study with children and adolescents aged 10-15 years observed that BMI and triceps skinfold thickness accurately predicted obesity (AUCs ranged

from 0.89 to 0.96 for girls and 0.61 to 0.98 for boys). Similarly, Sarria et al. [42] in their study with children and adolescents aged 7-16.9 years observed that triceps skinfold, BMI and WC accurately predicted adiposity (AUCs 0.90, 0.88 and 0.86, respectively).

The validity of BMI as a measure of BF has been extensively questioned. Freedman et al. [43] observed that a high BMI-for-age was associated with a moderately high (70-80%) sensitivity and positive predictive value, along with a high specificity (95%) in identifying an excess of BF in children. In Chinese adolescents, Ng et al. obtained BMI and WC thresholds that predict clustered CVD risk, obtaining similar AUCs than ours in girls (0.85 and 0.82, respectively) but lower AUCs in boys (0.76 and 0.78, respectively) [44]. Differences between studies may be due to methodological differences in data collection or even in data preparation (i.e. use of z-scores). In addition, our CVD risk score includes CRP [9], which may strengthen the associations.

Limitations and strengths

The main limitation of this study is its cross-sectional design that precludes determining any causality in the findings. The present study has, however, several strengths: the main one is the thorough standardization of the methods and collection of data throughout all the cities involved. In addition, a large number of body composition indices (including lean mass) as well as CVD risk factors (including an inflammatory marker such as CRP) have been considered.

CONCLUSIONS

Our results support an association between most of the assessed body composition indices and single and clustered CVD risk factors in European adolescents. In addition, lean mass is positively associated with clustered CVD risk independently of BF in girls, which is a novel

finding. Finally, moderate to highly accurate specific thresholds associated with an unhealthier clustered CVD risk are provided for the sum of four skinfolds, WHtR, BMI, WC and lean mass in boys and girls.

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Table 1. Descriptive characteristics of the population sample.

	Whole group n=1089		**Boys n=509**		**Girls n=580**		P
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	
Age (years)	14.8	1.2	14.8	1.2	14.8	1.2	.66
Weight (kg)	58.7	12.7	62	14.3	55.9	10.2	< ***.001***
Height (cm)	165.4	9.3	169.6	9.9	161.7	7	< ***.001***
BMI (kg/m^2)	21.4	3.7	21.4	4	21.3	3.4	.61
Lean mass (kg)	46.2	8.6	51.7	9	41.5	4.6	< ***.001***
Sum of four skinfolds (mm) [a]	51.9	26.2	44.3	27.2	58.6	23.5	< ***.001***
Central / peripheral skinfolds (mm) [b]	0.3	0.2	0.3	0.2	0.2	0.1	< ***.001***
WC (cm)	72.4	8.6	74.4	9.1	70.7	7.8	< ***.001***
HC (cm)	91.7	8.7	90.4	9	92.8	8.3	< ***.001***
WHtR (cm)	0.4	0.1	0.4	0.1	0.4	0.1	.51
WHR (cm)	0.8	0.1	0.8	0.1	0.8	0.1	< ***.001***
SBP (mm Hg)	116.2	13.3	120.5	13.8	112.5	11.6	< ***.001***
$VO_{2\ max}$ (ml/kg/min)	42.3	10.9	44.4	7.9	36.4	5.5	< ***.001***
HOMA-IR index	2.3	1.9	2.4	2.2	2.3	1.6	.68
TC/HDL-c (mg/dL)	3	0.7	3	0.7	3	0.6	.61

Triglycerides (mg/dL)	69.1	35	64.4	31.8	73.3	37.1	< ***.001***
CRP (mg/L) (n=748)	1.1	3.9	1.6	5.4	0.9	1.7	***0.07***
Cardiovascular risk score	0.01	0.56	-0.15	4.12	0.23	3.74	.19

Results shown as mean ± SD (standard deviation)

[a] Sum of: biceps, triceps, subscapular, suprailiac

[b] (subscapular + suprailiac) / (biceps + triceps)

Sex differences (ANOVA) are shown in **bold** ($p < 0.05$)

BMI (body mass index), CRP (C-reactive protein), HC (hip circumference), HOMA-IR (homeostasis model assessment-insulin resistance), SBP (systolic blood pressure), TC/HDL-c (total cholesterol/ high-density lipoprotein cholesterol), $VO_{2\ max}$ (maximal oxygen consumption), WC (waist circumference), WHR (waist-to-hip ratio), WHtR (waist-to-height ratio)

Table 2. Linear regression analyses of systolic blood pressure, maximal oxygen consumption and HOMA-IR index as regards to body composition indices in European adolescents.

	Boys				Girls			
	Adjusted R^2	β	SE	P	Adjusted R^2	β	SE	P
Indep. variables	**SBP (z) [a] (n=1089)**							
Height (z)	0.037	0.193	0.98	< ***.001***	0.008	0.094	0.99	< ***.001***
BMI (z)	0.139	0.375	0.93	< ***.001***	0.11	0.333	0.94	< ***.001***
Lean mass (z)	0.161	0.436	0.92	< ***.001***	0.105	0.49	0.94	< ***.001***
Sum of four skinfolds (z) [b]	0.053	0.235	0.97	< ***.001***	0.033	0.184	0.98	< ***.001***
C/P skinfolds (z) [c]	0.026	0.163	0.98	< ***.001***	0.004	0.07	0.99	***.003***
WC (z)	0.121	0.35	0.94	< ***.001***	0.092	0.303	0.95	< ***.001***
WHtR (z)	0.077	0.28	0.96	< ***.001***	0.068	0.259	0.96	< ***.001***
WHR (z)	0.024	0.157	0.99	< ***.001***	0.013	0.117	0.98	< ***.001***
	$VO_{2\ max}$ (z) [a] (n=1089)							
Height (z)	0.002	-0.049	0.99	.07	0.006	-0.08	0.99	***.002***
BMI (z)	0.173	0.419	0.91	< ***.001***	0.107	0.338	0.94	< ***.001***
Lean mass (z)	0.046	0.239	0.98	< ***.001***	0.019	0.206	0.99	< ***.001***
Sum of four skinfolds (z) [b]	0.156	0.396	0.92	< ***.001***	0.106	0.329	0.94	< ***.001***
C/P skinfolds (z) [c]	0.022	0.149	0.99	< ***.001***	0.02	0.145	0.99	< ***.001***
WC (z)	0.143	0.385	0.93	< ***.001***	0.069	0.264	0.97	< ***.001***
WHtR (z)	0.161	0.408	0.92	< ***.001***	0.084	0.297	0.96	< ***.001***
WHR (z)	0.049	0.222	0.98	< ***.001***	0.006	0.083	0.99	***.001***
	HOMA-IR index (z) [a] (n=1089)							
Height (z)	0.004	0.077	0.99	.08	-0.002	0.009	0.99	.83
BMI (z)	0.181	0.437	0.9	< ***.001***	0.089	0.315	0.95	< ***.001***
Lean mass (z)	0.112	0.363	0.94	< ***.001***	0.046	0.333	0.98	< ***.001***
Sum of four skinfolds (z) [b]	0.136	0.364	0.93	< ***.001***	0.079	0.296	0.96	< ***.001***
C/P skinfolds (z) [c]	0.016	0.049	0.99	< ***.001***	0.047	0.22	0.98	< ***.001***
WC (z)	0.149	0.407	0.92	< ***.001***	0.102	0.324	0.95	< ***.001***

WHtR (z)	0.131	0.384	0.93	< ***.001***	0.094	0.315	0.95	< ***.001***
WHR (z)	0.014	0.135	0.99	***.005***	0.034	0.173	0.99	< ***.001***

Significant results are in **bold**.

β is the estimated standardized regression coefficient; SE, standard error.

[a] It was entered as dependent variable and each independent variable (i.e. z-scores of height, BMI, lean mass, sum of four skinfolds, central-peripheral skinfolds, WC, WHtR and WHR) were entered separately in different models.

[b] Sum of: biceps, triceps, subscapular, suprailiac

[c] Central / peripheral skinfolds ratio: (subscapular + suprailiac) / (biceps + triceps)

BMI (body mass index), HC (hip circumference), HOMA-IR (homeostasis model assessment-insulin resistance), SBP (systolic blood pressure), $VO_{2\ max}$ (maximal oxygen consumption), WC (waist circumference), WHR (waist-to-hip ratio), WHtR (waist-to-height ratio)

Table 3. Linear regression analyses of total cholesterol/ high-density lipoprotein cholesterol, triglycerides and C-reactive protein as regards to body composition indices in European adolescents.

	Boys				Girls			
	Adjusted R^2	β	SE	P	Adjusted R^2	β	SE	P
Indep. variables	**TC/HDLc (z) [a] (n=1089)**							
Height (z)	<0.001	-0.047	0.99	.23	0.003	-0.069	0.99	.09
BMI (z)	0.106	0.323	0.94	< ***.001***	0.068	0.274	0.96	< ***.001***
Lean mass (z)	0.031	0.192	0.97	< ***.001***	0.015	0.192	0.98	***.002***
Sum of four skinfolds (z) [b]	0.053	0.229	0.97	< ***.001***	0.046	0.227	0.97	< ***.001***
C/P skinfolds (z) [c]	0.007	0.082	0.96	***.04***	0.026	0.165	0.98	< ***.001***
WC (z)	0.079	0.29	0.93	< ***.001***	0.066	0.259	0.96	< ***.001***
WHtR (z)	0.091	0.311	0.92	< ***.001***	0.078	0.284	0.95	< ***.001***
WHR (z)	0.021	0.158	0.96	***.001***	0.03	0.162	0.98	< ***.001***
	Triglycerides (z) [a] (n=1089)							
Height (z)	-0.002	-0.012	0.99	.78	-0.002	0.015	0.99	.72
BMI (z)	0.072	0.267	0.96	< ***.001***	0.014	0.132	0.99	***.002***
Lean mass (z)	0.03	0.188	0.97	< ***.001***	0.016	0.199	0.99	***.002***
Sum of four skinfolds (z) [b]	0.044	0.209	0.97	< ***.001***	0.001	0.05	0.99	.25
C/P skinfolds (z) [c]	0.016	0.119	0.97	***.003***	0.021	0.149	0.99	< ***.001***
WC (z)	0.071	`0.279	0.94	< ***.001***	0.022	0.153	0.99	< ***.001***
WHtR (z)	0.074	0.286	0.94	< ***.001***	0.018	0.143	0.99	***.001***
WHR (z)	0.015	0.137	0.97	***.003***	0.011	0.104	0.99	***.008***
	CRP (z) [a] (n=748)							
Height (z)	0.006	-0.087	0.99	***.05***	0.001	-0.039	1	.35
BMI (z)	0.074	0.276	0.96	< ***.001***	0.045	0.231	0.98	< ***.001***
Lean mass (z)	0.008	0.107	0.99	**0.03**	0.013	0.183	0.99	***0.005***
Sum of four skinfolds (z) [b]	0.062	0.251	0.97	< ***.001***	0.045	0.23	0.98	< ***.001***
C/P skinfolds (z) [c]	-0.001	0.031	1	.48	-0.002	-0.018	1	.67

WC (z)	0.059	0.26	0.97	< ***.001***	0.033	0.19	0.98	< ***.001***
WHtR (z)	0.078	0.302	0.96	< ***.001***	0.039	0.21	0.98	< ***.001***
WHR (z)	0.049	0.247	0.97	< ***.001***	0.007	0.095	0.99	***.04***

Significant results are in **bold**.

β is the estimated standardized regression coefficient; SE, standard error.

[a] It was entered as dependent variable and each independent variable (i.e. z-scores of height, BMI, lean mass, sum of four skinfolds, central-peripheral skinfolds, WC, WHtR and WHR) were entered separately in different models.

[b] Sum of: biceps, triceps, subscapular, suprailiac

[c] Central / peripheral skinfolds ratio: (subscapular + suprailiac) / (biceps + triceps)

BMI (body mass index), CRP (C-reactive protein), HC (hip circumference), TC/HDL-c (total cholesterol/ high-density lipoprotein cholesterol), WC (waist circumference), WHR (waist-to-hip ratio), WHtR (waist-to-height ratio)

Table 4. Linear regression analyses of the cardiovascular risk score [sum of SBP(z), VO_{2max} (z), HOMA-IR index (z), CRP (z), TC/HDLc (z) and triglycerides (z)] as regards to body composition indices in European adolescents.

	Boys				Girls			
	Adjusted R^2	β	SE	P	Adjusted R^2	β	SE	P
	Cardiovascular risk score [a] (n=748)							
Height (z)	-0.003	-0.038	4.12	.86	-0.002	0.1	3.74	.58
BMI (z)	0.523	3.122	2.84	**< *.001***	0.388	2.468	2.93	**< *.001***
Lean mass (z)	0.182	1.947	3.7	**< *.001***	0.167	2.262	3.42	**< *.001***
Sum of four skinfolds (z) [b]	0.589	3.15	2.64	**< *.001***	0.405	2.596	2.88	**< *.001***
C/P skinfolds (z) [c]	0.025	0.669	4.06	***.001***	0.072	0.984	3.6	**< *.001***
WC (z)	0.47	3.009	2.99	**< *.001***	0.348	2.166	3.02	**< *.001***
WHtR (z)	0.497	3.138	2.92	**< *.001***	0.339	2.208	3.04	**< *.001***
WHR (z)	0.158	1.795	3.78	**< *.001***	0.118	1.268	3.5	**< *.001***

Significant results are in **bold**.

β is the estimated standardized regression coefficient; SE, standard error.

[a] It was entered as dependent variable and each independent variable (i.e. z-scores of height, BMI, lean mass, sum of four skinfolds, central-peripheral skinfolds, WC, WHtR and WHR) were entered separately in different models.

[b] Sum of: biceps, triceps, subscapular, suprailiac

[c] Central / peripheral skinfolds ratio: (subscapular + suprailiac) / (biceps + triceps)

BMI (body mass index), HC (hip circumference), TC/HDL-c (total cholesterol/ high-density lipoprotein cholesterol), WC (waist circumference), WHR (waist-to-hip ratio), WHtR (waist-to-height ratio)

Table 5. Linear regression analyses of cardiovascular risk score [sum of SBP(z), VO_{2max} (z), HOMA-IR index (z), CRP (z), TC/HDLc (z) and triglycerides (z)] in European adolescents participating in the HELENA study as regards to lean mass and sum of 4 skinfolds.

	Boys				Girls			
	Adjusted R^2	β	SE	P	Adjusted R^2	β	SE	P
Independent variables	**Cardiovascular risk score [a] (n=748)**							
Lean mass (z)	0.336	0.061	0. 013	.65	0.569	0.378	0.015	***.02***
Sum of four skinfolds (z) [b]	0.729	1.366	0.013	< ***.001***	0.785	0.346	0. 011	< ***.001***

Significant results are in **bold**

β is the estimated standardized regression coefficient; SE, standard error

CRP (C-reactive protein), HOMA-IR (homeostasis model assessment-insulin resistance), SBP (systolic blood pressure), TC/HDL-c (total cholesterol/ high-density lipoprotein cholesterol), $VO_{2\,max}$ (maximal oxygen consumption)

[a] These analyses were adjusted by the residuals of the regression between lean and sum 4 skinfolds

[b] Sum of: biceps, triceps, subscapular, suprailiac

Table 6. Body composition indices-related thresholds associated with an unhealthier clustered CVD risk in European adolescents participating in the HELENA study

	BOYS (n=365)						GIRLS (n=383)					
Body composition indices	**z-score[a]**	**Threshold[b]**	**AUC**	**95% CI**	**Sens.**	**Spec.**	**z-score[a]**	**Threshold[b]**	**AUC**	**95% CI**	**Sens.**	**Spec.**
Body mass index	0.795	24.2	0.956	0.923-0.989	0.913	0.904	0.937	24.3	0.816	0.656-0.973	0.769	0.878
Sum of four skinfolds [c]	0.852	68.5	0.973	0.956-0.989	1.000	0.880	0.764	76.0	0.819	0.7-0.938	0.769	0.792
Waist circumference	1.057	83.5	0.951	0.91-0.992	0.913	0.915	1.340	80.9	0.817	0.665-0.97	0.692	0.924
Waist-to-height ratio	0.474	0.46	0.960	0.933- 0.987	0.955	0.860	0.467	0.43	0.854	0.702-0.949	0.750	0.819
Lean mass	1.365	63.5	0.822	0.717– 0.927	0.727	0.909	1.034	46.1	0.773	0.594- 0.953	0.692	0.900

[a] Z-score represents a specific threshold for having or not clustered CVD risk **(*all p< .001*)**

[b] The threshold below which adolescents present a healthier clustered CVD risk. This threshold was obtained using the equation: z = (value-mean) / SD

[c] Sum of: biceps, triceps, subscapular, suprailiac

AUC (Area Under the Curve), CI (Confidence Intervals), Sens (sensitivity), Spec (specificity)