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Neck circumference as an emergent predictor for clustered cardiovascular risk factors in children and adolescents

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ABSTRACT

Objective: Early detection of cardiovascular disease (CVD) risk factors, such as obesity, is crucial in order to prevent adverse long-term effects on individuals' health. Therefore, we aimed: (i) to examine the association of a number of body composition indices (including neck circumference (NC)) with single and clustered CVD risk factors, and (ii) to release gender-age-specific NC cut-off values to classify youths as overweight/obese.

Design: Cross-sectional study.

Setting: 23 primary schools and 17 secondary schools from Spain.

Participants: Grade 1-4 and 7-10 students

Measures: Pubertal development, body composition indices, systolic and diastolic blood pressure (SBP and DBP, respectively), cardiorespiratory fitness, blood sampling (triglycerides (TG), total-cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), glucose and inflammatory markers. Homeostasis model assessment (HOMA-IR), and cluster of CVD risk factors were calculated.

Results: NC was positively correlated with all body composition indices. NC was negatively associated with $VO2_{max}$ (R^2=0.231, p<0.001 for boys; R^2=0.018, p<0.001 for girls), and positively associated with SBP, DBP, TC/HDL-c, TG, HOMA, complement factors C-3 and C-4, leptin, adiponectin and clustered CVD risk factor in both genders (R^2 from 0.035 to 0.353, p<0.01 for boys; R^2 from 0.024 to 0.215, p<0.001 for girls). Moreover, NC was positively associated with serum C-reactive protein, LDL-c and visfatin only in boys (R^2 from 0.013 to 0.107, p<0.05).

Conclusion: NC is a simple, low-cost and practical screening tool of excess of upper-body obesity and CVD risk factors in children and adolescents. Pediatricians can easily use it as a preventative method for overweight/obesity in children and adolescents. For

this purpose, gender-and-age specific thresholds to classify children and adolescents as normalweight or overweight/obese are provided.

Keywords: Body composition, neck circumference, cardiometabolic risk and cardiovascular disease, inflammatory markers, youth.

Strengths and limitations of this study

- The use of a large number of body composition indices, as well as cardiometabolic risk factors and inflammatory markers is a strength of this study. To the best of our knowledge, no studies have been published analysing the associations between NC with inflammatory markers in children and adolescents.
- The study has important implications for early identification of overweight/obesity in children and adolescents using a simple and ease alternative such as NC.
- The study sample consisted of children and adolescents with different weight status, thus broadening the applicability of the findings. Overweight and obesity levels in our sample were comparable to those in the general population of children and adolescents of this age group in Spain
- The cross-sectional nature of the study limits the establishment of causality.
- The study group consisted of a single ethnic group (white Caucasican). Therefore, the cutoff values herein provided may not be valid for other ethnic groups.
- Finally, more accurate measures of overweight/obesity (e.g. those obtained with magnetic resonance imaging or dual energy X ray absorptiometry) might be used to assess their association with NC. However, this is not so feasible in studies with a relatively large sample of participants.

BACKGROUND

Obesity is a disease affecting people of all ages, gender, ethnicities, and socioeconomic levels [1] with serious public health implications. Particularly, overweight and obesity in children and adolescents are a pandemic worldwide, affecting not only developed but also developing countries [2]. Excess body fat in children and adolescents is associated with cardiovascular-related risk factors such as type II diabetes, insulin resistance, hypertension, elevated triglyceride and cholesterol levels [3]. The presence of cardiovascular disease (CVD) risk factors in childhood is associated with premature death in adulthood [4]. Consequently, early detection of CVD risk factors, such as obesity, is crucial in order to prevent adverse long-term effects on individuals' health [5].

Practical and simple body composition indices such as body mass index (BMI), waist circumference (WC), waist-to-hip ratio, waist-to-height ratio (WHtR) and body fat percentage (BFP) are widely used as indices of total and central obesity. Indeed, some of these indices have been taken into account in the ALPHA health-related fitness test battery, which is nowadays the most relevant health-related fitness test battery for children and adolescents [6].

Recent researchers have proposed the neck circumference (NC) as another screening tool of CVD risk factors (i.e. obesity) in children and adolescents [7-11]. However, these studies examine this association through single cardio-metabolic risk factors (i.e. insulin resistance, hypertension, and hyperlipidemia (triglyceride and cholesterol). Clustering of CVD risk factors seems to be a much stronger measure of cardiovascular health in children and adolescents than single risk factors, as a subject with cardiovascular risk may reflect high levels of several risk factors simultaneously [12]. Additionally, inflammatory markers have recently received close attention and are considered "emerging CVD risk factors" [13]. Previous studies have identified some inflammatory markers (such as complement factors C-3 and C-4, C-reactive protein, adiponectin,

leptin, interleukin 6, tumor necrosis factor alpha and visfatin) to play a key role in CVD development in children [14], adolescents [14] and adults [15]. However, whether NC is associated with inflammatory markers in children and adolescents is still unknown in spite of its clinical relevance as so it is the potential usefulness of NC as a predictor for CVD risk factors.

Therefore, we aimed: (i) to examine the association of a number of body composition indices (including NC) with single and clustered CVD risk factors (including inflammatory markers), and (ii) to release gender-age-specific NC cut-off values to classify youths as overweight/obese, based on prediction equations using BMI, WC and BFP.

METHODS

Study design, setting and participants

Participants selected for this study cross-sectional were enrolled in the UP&DOWN study [16]. In brief, the UP&DOWN study was a 3-year longitudinal study designed to assess the impact of physical activity and sedentary behaviors over time on health indicators as well as to identify the psycho-environmental and genetic determinants of physical activity in a Spanish sample of children and adolescents. Data from the present study were collected from September 2011 to June 2012. Children and adolescents were recruited from schools in Cádiz and Madrid, respectively. A total of 2,225 participants aged 6 to 18 years participated in the UP&DOWN study although the present study includes 2,198 participants (1060 girls) with complete data at baseline on body composition indices. Blood sampling was randomly performed in one-fourth of the recruited children and adolescents (514; 244 girls).

Parents and school supervisors were informed by letter about the nature and purpose of the study, and written informed consent was provided. The study protocols were 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).

Measurements

Pubertal development

After a brief visual observation, the participants self-classified in one of the five stages of pubertal development according to Tanner & Whitehouse [17]. To do this, breast development in girls and genital development in boys was used.

Body composition indices

Harmonization and standardization of body composition indices used to assess body composition in the UP&DOWN study were strictly controlled [16]. Anthropometric data included body weight, height, waist and NC, and triceps and subscapular skinfold thicknesses. Weight was measured with an electronic scale (Type SECA 861; range, 0.05 to 130 kg; precision, 0.05 kg), and height was measured in the Frankfort plane with a telescopic stature-measuring instrument (Type SECA 225; range, 60 to 200 cm; precision, 1 mm). WC was measured with a non-elastic tape (SECA 200; range, 0 to 150 cm; precision, 1 mm), at the level of the natural waist, in a horizontal plane, which is the narrowest part of the torso, as seen from a front view. NC was assessed with the participants standing in an erect position, hanging their arms freely and keeping their head aligned in the Frankfort horizontal plane. The superior border of a non-elastic tape measure (SECA 200; range, 0 to 150 cm; precision, 1 mm) was placed just below the laryngeal prominence and applied perpendicular to the long axis of the neck [18]. Triceps and subscapular skinfold thickness were measured 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 [19]. The measurements were carried out twice, but not consecutively, and the mean value of the two measurements was used in the analyses. BFP was calculated from triceps and subscapular skinfold thicknesses by using the equations developed by Slaughter et al. [20].

BMI was calculated as weight/height squared (kg/m^2) and participants were categorized as underweight/normalweight and overweight/obese following international cut-off points [21]. WHtR was computed from the original body composition index (waist/height). Fat mass index (FMI) was computed dividing BFP by height squared (m^2).

Blood pressure

Systolic and diastolic blood pressure (SBP and DBP, respectively) were measured by 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 [22]. The participants sat on a chair quietly for 5 minutes before the measurements were conducted on the left arm in an extended position. Two measures were taken 1-2 minutes apart. An additional measurement was performed if the first two readings differed in > 5 mm Hg, and the farthest value was removed. Average values (mm Hg) were calculated separately for SBP and DBP.

Cardiorespiratory fitness

20-m shuttle run test was used to assess cardiorespiratory fitness, according to the ALPHA health-related fitness test battery protocol [6]. The equation reported by Léger et al. [23] was used to estimate the maximum oxygen consumption (VO_{2max}, ml/kg/min).

Blood sampling

A fasting blood sample was obtained from the cubital vein early in the morning at the schools. 13.5 ml of blood were drawn from each subject, and 3.5 ml of them (anticoagulated blood in EDTA) were analyzed to obtain haemogram data. The remainder blood (dried gel and sodium citrate) was centrifuged; serum and plasma were removed and then frozen at -80º C to be analyzed later. Serum lipid triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), glucose and total proteins as CVD risk factors were analyzed by enzymatic colorimetric methods (Olympus AU2700 Analyzer; Olympus UK Ltd, Watford, UK).

The following inflammatory markers were analysed by turbidimetry (Olympus AU2700 Analyzer; Olympus UK Ltd, Watford, UK): serum complement factors C-3 (C3) C-4 (C4) and serum C-reactive protein (CRP). The coefficients of variation (inter-assay precision) were less than 2% for all proteins (1.39% for C3, 1.19% for C4 and 1.90% for CRP). Detection limits (sensitivity) for the analyses were 0.01 g/L for C3, 0.002 g/L for C4 and 0.007 mg/L for CRP. Adiponectin, leptin, interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) were quantified by multiple analyte profiling (xMAP) technology (xMAP Techonology; Luminex, Austin, TX, USA) using a kit (5+1) plex: 171-A7003M Bio-plex Pro Human Diabetes Adiponectin Assay; YB0000002Y Bio-Plex Human Diabetes 3-Plex Assay; 171D50001 Bio-Plex Human Cytokine Stds; 171B5006M Bio-Plex Human IL-6 set; 171B5026M Bio-Plex Human TNF-alpha set. Visfatin values were determined by enzyme-linked immunoSobent assay (ELISA) (Human visfatin Elisa kit; Cusabio Biotech, Wuhan, Hubei, China). Sensitivity for the analyses were 0.156 ng/ml, the coefficients of variation were less than 8% for intra-assay precision and less than 10% for inter-assay precision.

Insulin resistance was calculated through the homeostasis model assessment (HOMA) score as: [insulin (mLU/mL) X glucose (mmol/L)] / 22.5 [24].

Clustered CVD risk factors

A cluster of CVD risk factors by sex and age groups was constructed. For that purpose, we computed standardized normalized indices (z-score=[value-mean]/standard deviation) of the subsequent single CVD risk factors: metabolic syndrome risk factors (i.e. SBP, DBP, TC /HDL-c, and TG), HOMA, inflammatory markers (i.e. CRP, C-3, C-4, IL-6, leptin and adiponectin,) and VO_{2max}. The VO_{2max} z-score was inverted, since higher cardiorespiratory is associated with lower fatness. Finally, all the z-scores were

summed. LDL-c, TNF-α and visfatin were no included in the cluster because they did not show association with body composition indices.

Statistical analysis

Since body composition and cardiovascular markers are gender-dependent, boys and girls were analyzed separately. Descriptive data are shown as mean and standard deviation (SD) unless otherwise indicated. The Student's *t*-test was used to test differences in socio-demographic and clinical characteristics by gender, except for the Tanner stage, which was analyzed by χ^2 test. Pearson's correlation coefficients were obtained to analyze the association between NC and the other body composition indices (BMI, WC, WHtR, BFP and FMI), and the association between all body composition indices and single CVD risk factors. To test the association of single and cluster CVD risk factors with body composition indices we performed adjusted multiple linear regression analyses. Single and clustered CVD risk factors were entered as dependent variables and body composition indices as independent variables, in separate models.

To predict specific NC cut-off values for overweight and obesity, we performed a linear regression analysis following a stepwise selection procedure for the derivation of prediction equation models including NC as dependent variable and BMI, WC, and BFP as independent variables, in separate models. In the prediction equations, we used Cole's BMI [21], Katzmarzyk's WC [25] and McCarthy's BFP [26] cutoff values for overweight/obesity to obtain the corresponding NC cut-off values. These are optimal threshold values for predicting high-risk groups to be used in the white population worldwide. All models were controlled for age and Tanner stage. 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 statistical significance was set at $p<0.05$.

RESULTS

The characteristics of the study sample are presented in **table 1**. Boys presented higher values in Tanner stage, weight, height, NC, WC, WHtR, VO_{2max} and SBP than girls (all, $p<0.05$). However, BFP, FMI, DBP, HOMA, leptin and adiponectin were significantly higher in girls compared to boys (all, $p<0.05$). 32.7%, 38.7%, 16% and 37% of insulin, C-reactive protein, TNF- α and visfatin sample, respectively, were below detection threshold and were not included in the final analyses.

Table 2 shows correlation coefficients between NC and BMI, WC, WHtR, BFP and FMI by gender. NC was positively correlated with all body composition indices in both, boys and girls (all, $p\leq0.001$). Correlations were stronger with WC ($r=0.864$ in boys, $r=0.851$ in girls) and BMI ($r=0.754$ in boys, $r=0.799$ in girls).

Table 3 shows bivariate correlations between all body composition indices and CVD risk factors by gender. Overall, all body composition indices were correlated with most single CVD risk factors, with higher correlation coefficients in boys than girls (all, $p \leq0.05$). More specifically, NC was negatively correlated with VO_{2max} ($r = -0.481$, $p<0.001$ for boys; $r = -0.672$, $p<0.001$ for girls), positively correlated with SBP, DBP, TG, HOMA, C-3, C-4, leptin and adiponectin in both genders (r from 0.167 to 0.419; all, $p<0.01$), and with TC/HDL-c, LDL-c, CRP and visfatin only in boys (r from 0.243 to 0.388; all, $p<0.001$).

The associations between neck circumference and single and clustered CVD risk factors are presented in **table 4**. NC was negatively associated with $VO2_{max}$ ($R^2 = 0.231$, $p<0.001$ for boys; $R^2 = 0.018$, $p<0.001$ for girls), and positively associated with SBP, DBP, TC/HDL-c, TG, HOMA, C-3, C-4, leptin, adiponectin and clustered CVD risk factor in both genders (R^2 from 0.035 to 0.353, $p<0.01$ for boys; R^2 from 0.024 to 0.215, $p<0.001$ for girls). Moreover, NC was positively associated with CRP, LDL-c

and visfatin only in boys (R^2 from 0.013 to 0.107, $p<0.05$). Overall, the association of CVD risk factors with the other body composition indices presented similar results (see **supplementary tables** 1, 2, 3 and 4). For sensitivity-analysis purpose, we included those participants with values below the detection levels by assigning them the lowest value of the detection threshold. These new variables (i.e. insulin, C-reactive protein, TNF- α and visfatin) were natural log transformed and the analyses were repeated. The associations aforementioned remained unchanged (data not shown).

Stepwise regression analyses were conducted to get specific NC cutoff values for overweight/obesity based on BMI, WC and BFP values (**table 5**). Results showed very similar NC cutoff values for boys and girls with the same age, independently of the criteria used to classify children and adolescents as normalweight or overweight/obese. Moreover, cutoff values increase with age and are higher in boys than girls. The prediction equations for NC cutoff values are presented below:

Neck circumference = 13.631 + 0.466 x BMI + 0.668 x age (R^2 = 0.864 and SEE = 1.439) for boys.

Neck circumference = 15.656 + 0.452 x BMI + 0.365 x age (R^2 = 0.796 and SEE = 1.428) for girls.

Neck circumference = 10.735 + 0.210 x WC + 0.520 x age (R^2 = 0.877 and SEE = 1.367) for boys.

Neck circumference = 11.825 + 0.212 x WC + 0.315 x age (R^2 = 0.825 and SEE = 1.189) for girls.

Neck circumference = 18.364 + 0.106 x BFP + 0.872 x age (R^2 = 0.798 and SEE = 1.729) for boys.

Neck circumference = 19.107 + 0.172 x BFP + 0.480 x age (R^2 = 0.726 and SEE = 1.484) for girls.

DISCUSSION

The main findings indicate that: i) NC is strong and positively associated with well-known indices of body composition, such as BMI, WC, BFP, WHtR and FMI; ii) NC is one of the strongest indices associated with single and clustered CVD risk factors; iii) gender-and-age specific NC cutoff values are provided to classify children and adolescents as normalweight or overweight/obese.

Comparison of NC with well-known body composition indices

BMI is the most common body composition index used to determine overweight and obesity not only in children and adolescents but also in adults. BMI has become a very popular screening tool for overweight and obesity due to its simplicity and ease. However, it is becoming increasingly clear that it is not a good proxy for regional adiposity [27]. Regional deposition of fat is a better predictor of some obesity related complications, such as metabolic disorders and CVD risk factors [28]. In this regard, WC seems to be a better body composition index in children and adolescents [29]. On the other hand, WHtR [30] and FMI [31] have been proposed as a marker of adiposity in children and adolescents, however, these indices have been built in analogy with BMI.

Recently, NC has been proposed as a surrogate marker of regional obesity in children and adolescents [7 8]. These studies reported strong correlations between NC and BMI and WC ($r > 0.7$). Concurring with these studies, we found that NC was correlated with a larger number of body composition indices (i.e. BMI, WC, WHtR, BFP and FMI). More specifically, NC was highly correlated with WC and BMI and in both genders, which confirms its use as a screening tool for identifying overweight/obese children and adolescents. NC is a simple, quick and low-cost method that requires less effort from both the examiner and the examinee than other body composition methods. NC has shown very good inter- and intra-rater reliability [32] and unlike WC, NC is not influenced

by the timing of measurement (e.g. pre-prandial and postprandial period; measurement at the end of a gentle expiration as suggested for WC). Additionally, this technique does not require the patient to remove his/her clothing in order to obtain a more accurate measurement. Finally, the measurement of NC may be more socially acceptable and convenient for overweight and obese children, thus making this measurement more tolerable for them.

NC and single CVD risk factors

There is evidence to support that NC is a screening tool for identifying CVD in adults in worldwide [33]. However, little is known about the association between NC and CVD risk factors in children and adolescents. In our study, we found that NC, as well as all other body composition indices, was associated with cardio-metabolic syndrome risk factors. Androutsos et al. [10] and Kurtoglu et al. [9] also observed that increases in NC were associated with cardio-metabolic syndrome risk factors in Greek and Turkish normal or overweight/obese children and adolescents. Moreover, Guo et al. [34] showed that NC could predict pre-hypertension in Chinese normal-weight children and adolescents, but not in those overweight and obese. It is widely accepted that obesity is associated with metabolic disorders and CVD risk factors both in youth and adulthood [35]. Free fatty acid concentration is related with the development of CVD risk factors. It has been demonstrated that upper-body subcutaneous fat is responsible for a much larger proportion of systemic free fatty acid release than visceral fat, particularly in obese individuals [36]. Moreover, The Framingham Heart Study showed that NC was associated with CVD risk factors even after adjustment for visceral adipose tissue and BMI, suggesting that upper-body subcutaneous fat may be a unique fat depot conferring additional cardiovascular risk above and beyond central body fat [37]. These previous

results are in line with ours suggesting that thicker NC is associated with greater risk of CVD.

Inflammatory markers have been proposed as new emerging CVD risk factors [38]. Indeed, persistent low-grade inflammation in children, especially in obese, may increase the risk of metabolic and cardiovascular events in later life and play a role in the pathogenesis of atherosclerosis [39]. Thus, inflammatory markers have a potential interest for pediatric CVD risk factor control and future preventive strategies. Overall, obesity in children and adolescents is associated with increased CRP, C-3, C-4, and leptin levels, and decreased adiponectin levels [39-41], which concurs with the results of the present study. To the best of our knowledge, no studies have been published analyzing the associations between NC and inflammatory markers in children and adolescents. In addition, data with WHtR, BFP and FMI are sparse. In the present study, similar associations were found among all body composition indices and inflammatory markers. There is controversy in the relationship between some inflammatory markers (i.e. plasma IL-6, TNF-α and visfatin) and obesity, with some studies reporting positive [40 42] or no associations [40 41] with obesity. In our study, IL-6 and visfatin were associated with WHtR and FMI in both genders, and only with NC in boys. However, no associations among all body composition indices and TNF- α. Differences among studies might be due, as Jaalel et al. [40] suggested, to differences in degrees of maturation, gender distribution and ethnic specificity. Further studies are needed to clarify this association.

NC and clustered CVD risk factors

A clustered CVD risk factors reflects cardiovascular health better than single CVD risk factors, and might, to some extent, compensate for the day-to-day fluctuations in the single risk factors [43]. Even if none of the participants suffer from clinical disease,

clustered risk is certainly an undesirable condition, and it has been shown to track into young adulthood [44]. Our results showed that NC was associated with clustered CVD risk factors in boys and girls.

Previous studies found a strong association between some body composition indices and clustered CVD risk factors [11 14 43]. However, to the best of our knowledge this is the first study in examining the association between NC and a cluster of CVD risk factors, and also the first study in including a large number of inflammatory markers (i.e. CRP, C-3, C-4, IL-6, leptin and adiponectin) as part of the cluster, as recently suggested, due to its relation to clustering of CVD risk factors at early ages [38]. In addition, VO_{2max} was also included in the cluster as performed in previous studies [43] as it is considered a CVD risk factor in children and adolescents. Andersen et al. [43] previously followed this strategy and it is noteworthy that their findings were consistent with those of the present study.

Establishment of NC cutoffs to identify children and adolescents with overweight/obesity

Results from the present study showed that NC could be used as a screening tool to identify children and adolescents at CVD risk. Therefore, providing with gender-and-age specific cutoff values to classify children and adolescents as normalweight or overweight/obese is of relevance from a public health and clinical perspective. The criteria used to classify children and adolescents as normalweight or overweight /obese were based on BMI [21], WC [25], and BFP [26] widely accepted cutoff values in children and adolescents. The explained variances of the full models were high (86% for boys and 80% for girls with BMI; 88% for boys and 83% for girls with WC; and 80% for boys and 73% for girls with BFP), suggesting that NC cutoff values can be established according to BMI, WC and BFP values. Our results show NC cutoffs that increase with age and are higher in boys than girls. In addition, cutoffs for the same age are very

similar independently of the criteria used to classify children and adolescents as normalweight or overweight /obese. It is known that body fat distribution and its metabolic effects are different in men and women with sex hormones playing a role in this difference. However, a definite cause has not been established [45]. These results are consistent with those reported in other study based only in BMI cutoff values [7 8]. To note is that NC cutoff values from the current study were slightly lower than those of previous studies [7 8], which may be due to ethnic differences. To our knowledge, this is the first study that establishes NC cutoffs for overweight/obesity according to BMI, WC and BFP values in the same sample.

CONCLUSIONS

In conclusion, NC was positively associated with other body composition indices, single and clustered CVD risk factors. NC is a simple, low-cost and practical screening tool of excess of upper-body obesity and CVD risk factors in children and adolescents. Pediatricians can easily use it as a preventative method for overweight/obesity in children and adolescents. For this purpose, gender-and-age specific thresholds to classify children and adolescents as normalweight or overweight/obese are provided.

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Contributors JCP, ADA, LGM and VSJ contributed to the concept and design of the study. JCP, LGM and VSJ contributed to the analysis and interpretation of the data. JCP, ADA, and VSJ contributed to drafting the manuscript. JCP, ADA, LGM, SGM, IEC, OLV, AM and VSJ contributed to the conduct of the study and critically reviewd the manuscript. Final approval of the version to be published was obtained from each of the authors.

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Competing interests None declared.

Ethics approval The study protocol was carried out according to 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).

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Table 1. Descriptive characteristics of the population sample.

	Boys n=1138		Girls n=1060		P
	Mean	*SD*	*Mean*	*SD*	
Age (years)	10.8	3.4	10.9	3.3	0.449
Tanner stage (n (%))					
1	321 (27.6)		329 (30.4)		**0.010**
2	303 (26.1)		233 (21.4)		
3	195 (16.8)		250 (23.0)		
4	190 (16.3)		216 (19.9)		
5	129 (11.0)		32 (2.9)		
Weight (kg)	42.8	17.4	41.2	16.1	**0.018**
Height (cm)	144.8	19.9	142.8	17.0	**0.014**
Body mass index (kg/m^2)	19.5	3.7	19.5	3.6	0.895
Neck circumference (cm)	30.0	3.6	28.5	2.7	**<0.001**
Waist Circumference (cm)	64.7	9.9	62.2	8.6	**<0.001**
Waist-to-height ratio (cm)	0.5	0.0	0.4	0.0	**<0.001**
Body fat (%)	19.9	10.3	23.7	7.6	**<0.001**
Fat mass index	9.9	5.1	11.8	3.9	**<0.001**
$VO_{2\,max}$ (ml/kg/min)	38.4	6.7	32.3	7.7	**<0.001**
Systolic blood pressure (mm Hg)	106.7	13.1	103,5	11.1	**<0.001**
Diastolic blood pressure (mm Hg)	65.6	8.1	67.0	8.7	**<0.001**
	Boys n=270		**Girls n=244**		P
TC/HDL-c (mg/dL)	2.9	0.6	3.0	0.7	0.214
LDL-c (mg/dL)	70.9	23.4	73.4	23.4	0.221
Triglycerides (mg/dL)	48.1	30.7	52.5	25.5	0.085
Insulin resistance (HOMA score) *	2.1	2.1	2.5	2.5	**0.047**
C-reactive protein (mg/dL) *	8.8	20.5	9.9	23.1	0.828
C-3 protein (mg/dL)	93.4	31.0	93.7	28.7	0.903

C-4 protein (mg/dL)	20.3	9.9	20.4	9.8	0.944
Interleukin-6 (pg/mL)	34.4	37.5	43.0	62.1	0.061
Leptin (ng/mL)	6.7	6.7	11.7	9.2	**<0.001**
Adiponectin (µg/mL)	13.3	7.2	14.8	7.5	**0.023**
TNF- α (pg/mL)*	77.1	66.2	72.8	57.1	0.445
Visfatin (ng/mL)*	1.5	1.3	1.4	1.2	0.589
Cardiovascular risk score	0.05	1.0	-0.07	1.0	0.269

Results are showed as mean ± SD (standard deviation).

VO_{2max} indicates maximum oxygen consumption; TC/HDL, total cholesterol/ high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA, homeostasis model assessment; TNF- α, tumor necrosis factor alpha.

*32.7%, 38.7%, 16% and 37% of insulin, C-reactive protein, TNF- α and visfatin sample, respectively, were below detection threshold and were not included in the analyses.

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

Table 2. Correlations coefficients of neck circumference with BMI, WC, WtHR, BFP and FMI.

	Neck Circumference			
	Boys (n= 1138)		Girls (n= 1060)	
	r	P	r	P
BMI (kg/m^2)	0.754	**<0.001**	0.799	**<0.001**
WC (cm)	0.864	**<0.001**	0.851	**<0.001**
WHtR (cm)	0.610	**0.001**	0.621	**<0.001**
BFP (%)	0.552	**<0.001**	0.648	**<0.001**
FMI	0.494	**<0.001**	0.474	**<0.001**

BMI indicates body mass index; WC, waist circumference; WHtR, waist to height ratio; BFP, body fat percentage; FMI, fat mass index.

Significant results are in **bold** ($p < 0.05$).

Table 3. Correlation coefficients between anthropometric variables and cardiovascular risk factor in subsample (270 boys and 244 girls).

		VO_{2max} (ml/kg/min)	SBP (mm Hg)	DBP (mm Hg)	TC /HDL-c	LDL-c (mg/dL)	TG (mg/dL)	HOMA (mg/dL)	CRP (mg/dL)	C-3 (mg/dL)	C-4 (mg/dL)	IL-6 (pg/mL)	Leptin (ng/mL)	Adiponectin (µg/mL)	TNF-α (ng/mL)	Visfatin (ng/mL)
NC (cm)	Boys	-0.481***	0.511***	0.335***	0.388***	0.243***	0.372***	0.255***	0.243***	0.194***	0.200***	-0.021	0.295***	-0.228***	-0.040	-0.338***
	Girls	-0.672***	0.419***	0.301***	0.056	0.016	0.284***	0.274***	0.079	0.172**	0.167**	-0.082	0.427***	-0.198**	-0.004	-0.143
BMI (kg/m^2)	Boys	-0.509***	0.448***	0.363***	0.388***	0.284***	0.358***	0.252***	0.180**	0.301***	0.253***	-0.010	0.409***	-0.233***	-0.012	-0.185*
	Girls	-0.563***	0.379***	0.312***	0.216***	0.054	0.256***	0.194***	0.173*	0.231***	0.257***	-0.022	0.613***	-0.213***	0.011	-0.075
WC (cm)	Boys	-0.496***	0.486***	0.355***	0.330***	0.236***	0.394***	0.319***	0.194**	0.256***	0.210***	-0.008	0.303***	-0.262***	0.036	-0.317***
	Girls	-0.566***	0.425***	0.327***	0.184***	0.022	0.273***	0.167***	0.094	0.198**	0.186**	-0.065	0.521***	-0.203**	-0.017	-0.045
WHtR (cm)	Boys	-0.386***	0.080**	0.193***	0.474***	0.277***	0.321***	-0.001	0.251***	0.347***	0.264***	0.177**	0.537***	-0.179**	0.010	0.131
	Girls	-0.057	0.125***	0.186***	0.354***	0.149*	0.183**	-0.007	0.255***	0.364***	0.320***	0.071	0.460***	-0.200**	0.053	0.142
BFP (%)	Boys	-0.619***	0.196***	0.255***	0.478***	-0.118*	0.356***	0.072	0.282***	0.378***	0.348***	0.043	0.495***	-0.138*	-0.019	0.007
	Girls	-0.500***	0.326***	0.305***	0.291***	0.068	0.283***	0.220**	0.174*	0.304***	0.315***	-0.008	0.608***	-0.224***	0.008	-0.033
FMI	Boys	-0.435***	0.034	0.136***	0.466***	0.286***	0.252***	-0.100	0.264***	0.347***	0.317***	0.124*	0.540***	-0.066	-0.036	0.238**
	Girls	-0.060	0.058	0.162***	0.385***	0.154*	0.198**	0.072	0.292***	0.401***	0.385***	0.102	0.514***	-0.188**	0.068	0.101

NC indicates neck circumference; BMI, body mass index; WC, waist circumference; WHtR, waist to height ratio; BFP, body fat percentage; FMI, fat mass index; VO_{2max}, maximum oxygen consumption; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC/HDL-c, total cholesterol/ high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; HOMA, homeostasis model assessment; CRP, C-reactive protein; C-3, C3 protein; C4, C-4 protein; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha.

*p<0.05, ** p<0.01, *** p<0.001.

Tabla 4. Multiple linear regression analyses of single and cluster cardiovascular risk score with neck circumference.

		Boys				Girls			
	n	Adjusted R^2	β	SE	P	Adjusted R^2	β	SE	P
Dependent variables					**Neck circumference**				
$VO_{2\ max}$ (ml/kg/min)	2146	0.231	-1.173	0.062	**<0.001**	0.018	-0.467	0.081	**<0.001**
SBP (mm Hg)	2191	0.353	2.526	0.155	**<0.001**	0.353	2.526	0.155	**<0.001**
DBP (mm Hg)	2191	0.113	1.195	0.111	**<0.001**	0.089	1.216	0.133	**<0.001**
TC/HDL-c (mg/dL)	514	0.098	0.098	0.015	**<0.001**	0.048	0.073	0.019	**<0.001**
LDL-c (mg/dL)	513	0.013	1.377	0.663	**0.039**	0.007	-0.844	0.516	0.103
Triglycerides (mg/dL)	514	0.038	1.669	0.490	**<0.001**	0.042	1.799	0.553	**<0.001**
HOMA score	342	0.169	0.256	0.043	**<0.001**	0.073	0.604	0.160	**<0.001**
C-reactive protein	315	0.055	0.351	0.094	**<0.001**	<0.001	-0.092	0.094	0.328
C-3 protein (mg/dL)	514	0.035	3.164	0.728	**<0.001**	0.026	1.630	0.557	**0.004**
C-4 protein (mg/dL)	514	0.037	0.478	0.129	**<0.001**	0.024	0.539	0.189	**0.005**
Interleukin-6 (pg/mL)	502	<0.001	-0.200	0.527	0.705	0.003	-1.552	1.139	0.174
Leptin (ng/mL)	499	0.060	713.14	155.007	**<0.001**	0.179	1927.779	252.179	**<0.001**
Adiponectin (µg/mL)	506	0.049	-0.357	0.084	**<0.001**	0.036	-0.463	0.138	**<0.001**
TNF- α (pg/mL)	478	<0.001	1.058	1.182	0.539	0.016	-2.052	3.976	0.413
Visfatin (ng/mL)	324	0.107	-0.100	0.025	**<0.001**	0.012	-0.046	0.030	0.124
Cardiovascular risk	315	0.130	0.287	0.103	**<0.001**	0.207	0.834	0.248	**<0.001**

VO_{2max} indicates maximum oxygen consumption; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC/HDL-c, total cholesterol/ high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; HOMA, homeostasis model assessment; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha.

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

Table 5. Neck circumference cutoff values for determining overweight/obese youth with BMI, WC and BFP.

	Using Cole´s BMI cutoff values		Using Katzmarzyk´s WC cutoff values		Using McCarthy´s BFP cutoff values	
AGE	BOYS	GIRLS	BOYS	GIRLS	BOYS	GIRLS
6	25.8	25.7	25.3	25.0	25.6	25.9
7	26.7	26.2	26.2	25.7	26.6	26.6
8	27.6	26.9	27.1	26.6	27.6	27.3
9	28.5	27.5	28.3	27.5	28.5	28.0
10	29.5	28.3	29.5	28.3	29.5	28.7
11	30.5	29.0	30.6	29.2	30.4	29.3
12	31.5	29.8	31.8	29.9	31.3	29.9
13	32.4	30.6	32.9	30.6	32.1	30.5
14	33.4	31.3	33.9	31.3	32.9	31.0
15	34.4	31.9	34.7	31.6	33.7	31.5
16	35.3	32.5	35.4	32.0	34.5	32.0
17	36.2	32.9	36.1	32.2	35.4	32.5
18	37.1	33.5	36.7	32.5	36.3	33.1

BMI indicates body mass index; WC, waist circumference; BFP, body fat percentage.

Supplementary table 1. Multiple linear regression analyses of maximal oxygen consumption (VO_{2max}, ml/kg/min), systolic blood pressure (SBP, mm Hg), diastolic blood pressure (DBP, mm Hg), total cholesterol/ high-density lipoprotein cholesterol (TC/HDL, mg/dL) and low-density lipoprotein cholesterol (LDL, mg/dL) with body composition indices.

	Boys				Girls			
	Adjusted R^2	β	SE	P	Adjusted R^2	β	SE	P
Independent variables				**$VO_{2\ max}$ (n=2146)**				
Neck circumference	0.231	-1.173	0.062	**<0.001**	0.018	-0.467	0.081	**<0.001**
Body mass index	0.253	-0.910	0.047	**<0.001**	0.044	-0.497	0.044	**<0.001**
Waist circumference	0.231	-0.379	0.022	**<0.001**	0.037	-0.197	0.019	**<0.001**
Waist-to-height ratio	0.113	-61.143	3.823	**<0.001**	0.039	-31.074	3.138	**<0.001**
Body fat percentage	0.285	-0.339	0.016	**<0.001**	0.066	-0.266	0.018	**<0.001**
Fat mass index	0.111	-0.684	0.036	**<0.001**	0.058	-0.492	0.039	**<0.001**
				SBP (n=2191)				
Neck circumference	0.353	2.526	0.155	**<0.001**	0.353	2.526	0.155	**<0.001**
Body mass index	0.239	1.237	0.100	**<0.001**	0.168	1.059	0.096	**<0.001**
Waist circumference	0.281	0.552	0.042	**<0.001**	0.200	0.515	0.042	**<0.001**
Waist-to-height ratio	0.053	66.232	7.317	**<0.001**	0.042	57.874	6.994	**<0.001**
Body fat percentage	0.047	0.279	0.033	**<0.001**	0.127	0.436	0.042	**<0.001**
Fat mass index	0.033	0.531	0.073	**<0.001**	0.026	0.603	0.090	**<0.001**
				DBP (n=2191)				
Neck circumference	0.113	1.195	0.111	**<0.001**	0.089	1.216	0.133	**<0.001**
Body mass index	0.132	0.793	0.061	**<0.001**	0.095	0.741	0.071	**<0.001**
Waist circumference	0.126	0.293	0.023	**<0.001**	0.105	0.328	0.030	**<0.001**
Waist-to-height ratio	0.058	46.204	4.984	**<0.001**	0.035	47.497	5.554	**<0.001**
Body fat percentage	0.065	0.198	0.022	**<0.001**	0.092	0.323	0.035	**<0.001**
Fat mass index	0.061	0.434	0.049	**<0.001**	0.047	0.588	0.072	**<0.001**
				Total cholesterol/HDL cholesterol (n=514)				
Neck circumference	0.098	0.098	0.015	**<0.001**	0.048	0.073	0.019	**<0.001**
Body mass index	0.148	1.715	0.183	**<0.001**	0.045	0.003	0.010	**<0.001**
Waist circumference	0.106	0.022	0.003	**<0.001**	0.030	0.027	0.005	**<0.001**
Waist-to-height ratio	0.222	5.999	0.617	**0.001**	0.126	4.562	0.688	**<0.001**
Body fat percentage	0.226	0.025	0.003	**<0.001**	0.081	0.032	0.005	**<0.001**
Fat mass index	0.143	0.062	0.009	**<0.001**	0.143	0.062	0.009	**<0.001**
				LDL cholesterol (n=513)				
Neck circumference	0.013	1.377	0.663	**0.039**	0.007	-0.844	0.516	0.103
Body mass index	0.001	0.445	0.407	0.275	<0.001	-0.400	0.390	0.306
Waist circumference	<0.001	0.094	0.152	0.535	0.002	-0.205	0.169	0.225
Waist-to-height ratio	0.002	39.593	30.87	0.201	<0.001	25.630	31.298	0.414
Body fat percentage	0.002	0.186	0.147	0.209	<0.001	-0.046	0.187	0.806
Fat mass index	0.002	0.354	0.286	0.216	0.004	0.560	0.400	0.162

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

Supplementary table 2. Multiple linear regression analyses of triglycerides (mg/dL), of insulin resistance (HOMA score), C-reactive protein (CRP, mg/dL), C3-protein (C-3, mg/dL) and C4-protein (C-4, mg/dL) with body composition indices.

	Boys				Girls			
	Adjusted	β	SE	P	Adjusted R^2	β	SE	P
Independent variables				**Triglycerides (n=514)**				
Neck circumference	0.038	1.669	0.490	**<0.001**	0.042	1.799	0.553	**<0.001**
Body mass index	0.033	1.669	0.524	**<0.001**	0.037	1.262	0.416	**<0.001**
Waist circumference	0.053	0.748	0.193	**<0.001**	0.042	0.590	0.181	**0.001**
Waist-to-height ratio	0.019	97.477	41.998	**0.003**	<0.001	0.108	1.690	0.108
Body fat percentage	0.015	0.382	0.190	**0.045**	0.036	0.637	0.200	**0.002**
Fat mass index	<0.001	0.802	0.418	0.056	0.01	1.037	0.047	**0.021**
				HOMA (n=342)				
Neck circumference	0.169	0.256	0.043	**<0.001**	0.073	0.604	0.160	**<0.001**
Body mass index	0.117	0.151	0.043	**0.001**	0.029	0.124	0.050	**0.015**
Waist circumference	0.162	0.066	0.019	**<0.001**	0.009	0.037	0.024	0.119
Waist-to-height ratio	0.032	8.029	3.110	**0.011**	0.003	-4.321	3.612	0.233
Body fat percentage	0.044	0.043	0.014	**0.003**	0.015	0.045	0.024	0.060
Fat mass index	0.021	0.069	0.033	**0.039**	<0.001	0.009	0.047	0.847
				CRP (n=315)				
Neck circumference	0.055	0.351	0.094	**<0.001**	<0.001	-0.092	0.094	0.328
Body mass index	0.028	0.291	0.106	**0.007**	0.025	0.221	0.069	**0.002**
Waist circumference	0.033	0.120	0.040	**0.003**	0.002	-0.034	0.030	0.257
Waist-to-height ratio	0.015	8.941	4.762	**0.047**	<0.001	5.088	5.291	0.338
Body fat percentage	0.059	29.624	7.558	**<0.001**	0.060	15.668	4.388	**<0.001**
Fat mass index	0.065	0.226	0.059	**<0.001**	0.080	0.236	0.057	**<0.001**
				C-3 (n=514)				
Neck circumference	0.035	3.164	0.728	**<0.001**	0.026	1.630	0.557	**0.004**
Body mass index	0.088	2.364	0.413	**<0.001**	0.050	1.644	0.412	**<0.001**
Waist circumference	0.063	1.133	0.220	**<0.001**	0.039	0.648	0.191	**0.001**
Waist-to-height ratio	0.118	206.454	30.784	**<0.001**	0.130	193.054	30.910	**<0.001**
Body fat percentage	0.140	0.915	0.129	**<0.001**	0.089	1.024	0.192	**<0.001**
Fat mass index	0.118	1,884	0.285	**<0.001**	0.158	2.784	0.382	**<0.001**
				C-4 (n=514)				
Neck circumference	0.037	0.478	0.129	**<0.001**	0.024	0.539	0.189	**0.005**
Body mass index	0.061	0.671	0.141	**<0.001**	0.063	0.619	0.139	**0.002**
Waist circumference	0.041	0.241	0.055	**<0.001**	0.031	0.271	0.065	**<0.001**
Waist-to-height ratio	0.067	53.401	10.570	**<0.001**	0.99	56.768	10.655	**<0.001**
Body fat percentage	0.118	0.277	0.043	**<0.001**	0.99	0.36	0.065	**<0.001**
Fat mass index	0.098	0.574	0.094	**<0.001**	0.145	0.869	0.132	**<0.001**

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

Supplementary table 3. Multiple linear regression analyses of interleukin 6 (IL-6, pg/mJ) leptin (ng/mL), adiponectin (µg/mL), and visfatin (ng/mL) with body composition indices.

	Boys				Girls			
	Adjusted	β	SE	P	Adjusted	β	SE	P
Independent variables				**Il-6 (n=502)**				
Neck circumference	<0.001	-0.200	0.527	0.705	0.003	-1.552	1.139	0.174
Body mass index	<0.001	-0.106	0.590	0.857	<0.001	-0.753	1.001	0.453
Waist circumference	<0.001	-0.030	0.225	0.893	0.001	-0.425	0.391	0.279
Waist-to-height ratio	0.028	139.795	43.662	**0.002**	0.001	77.657	65.893	0.240
Body fat percentage	<0.001	0.255	0.183	0.164	<0.001	0.325	0.448	0.469
Fat mass index	0.012	0.739	0.384	**0.048**	0.007	1.402	0.831	0.093
				Leptin (n=499)				
Neck circumference	0.060	713.142	155.007	**<0.001**	0.179	1927.779	252.179	**<0.001**
Body mass index	0.165	1158.997	89.388	**<0.001**	0.374	1747.644	139.514	**<0.001**
Waist circumference	0.089	495.536	40.142	**<0.001**	0.269	554.476	54.713	**<0.001**
Waist-to-height ratio	0.286	66804.453	5902.475	**<0.001**	0.208	84803.472	9319.467	**<0.001**
Body fat percentage	0.242	300.358	26.911	**<0.001**	0.368	668.546	52.889	**<0.001**
Fat mass index	0.289	612.946	56.721	**<0.001**	0.261	1183.717	113.155	**<0.001**
				Adiponectin (n=506)				
Neck circumference	0.049	-0.357	0.084	**<0.001**	0.036	-0.463	0.138	**<0.001**
Body mass index	0.051	-0.282	0.110	**0.010**	0.042	-0.371	0.102	**<0.001**
Waist circumference	0.066	-0.175	0.036	**<0.001**	0.038	-0.162	0.047	**0.001**
Waist-to-height ratio	0.030	-23.791	7.015	**0.001**	0.036	-26.967	7.972	**0.001**
Body fat percentage	0.016	-0.045	0.032	**0.016**	0.047	-0.186	0.049	**<0.001**
Fat mass index	0.001	-0.100	0-0067	0.135	0.032	-0.319	0.101	**0.002**
				TNF- α (n=478)				
Neck circumference	<0.001	1.058	1.182	0.539	0.016	-2.052	3.976	0.413
Body mass index	0.001	2.915	1.199	0.628	0.001	-5.176	1.887	0.459
Waist circumference	0.003	0.366	0.526	0.541	0.003	-0,539	0.968	0.409
Waist-to-height ratio	<0.001	11.347	7.602	0.881	<0.001	8.959	6.398	0.926
Body fat percentage	<0.001	-0.104	0.101	0.743	0.002	-0.466	4.619	0.460
Fat mass index	0.002	-0.290	0.632	0.662	0.001	0.114	1.840	0.924
				Visfatin (n=324)				
Neck circumference	0.107	-0.100	0.025	**<0.001**	0.012	-0.046	0.030	0.124
Body mass index	0.026	-0.005	0.033	0.876	<0.001	-0.019	0.024	0.421
Waist circumference	0.093	-0.017	0.015	0.264	<0.001	-0.005	0.011	0.627
Waist-to-height ratio	0.010	1.263	2.136	0.555	0.012	2.689	1.736	0.124
Body fat percentage	<0.001	0.006	0,011	0.606	<0.001	-0.004	0.011	0.722
Fat mass index	0.057	0.025	0.022	0.273	0.002	0.022	0.024	0.278

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

Supplementary table 4. Multiple linear regression analyses of cardiovascular risk score with body composition indices.

	Boys				Girls			
	Adjusted	β	SE	P	Adjusted	β	SE	P
Independent variables	**Clustered CVD risk factor (n=315)**							
Neck circumference	0.130	0.287	0.103	**<0.001**	0.207	0.834	0.248	**<0.001**
Body mass index	0.095	0.201	0.104	**<0.001**	0.111	0.432	0.095	**<0.001**
Waist circumference	0.125	0.175	0.027	**<0.001**	0.073	0.141	0.028	**<0.001**
Waist-to-height ratio	0.175	34.634	7.176	**<0.001**	0.174	21.415	6.394	**<0.001**
Body fat percentage	0.113	0.119	0.033	**<0.001**	0.143	0.194	0.042	**<0.001**
Fat mass index	0.109	0.268	0.076	**<0.001**	0.290	0.419	0.088	**<0.001**

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any pre-specified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7-10*
Bias	9	Describe any efforts to address potential sources of bias	11
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10-11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	11

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-14
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	4
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-19
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	20

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Neck circumference and clustered cardiovascular risk factors in children and adolescents: a cross-sectional study

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Neck circumference and clustered cardiovascular risk factors in children and adolescents: a cross-sectional study

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ABSTRACT

Objective: Early detection of cardiovascular disease (CVD) risk factors, such as obesity, is crucial in order to prevent adverse long-term effects on individuals' health. Therefore, the aims were: (i) to explore the robustness of neck circumference (NC) as a predictor of CVD and examine its association with numerous anthropometric and body composition indices, and (ii) to release sex-age-specific NC cut-off values to classify youths as overweight/obese.

Design: Cross-sectional study.

Setting: 23 primary schools and 17 secondary schools from Spain.

Participants: 2,198 students (1060 girls), grade 1-4 and 7-10.

Measures: Pubertal development, anthropometric and body composition indices, systolic and diastolic blood pressure (SBP and DBP, respectively), cardiorespiratory fitness, blood sampling (triglycerides (TG), total-cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), glucose and inflammatory markers. Homeostasis model assessment (HOMA-IR), and cluster of CVD risk factors were calculated.

Results: NC was positively correlated with all anthropometric and body composition indices. NC was negatively associated with VO_{2max} (R^2=0.231, p<0.001 for boys; R^2=0.018, p<0.001 for girls), and positively associated with SBP, DBP, TC/HDL-c, TG, HOMA, complement factors C-3 and C-4, leptin, adiponectin and clustered CVD risk factor in both sexes (R^2 from 0.035 to 0.353, p<0.01 for boys; R^2 from 0.024 to 0.215, p<0.001 for girls). Moreover, NC was positively associated with serum C-reactive protein, LDL-c and visfatin only in boys (R^2 from 0.013 to 0.107, p<0.05).

Conclusion: NC is a simple, low-cost and practical screening tool of excess of upper-body obesity and CVD risk factors in children and adolescents. Pediatricians can easily use it as a screening tool for overweight/obesity in children and adolescents. For this

purpose, sex-and-age specific thresholds to classify children and adolescents as normal weight or overweight/obese are provided.

Keywords: Anthropometry and body composition, neck circumference, cardiometabolic risk and cardiovascular disease, inflammatory markers, youth.

Strengths and limitations of this study

- The use of a large number of anthropometric and body composition indices, as well as cardiometabolic risk factors and inflammatory markers is a strength of this study.
- The study sample consisted of children and adolescents with different weight status, thus broadening the applicability of the findings.
- The cross-sectional nature of the study limits the establishment of causality.
- The study group consisted of a single ethnic group (white Caucasian) and the cutoff values herein provided may not be valid for other ethnic groups.
- More accurate measures of overweight/obesity (e.g. those obtained with magnetic resonance imaging or dual energy X ray absorptiometry) might be used to assess the association with NC.

BACKGROUND

Obesity is a disease affecting people of all ages, sex, ethnicities, and socioeconomic levels [1] with serious public health implications. Particularly, overweight and obesity in children and adolescents are a pandemic worldwide, affecting not only developed but also developing countries [2]. Excess body fat in children and adolescents is associated with cardiovascular-related risk factors such as type II diabetes, insulin resistance, hypertension, elevated triglyceride and cholesterol levels [3]. The presence of cardiovascular disease (CVD) risk factors in childhood is associated with premature death in adulthood [4]. Consequently, early detection of CVD risk factors, such as obesity, is crucial in order to prevent adverse long-term effects on individuals' health [5]. Practical and simple anthropometric and body composition indices such as body mass index (BMI), waist circumference (WC), waist-to-hip ratio, waist-to-height ratio (WHtR) and body fat percentage (BFP) are widely used as indices of total and central obesity. Indeed, some of these indices have been taken into account in the ALPHA (Assessing Levels of PHysical Activity) health-related fitness test battery, which is nowadays the most relevant health-related fitness test battery for children and adolescents [6].

Recent researchers have proposed the neck circumference (NC) as another screening tool of CVD risk factors (i.e. obesity) in children and adolescents [7-11]. However, these studies examine this association through single cardio-metabolic risk factors (i.e. insulin resistance, hypertension, and hyperlipidemia (triglyceride and cholesterol). Clustering of CVD risk factors seems to be a much stronger measure of cardiovascular health in children and adolescents than single risk factors, as a subject with cardiovascular risk may reflect high levels of several risk factors simultaneously [12]. Additionally, inflammatory markers have recently received close attention and are considered "emerging CVD risk factors" [13]. Previous studies have identified some inflammatory

markers (such as complement factors C-3 and C-4, C-reactive protein, adiponectin, leptin, interleukin 6, tumor necrosis factor alpha and visfatin) to play a key role in CVD development in children [14], adolescents [14] and adults [15]. However, whether NC is associated with inflammatory markers in children and adolescents is still unknown in spite of its clinical relevance as so it is the potential usefulness of NC as a predictor for CVD risk factors.

Therefore, the aims were: (i) to explore the robustness of NC as a predictor of CVD and examine its association with numerous anthropometric and body composition indices, and (ii) to release sex-age-specific NC cut-off values to classify youths as overweight/obese, based on prediction equations using BMI, WC and BFP.

METHODS

Study design, setting and participants

Participants selected for this study cross-sectional were enrolled in the UP&DOWN study [16]. In brief, the UP&DOWN study was a 3-year longitudinal study designed to assess the impact of physical activity and sedentary behaviors over time on health indicators as well as to identify the psycho-environmental and genetic determinants of physical activity in a Spanish sample of children and adolescents. Data from the present study were collected from September 2011 to June 2012. Children and adolescents were recruited from schools in Cádiz and Madrid, respectively. A total convenience sample of 2,225 participants aged 6 to 18 years participated in the UP&DOWN study although the present study includes 2,198 participants (1060 girls) with complete data at baseline on anthropometric and body composition indices. According to the database of the Spanish Institute of National Statistics, our sample size represented the 50% (n=1179) and 5% (n=1019) of the total population size of school children and adolescents, respectively; with a 3% percentage of error for both sample sizes. Blood sampling was randomly performed in one-fourth of the recruited children and adolescents (514; 244 girls).

Parents and school supervisors were informed by letter about the nature and purpose of the study, and written informed consent was provided. The study protocols were 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).

Measurements

Pubertal development

After a brief visual observation, the participants self-classified in one of the five stages of pubertal development according to Tanner & Whitehouse [17]. To do this, breast development in girls and genital development in boys was used.

Anthropometric and body composition indices

Harmonization and standardization of anthropometric and body composition indices used to assess body composition in the UP&DOWN study were strictly controlled [16]. Anthropometric data included body mass, height, waist and NC, and triceps and subscapular skinfold thicknesses. Body mass was measured with an electronic scale (Type SECA 861; range, 0.05 to 130 kg; precision, 0.05 kg), and height was measured in the Frankfort plane with a telescopic stature-measuring instrument (Type SECA 225; range, 60 to 200 cm; precision, 1 mm). WC was measured with a non-elastic tape (SECA 200; range, 0 to 150 cm; precision, 1 mm), at the level of the natural waist, in a horizontal plane, which is the narrowest part of the torso, as seen from a front view. NC was assessed with the participants standing in an erect position, hanging the arms freely and keeping the head aligned in the Frankfort horizontal plane. The superior border of a non-elastic tape measure (SECA 200; range, 0 to 150 cm; precision, 1 mm) was placed just below the laryngeal prominence and applied perpendicular to the long axis of the neck [18]. Triceps and subscapular skinfold thickness were measured 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 [19]. The measurements were carried out twice, but not consecutively, and the mean value of the two measurements was used in the analyses. BFP was calculated from triceps and subscapular skinfold thicknesses by using the equations developed by Slaughter et al. [20].

BMI was calculated as body mass/height squared (kg/m^2) and participants were categorized as underweight/normal weight and overweight/obese following

international cut-off points [21]. WHtR was computed from the original anthropometric index (waist/height). Fat mass index (FMI) was computed dividing BFP by height squared (m^2).

Blood pressure

Systolic and diastolic blood pressure (SBP and DBP, respectively) were measured by 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 [22]. The participants sat on a chair quietly for 5 minutes before the measurements were conducted on the left arm in an extended position. Two measures were taken 1-2 minutes apart. An additional measurement was performed if the first two readings differed in > 5 mm Hg, and the farthest value was removed. Average values (mm Hg) were calculated separately for SBP and DBP.

Cardiorespiratory fitness

20-m shuttle run test was used to assess cardiorespiratory fitness, according to the ALPHA health-related fitness test battery protocol [6]. The equation reported by Léger et al. [23] was used to estimate the maximum oxygen consumption (VO_{2max}, ml/kg/min).

Blood sampling

A fasting blood sample was obtained from the cubital vein early in the morning at the schools. 13.5 ml of blood were drawn from each subject, and 3.5 ml of them (anticoagulated blood in EDTA) were analyzed to obtain haemogram data. The remainder blood (dried gel and sodium citrate) was centrifuged; serum and plasma were removed and then frozen at -80º C to be analyzed later. Serum lipid triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), glucose and total proteins as CVD risk factors were

analyzed by enzymatic colorimetric methods (Olympus AU2700 Analyzer; Olympus UK Ltd, Watford, UK).

The following inflammatory markers were analysed by turbidimetry (Olympus AU2700 Analyzer; Olympus UK Ltd, Watford, UK): serum complement factors C-3 (C3) C-4 (C4) and serum C-reactive protein (CRP). The coefficients of variation (inter-assay precision) were less than 2% for all proteins (1.39% for C3, 1.19% for C4 and 1.90% for CRP). Detection limits (sensitivity) for the analyses were 0.01 g/L for C3, 0.002 g/L for C4 and 0.007 mg/L for CRP. Adiponectin, leptin, interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) were quantified by multiple analyte profiling (xMAP) technology (xMAP Techonology; Luminex, Austin, TX, USA) using a kit (5+1) plex: 171-A7003M Bio-plex Pro Human Diabetes Adiponectin Assay; YB0000002Y Bio-Plex Human Diabetes 3-Plex Assay; 171D50001 Bio-Plex Human Cytokine Stds; 171B5006M Bio-Plex Human IL-6 set; 171B5026M Bio-Plex Human TNF-alpha set. Visfatin values were determined by enzyme-linked immunoSobent assay (ELISA) (Human visfatin Elisa kit; Cusabio Biotech, Wuhan, Hubei, China). Sensitivity for the analyses were 0.156 ng/ml, the coefficients of variation were less than 8% for intra-assay precision and less than 10% for inter-assay precision.

Insulin resistance was calculated through the homeostasis model assessment (HOMA) score as: [insulin (mLU/mL) X glucose (mmol/L)] / 22.5 [24].

Clustered CVD risk factors

A cluster of CVD risk factors by sex and age groups was constructed. For that purpose, standardized normalized indices (z-score=[value-mean]/standard deviation) of the subsequent single CVD risk factors were computed: metabolic syndrome risk factors (i.e. SBP, DBP, TC /HDL-c, and TG), HOMA, inflammatory markers (i.e. CRP, C-3, C-4, IL-6, leptin and adiponectin,) and VO_{2max}. The VO_{2max} z-score was inverted,

because higher cardiorespiratory fitness is associated with lower fatness. Finally, all the z-scores were summed. LDL-c, TNF-α and visfatin were not included in the cluster because they did not show association with anthropometric nor body composition indices.

Statistical analysis

Antrhropometric and body composition indices and cardiovascular markers are sex-dependent; therefore, boys and girls were analyzed separately. Descriptive data are shown as mean and standard deviation (SD) unless otherwise indicated. The Student's *t*-test was used to test differences in socio-demographic and clinical characteristics by sex, except for the Tanner stage, which was analyzed by χ^2 test. Pearson's correlation coefficients were obtained to analyze the association between NC and the other anthropometric and body composition indices (BMI, WC, WHtR, BFP and FMI), and the association between all anthropometric and body composition indices and single CVD risk factors. To test the association of single and cluster CVD risk factors with anthropometric and body composition indices adjusted multiple linear regression analyses were performed. Single and clustered CVD risk factors were entered as dependent variables and anthropometric and body composition indices as independent variables, in separate models.

To predict specific NC cut-off values for overweight and obesity, a linear regression analysis following a stepwise selection procedure for the derivation of prediction equation models including NC as dependent variable and BMI, WC, and BFP as independent variables, in separate models were performed. In the prediction equations, Cole's BMI [21], Katzmarzyk's WC [25] and McCarthy's BFP [26] cutoff values for overweight/obesity to obtain the corresponding NC cut-off values were used. These are optimal threshold values for predicting high-risk groups to be used in the white

population worldwide. All models were controlled for age and Tanner stage. 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 statistical significance was set at $p<0.05$.

RESULTS

The characteristics of the study sample are presented in **table 1**. Boys presented higher values in Tanner stage, body mass, height, NC, WC, WHtR, VO_{2max} and SBP than girls (all, $p<0.05$). However, BFP, FMI, DBP, HOMA, leptin and adiponectin were significantly higher in girls compared to boys (all, $p<0.05$). 32.7%, 38.7%, 16% and 37% of insulin, C-reactive protein, TNF- α and visfatin sample, respectively, were below detection threshold and were not included in the final analyses.

Table 2 shows correlation coefficients between NC and BMI, WC, WHtR, BFP and FMI by sex. NC was positively correlated with all anthropometric and body composition indices in both, boys and girls (all, $p\leq0.001$). Correlations were stronger with WC ($r=0.864$ in boys, $r=0.851$ in girls) and BMI ($r=0.754$ in boys, $r=0.799$ in girls).

Table 3 shows bivariate correlations between all anthropometric and body composition indices and CVD risk factors by sex. Overall, all anthropometric and body composition indices were correlated with most single CVD risk factors, with higher correlation coefficients in boys than girls (all, $p \leq0.05$). More specifically, NC was negatively correlated with VO_{2max} ($r = -0.481$, $p<0.001$ for boys; $r = -0.672$, $p<0.001$ for girls), positively correlated with SBP, DBP, TG, HOMA, C-3, C-4, leptin and adiponectin in both sexes (r from 0.167 to 0.419; all, $p<0.01$), and with TC/HDL-c, LDL-c, CRP and visfatin only in boys (r from 0.243 to 0.388; all, $p<0.001$).

The associations between neck circumference and single and clustered CVD risk factors are presented in **table 4**. NC was negatively associated with VO_{2max} ($R^2 = 0.231$, $p<0.001$ for boys; $R^2 = 0.018$, $p<0.001$ for girls), and positively associated with SBP, DBP, TC/HDL-c, TG, HOMA, C-3, C-4, leptin, adiponectin and clustered CVD risk factor in both sexes (R^2 from 0.035 to 0.353, $p<0.01$ for boys; R^2 from 0.024 to 0.215,

p<0.001 for girls). Moreover, NC was positively associated with CRP, LDL-c and visfatin only in boys (R^2 from 0.013 to 0.107, p<0.05). Overall, the association of CVD risk factors with the other anthropometric and body composition indices presented similar results (see **supplementary tables** 1, 2, 3 and 4). When the variables with values below the detection levels (i.e. insulin, C-reactive protein, TNF- α and visfatin) were included in the analyses of sensitivity, before natural log transformation, the associations aforementioned remained unchanged (data not shown). The associations Stepwise regression analyses were conducted to get specific NC cutoff values for overweight/obesity based on BMI, WC and BFP values (**table 5**). Results showed very similar NC cutoff values for boys and girls with the same age, independently of the criteria used to classify children and adolescents as normal weight or overweight/obese. Moreover, cutoff values increase with age and are higher in boys than girls. The prediction equations for NC cutoff values are presented below:

Neck circumference = 13.631 + 0.466 x BMI + 0.668 x age (R^2 = 0.864 and SEE = 1.439) for boys.

Neck circumference = 15.656 + 0.452 x BMI + 0.365 x age (R^2 = 0.796 and SEE = 1.428) for girls.

Neck circumference = 10.735 + 0.210 x WC + 0.520 x age (R^2 = 0.877 and SEE = 1.367) for boys.

Neck circumference = 11.825 + 0.212 x WC + 0.315 x age (R^2 = 0.825 and SEE = 1.189) for girls.

Neck circumference = 18.364 + 0.106 x BFP + 0.872 x age (R^2 = 0.798 and SEE = 1.729) for boys.

Neck circumference = 19.107 + 0.172 x BFP + 0.480 x age (R^2 = 0.726 and SEE = 1.484) for girls.

DISCUSSION

The main findings indicate that: i) NC is strong and positively associated with well-known indices of anthropometric and body composition, such as BMI, WC, BFP, WHtR and FMI; ii) NC is one of the strongest indices associated with single and clustered CVD risk factors; iii) sex-and-age specific NC cutoff values are provided to classify children and adolescents as normal weight or overweight/obese.

Comparison of NC with well-known anthropometric and body composition indices

BMI is the most common anthropometric index used to determine overweight and obesity not only in children and adolescents but also in adults. BMI has become a very popular screening tool for overweight and obesity due to its simplicity and ease. However, it is becoming increasingly clear that it is not a good proxy for regional adiposity [27]. Regional deposition of fat is a better predictor of some obesity related complications, such as metabolic disorders and CVD risk factors [28]. In this regard, WC seems to be a better anthropometric index in children and adolescents [29]. On the other hand, WHtR [30] and FMI [31] have been proposed as a marker of adiposity in children and adolescents, however, these indices have been built in analogy with BMI.

Recently, NC has been proposed as a surrogate marker of regional obesity in children and adolescents [7 8]. These studies reported strong correlations between NC and BMI and WC ($r > 0.7$). Concurring with these studies, NC was correlated with a larger number of anthropometric and body composition indices (i.e. BMI, WC, WHtR, BFP and FMI). More specifically, NC was highly correlated with WC and BMI and in both sexes, which confirms its use as a screening tool for identifying overweight/obese children and adolescents. NC is a simple, quick and low-cost method that requires less effort from both the examiner and the examinee than other anthropometric and body composition methods. NC has shown very good inter- and intra-rater reliability [32] and unlike WC,

NC is not influenced by the timing of measurement (e.g. pre-prandial and postprandial period; measurement at the end of a gentle expiration as suggested for WC). Additionally, this technique does not require the patient to remove his/her clothing in order to obtain a more accurate measurement. Finally, the measurement of NC may be more socially acceptable and convenient for overweight and obese children, thus making this measurement more tolerable for them.

NC and single CVD risk factors

There is evidence to support that NC is a screening tool for identifying CVD in adults in worldwide [33]. However, little is known about the association between NC and CVD risk factors in children and adolescents. In the present study, NC, as well as all other anthropometric and body composition indices, was associated with cardio-metabolic syndrome risk factors. Androutsos et al. [10] and Kurtoglu et al. [9] also observed that increases in NC were associated with cardio-metabolic syndrome risk factors in Greek and Turkish normal or overweight/obese children and adolescents. Moreover, Guo et al. [34] showed that NC could predict pre-hypertension in Chinese normal weight children and adolescents, but not in those overweight and obese. It is widely accepted that obesity is associated with metabolic disorders and CVD risk factors both in youth and adulthood [35]. Free fatty acid concentration is related with the development of CVD risk factors. It has been demonstrated that upper-body subcutaneous fat is responsible for a much larger proportion of systemic free fatty acid release than visceral fat, particularly in obese individuals [36]. Moreover, The Framingham Heart Study showed that NC was associated with CVD risk factors even after adjustment for visceral adipose tissue and BMI, suggesting that upper-body subcutaneous fat may be a unique fat depot conferring additional cardiovascular risk above and beyond central body fat [37]. These previous

results are in line with this study findings, suggesting that thicker NC is associated with greater risk of CVD.

Inflammatory markers have been proposed as new emerging CVD risk factors [38]. Indeed, persistent low-grade inflammation in children, especially in obese, may increase the risk of metabolic and cardiovascular events in later life and play a role in the pathogenesis of atherosclerosis [39]. Thus, inflammatory markers have a potential interest for pediatric CVD risk factor control and future preventive strategies. Overall, obesity in children and adolescents is associated with increased CRP, C-3, C-4, and leptin levels, and decreased adiponectin levels [39-41], which concurs with the results of the present study. To the best of our knowledge, no studies have been published analyzing the associations between NC and inflammatory markers in children and adolescents. In addition, data with WHtR, BFP and FMI are sparse. In the present study, similar associations were found among all anthropometric and body composition indices and inflammatory markers. There is controversy in the relationship between some inflammatory markers (i.e. plasma IL-6, TNF-α and visfatin) and obesity, with some studies reporting positive [40 42] or no associations [40 41] with obesity. In the present study, IL-6 and visfatin were associated with WHtR and FMI in both sexes, and only with NC in boys. However, no associations among all anthropometric and body composition indices and TNF- α. Differences among studies might be due, as Jaalel et al. [40] suggested, to differences in degrees of maturation, sex distribution and ethnic specificity. Further studies are needed to clarify this association.

NC and clustered CVD risk factors

A clustered CVD risk factors reflects cardiovascular health better than single CVD risk factors, and might, to some extent, compensate for the day-to-day fluctuations in the single risk factors [43]. Even if none of the participants suffer from clinical disease,

clustered risk is certainly an undesirable condition, and it has been shown to track into young adulthood [44]. The results of the current study showed that NC was associated with clustered CVD risk factors in boys and girls.

Previous studies found a strong association between some anthropometric and body composition indices and clustered CVD risk factors [11 14 43]. However, to the best of our knowledge this is the first study in examining the association between NC and a cluster of CVD risk factors, and also the first study in including a large number of inflammatory markers (i.e. CRP, C-3, C-4, IL-6, leptin and adiponectin) as part of the cluster, as recently suggested, due to its relation to clustering of CVD risk factors at early ages [38]. In addition, VO_{2max} was also included in the cluster as performed in previous studies [43] as it is considered a CVD risk factor in children and adolescents. Andersen et al. [43] previously followed this strategy and it is noteworthy that their findings were consistent with those of the present study.

Establishment of NC cutoffs to identify children and adolescents with overweight/obesity

Results from the present study showed that NC could be used as a screening tool to identify children and adolescents at CVD risk. Therefore, providing with sex-and-age specific cutoff values to classify children and adolescents as normal weight or overweight/obese is of relevance from a public health and clinical perspective. The criteria used to classify children and adolescents as normal weight or overweight /obese were based on BMI [21], WC [25], and BFP [26] widely accepted cutoff values in children and adolescents. The explained variances of the full models were high (86% for boys and 80% for girls with BMI; 88% for boys and 83% for girls with WC; and 80% for boys and 73% for girls with BFP), suggesting that NC cutoff values can be established according to BMI, WC and BFP values. The results show NC cutoffs that increase with

age and are higher in boys than girls. In addition, cutoffs for the same age are very similar independently of the criteria used to classify children and adolescents as normal weight or overweight /obese. It is known that body fat distribution and its metabolic effects are different in men and women with sex hormones playing a role in this difference. However, a definite cause has not been established [45]. These results are consistent with those reported in other study based only in BMI cutoff values [7 8]. To note is that NC cutoff values from the current study were slightly lower than those of previous studies [7 8], which may be due to ethnic differences. To our knowledge, this is the first study that establishes NC cutoffs for overweight/obesity according to BMI, WC and BFP values in the same sample.

CONCLUSIONS

In conclusion, NC was positively associated with other anthropometric and body composition indices, single and clustered CVD risk factors. NC is a simple, low-cost and practical screening tool of excess of upper-body obesity and CVD risk factors in children and adolescents. For this purpose, sex-and-age specific thresholds to classify children and adolescents as normal weight or overweight/obese are provided. It might be interesting to test in prospective studies whether NC cut-off points presented in the current study are predictors of cardiovascular disease risk factors in adulthood.

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Competing interests None declared.

Ethics approval The study protocol was carried out according to 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).

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Table 1. Descriptive characteristics of the population sample.

	Boys n=1138		Girls n=1060		P
	Mean	*SD*	*Mean*	*SD*	
Age (years)	10.8	3.4	10.9	3.3	0.449
Tanner stage (n (%))					
1	321 (27.6)		329 (30.4)		**0.010**
2	303 (26.1)		233 (21.4)		
3	195 (16.8)		250 (23.0)		
4	190 (16.3)		216 (19.9)		
5	129 (11.0)		32 (2.9)		
Body mass (kg)	42.8	17.4	41.2	16.1	**0.018**
Height (cm)	144.8	19.9	142.8	17.0	**0.014**
Body mass index (kg/m^2)	19.5	3.7	19.5	3.6	0.895
Neck circumference (cm)	30.0	3.6	28.5	2.7	**<0.001**
Waist Circumference (cm)	64.7	9.9	62.2	8.6	**<0.001**
Waist-to-height ratio (cm)	0.5	0.0	0.4	0.0	**<0.001**
Body fat (%)	19.9	10.3	23.7	7.6	**<0.001**
Fat mass index	9.9	5.1	11.8	3.9	**<0.001**
$VO_{2\,max}$ (ml/kg/min)	38.4	6.7	32.3	7.7	**<0.001**
Systolic blood pressure (mm Hg)	106.7	13.1	103,5	11.1	**<0.001**
Diastolic blood pressure (mm Hg)	65.6	8.1	67.0	8.7	**<0.001**
	Boys n=270		**Girls n=244**		P
TC/HDL-c (mg/dL)	2.9	0.6	3.0	0.7	0.214
LDL-c (mg/dL)	70.9	23.4	73.4	23.4	0.221
Triglycerides (mg/dL)	48.1	30.7	52.5	25.5	0.085
Insulin resistance (HOMA score) *	2.1	2.1	2.5	2.5	**0.047**
C-reactive protein (mg/dL) *	8.8	20.5	9.9	23.1	0.828
C-3 protein (mg/dL)	93.4	31.0	93.7	28.7	0.903

C-4 protein (mg/dL)	20.3	9.9	20.4	9.8	0.944
Interleukin-6 (pg/mL)	34.4	37.5	43.0	62.1	0.061
Leptin (ng/mL)	6.7	6.7	11.7	9.2	**<0.001**
Adiponectin (µg/mL)	13.3	7.2	14.8	7.5	**0.023**
TNF- α (pg/mL)*	77.1	66.2	72.8	57.1	0.445
Visfatin (ng/mL)*	1.5	1.3	1.4	1.2	0.589
Cardiovascular risk score	0.05	1.0	-0.07	1.0	0.269

Results are showed as mean ± SD (standard deviation).

VO_{2max} indicates maximum oxygen consumption; TC/HDL, total cholesterol/ high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA, homeostasis model assessment; TNF- α, tumor necrosis factor alpha.

*32.7%, 38.7%, 16% and 37% of insulin, C-reactive protein, TNF- α and visfatin sample, respectively, were below detection threshold and were not included in the analyses.

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

Table 2. Correlations coefficients of neck circumference with BMI, WC, WtHR, BFP and FMI.

	Neck Circumference			
	Boys (n= 1138)		Girls (n= 1060)	
	r	P	r	P
BMI (kg/m^2)	0.754	**<0.001**	0.799	**<0.001**
WC (cm)	0.864	**<0.001**	0.851	**<0.001**
WHtR (cm)	0.610	**0.001**	0.621	**<0.001**
BFP (%)	0.552	**<0.001**	0.648	**<0.001**
FMI	0.494	**<0.001**	0.474	**<0.001**

BMI indicates body mass index; WC, waist circumference; WHtR, waist to height ratio; BFP, body fat percentage; FMI, fat mass index.

Significant results are in **bold** ($p < 0.05$).

Table 3. Correlation coefficients between anthropometric variables and cardiovascular risk factor in subsample (270 boys and 244 girls).

		VO_{2max} (ml/kg/min)	SBP (mm Hg)	DBP (mm Hg)	TC /HDL-c	LDL-c (mg/dL)	TG (mg/dL)	HOMA (mg/dL)	CRP (mg/dL)	C-3 (mg/dL)	C-4 (mg/dL)	IL-6 (pg/mL)	Leptin (ng/mL)	Adiponectin (µg/mL)	TNF-α (ng/mL)	Visfatin (ng/mL)
NC (cm)	Boys	-0.481***	0.511***	0.335***	0.388***	0.243***	0.372***	0.255***	0.243***	0.194***	0.200***	-0.021	0.295***	-0.228***	-0.040	-0.338***
	Girls	-0.672***	0.419***	0.301***	0.056	0.016	0.284***	0.274***	0.079	0.172**	0.167**	-0.082	0.427***	-0.198**	-0.004	-0.143
BMI (kg/m^2)	Boys	-0.509***	0.448***	0.363***	0.388***	0.284***	0.358***	0.252***	0.180**	0.301***	0.253***	-0.010	0.409***	-0.233***	-0.012	-0.185*
	Girls	-0.563***	0.379***	0.312***	0.216***	0.054	0.256***	0.194***	0.173*	0.231***	0.257***	-0.022	0.613***	-0.213***	0.011	-0.075
WC (cm)	Boys	-0.496***	0.486***	0.355***	0.330***	0.236***	0.394***	0.319***	0.194**	0.256***	0.210***	-0.008	0.303***	-0.262***	0.036	-0.317***
	Girls	-0.566***	0.425***	0.327***	0.184***	0.022	0.273***	0.167***	0.094	0.198**	0.186**	-0.065	0.521***	-0.203**	-0.017	-0.045
WHtR (cm)	Boys	-0.386***	0.080**	0.193***	0.474***	0.277***	0.321***	-0.001	0.251***	0.347***	0.264***	0.177**	0.537***	-0.179**	0.010	0.131
	Girls	-0.057	0.125***	0.186***	0.354***	0.149*	0.183**	-0.007	0.255***	0.364***	0.320***	0.071	0.460***	-0.200**	0.053	0.142
BFP (%)	Boys	-0.619***	0.196***	0.255***	0.478***	-0.118*	0.356***	0.072	0.282***	0.378***	0.348***	0.043	0.495***	-0.138*	-0.019	0.007
	Girls	-0.500***	0.326***	0.305***	0.291***	0.068	0.283***	0.220**	0.174*	0.304***	0.315***	-0.008	0.608***	-0.224***	0.008	-0.033
FMI	Boys	-0.435***	0.034	0.136***	0.466***	0.286***	0.252***	-0.100	0.264***	0.347***	0.317***	0.124*	0.540***	-0.066	-0.036	0.238**
	Girls	-0.060	0.058	0.162***	0.385***	0.154*	0.198**	0.072	0.292***	0.401***	0.385***	0.102	0.514***	-0.188**	0.068	0.101

NC indicates neck circumference; BMI, body mass index; WC, waist circumference; WHtR, waist to height ratio; BFP, body fat percentage; FMI, fat mass index; VO_{2max}, maximum oxygen consumption; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC/HDL-c, total cholesterol/ high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; HOMA, homeostasis model assessment; CRP, C-reactive protein; C-3, C3 protein; C4, C-4 protein; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha.

*p<0.05, ** p<0.01, *** p<0.001.

Tabla 4. Multiple linear regression analyses of single and cluster cardiovascular risk score with neck circumference.

		Boys				Girls			
	n	Adjusted R^2	β	SE	P	Adjusted R^2	β	SE	P
Dependent variables					**Neck circumference**				
$VO_{2\ max}$ (ml/kg/min)	2146	0.231	-1.173	0.062	**<0.001**	0.018	-0.467	0.081	**<0.001**
SBP (mm Hg)	2191	0.353	2.526	0.155	**<0.001**	0.353	2.526	0.155	**<0.001**
DBP (mm Hg)	2191	0.113	1.195	0.111	**<0.001**	0.089	1.216	0.133	**<0.001**
TC/HDL-c (mg/dL)	514	0.098	0.098	0.015	**<0.001**	0.048	0.073	0.019	**<0.001**
LDL-c (mg/dL)	513	0.013	1.377	0.663	**0.039**	0.007	-0.844	0.516	0.103
Triglycerides (mg/dL)	514	0.038	1.669	0.490	**<0.001**	0.042	1.799	0.553	**<0.001**
HOMA score	342	0.169	0.256	0.043	**<0.001**	0.073	0.604	0.160	**<0.001**
C-reactive protein	315	0.055	0.351	0.094	**<0.001**	<0.001	-0.092	0.094	0.328
C-3 protein (mg/dL)	514	0.035	3.164	0.728	**<0.001**	0.026	1.630	0.557	**0.004**
C-4 protein (mg/dL)	514	0.037	0.478	0.129	**<0.001**	0.024	0.539	0.189	**0.005**
Interleukin-6 (pg/mL)	502	<0.001	-0.200	0.527	0.705	0.003	-1.552	1.139	0.174
Leptin (ng/mL)	499	0.060	713.14	155.007	**<0.001**	0.179	1927.779	252.179	**<0.001**
Adiponectin (µg/mL)	506	0.049	-0.357	0.084	**<0.001**	0.036	-0.463	0.138	**<0.001**
TNF- α (pg/mL)	478	<0.001	1.058	1.182	0.539	0.016	-2.052	3.976	0.413
Visfatin (ng/mL)	324	0.107	-0.100	0.025	**<0.001**	0.012	-0.046	0.030	0.124
Cardiovascular risk	315	0.130	0.287	0.103	**<0.001**	0.207	0.834	0.248	**<0.001**

VO_{2max} indicates maximum oxygen consumption; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC/HDL-c, total cholesterol/ high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; HOMA, homeostasis model assessment; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha.

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

Table 5. Neck circumference cutoff values for determining overweight/obese youth with BMI, WC and BFP.

	Using Cole´s BMI cutoff values		Using Katzmarzyk´s WC cutoff values		Using McCarthy´s BFP cutoff values	
AGE	BOYS	GIRLS	BOYS	GIRLS	BOYS	GIRLS
6	25.8	25.7	25.3	25.0	25.6	25.9
7	26.7	26.2	26.2	25.7	26.6	26.6
8	27.6	26.9	27.1	26.6	27.6	27.3
9	28.5	27.5	28.3	27.5	28.5	28.0
10	29.5	28.3	29.5	28.3	29.5	28.7
11	30.5	29.0	30.6	29.2	30.4	29.3
12	31.5	29.8	31.8	29.9	31.3	29.9
13	32.4	30.6	32.9	30.6	32.1	30.5
14	33.4	31.3	33.9	31.3	32.9	31.0
15	34.4	31.9	34.7	31.6	33.7	31.5
16	35.3	32.5	35.4	32.0	34.5	32.0
17	36.2	32.9	36.1	32.2	35.4	32.5
18	37.1	33.5	36.7	32.5	36.3	33.1

BMI indicates body mass index; WC, waist circumference; BFP, body fat percentage.

Supplementary table 1. Multiple linear regression analyses of maximal oxygen consumption (VO_{2max}, ml/kg/min), systolic blood pressure (SBP, mm Hg), diastolic blood pressure (DBP, mm Hg), total cholesterol/ high-density lipoprotein cholesterol (TC/HDL, mg/dL) and low-density lipoprotein cholesterol (LDL, mg/dL) with anthropometric and body composition indices.

	Boys				Girls			
	Adjusted R^2	β	SE	P	Adjusted R^2	β	SE	P
Independent variables				**$VO_{2\ max}$ (n=2146)**				
Neck circumference	0.231	-1.173	0.062	**<0.001**	0.018	-0.467	0.081	**<0.001**
Body mass index	0.253	-0.910	0.047	**<0.001**	0.044	-0.497	0.044	**<0.001**
Waist circumference	0.231	-0.379	0.022	**<0.001**	0.037	-0.197	0.019	**<0.001**
Waist-to-height ratio	0.113	-61.143	3.823	**<0.001**	0.039	-31.074	3.138	**<0.001**
Body fat percentage	0.285	-0.339	0.016	**<0.001**	0.066	-0.266	0.018	**<0.001**
Fat mass index	0.111	-0.684	0.036	**<0.001**	0.058	-0.492	0.039	**<0.001**
				SBP (n=2191)				
Neck circumference	0.353	2.526	0.155	**<0.001**	0.353	2.526	0.155	**<0.001**
Body mass index	0.239	1.237	0.100	**<0.001**	0.168	1.059	0.096	**<0.001**
Waist circumference	0.281	0.552	0.042	**<0.001**	0.200	0.515	0.042	**<0.001**
Waist-to-height ratio	0.053	66.232	7.317	**<0.001**	0.042	57.874	6.994	**<0.001**
Body fat percentage	0.047	0.279	0.033	**<0.001**	0.127	0.436	0.042	**<0.001**
Fat mass index	0.033	0.531	0.073	**<0.001**	0.026	0.603	0.090	**<0.001**
				DBP (n=2191)				
Neck circumference	0.113	1.195	0.111	**<0.001**	0.089	1.216	0.133	**<0.001**
Body mass index	0.132	0.793	0.061	**<0.001**	0.095	0.741	0.071	**<0.001**
Waist circumference	0.126	0.293	0.023	**<0.001**	0.105	0.328	0.030	**<0.001**
Waist-to-height ratio	0.058	46.204	4.984	**<0.001**	0.035	47.497	5.554	**<0.001**
Body fat percentage	0.065	0.198	0.022	**<0.001**	0.092	0.323	0.035	**<0.001**
Fat mass index	0.061	0.434	0.049	**<0.001**	0.047	0.588	0.072	**<0.001**
				Total cholesterol/HDL cholesterol (n=514)				
Neck circumference	0.098	0.098	0.015	**<0.001**	0.048	0.073	0.019	**<0.001**
Body mass index	0.148	1.715	0.183	**<0.001**	0.045	0.003	0.010	**<0.001**
Waist circumference	0.106	0.022	0.003	**<0.001**	0.030	0.027	0.005	**<0.001**
Waist-to-height ratio	0.222	5.999	0.617	**0.001**	0.126	4.562	0.688	**<0.001**
Body fat percentage	0.226	0.025	0.003	**<0.001**	0.081	0.032	0.005	**<0.001**
Fat mass index	0.143	0.062	0.009	**<0.001**	0.143	0.062	0.009	**<0.001**
				LDL cholesterol (n=513)				
Neck circumference	0.013	1.377	0.663	**0.039**	0.007	-0.844	0.516	0.103
Body mass index	0.001	0.445	0.407	0.275	<0.001	-0.400	0.390	0.306
Waist circumference	<0.001	0.094	0.152	0.535	0.002	-0.205	0.169	0.225
Waist-to-height ratio	0.002	39.593	30.87	0.201	<0.001	25.630	31.298	0.414
Body fat percentage	0.002	0.186	0.147	0.209	<0.001	-0.046	0.187	0.806
Fat mass index	0.002	0.354	0.286	0.216	0.004	0.560	0.400	0.162

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

Supplementary table 2. Multiple linear regression analyses of triglycerides (mg/dL), of insulin resistance (HOMA score), C-reactive protein (CRP, mg/dL), C3-protein (C-3, mg/dL) and C4-protein (C-4, mg/dL) with anthropometric and body composition indices.

	Boys				Girls			
	Adjusted	β	SE	P	Adjusted R^2	β	SE	P
Independent variables				**Triglycerides (n=514)**				
Neck circumference	0.038	1.669	0.490	**<0.001**	0.042	1.799	0.553	**<0.001**
Body mass index	0.033	1.669	0.524	**<0.001**	0.037	1.262	0.416	**<0.001**
Waist circumference	0.053	0.748	0.193	**<0.001**	0.042	0.590	0.181	**0.001**
Waist-to-height ratio	0.019	97.477	41.998	**0.003**	<0.001	0.108	1.690	0.108
Body fat percentage	0.015	0.382	0.190	**0.045**	0.036	0.637	0.200	**0.002**
Fat mass index	<0.001	0.802	0.418	0.056	0.01	1.037	0.047	**0.021**
				HOMA (n=342)				
Neck circumference	0.169	0.256	0.043	**<0.001**	0.073	0.604	0.160	**<0.001**
Body mass index	0.117	0.151	0.043	**0.001**	0.029	0.124	0.050	**0.015**
Waist circumference	0.162	0.066	0.019	**<0.001**	0.009	0.037	0.024	0.119
Waist-to-height ratio	0.032	8.029	3.110	**0.011**	0.003	-4.321	3.612	0.233
Body fat percentage	0.044	0.043	0.014	**0.003**	0.015	0.045	0.024	0.060
Fat mass index	0.021	0.069	0.033	**0.039**	<0.001	0.009	0.047	0.847
				CRP (n=315)				
Neck circumference	0.055	0.351	0.094	**<0.001**	<0.001	-0.092	0.094	0.328
Body mass index	0.028	0.291	0.106	**0.007**	0.025	0.221	0.069	**0.002**
Waist circumference	0.033	0.120	0.040	**0.003**	0.002	-0.034	0.030	0.257
Waist-to-height ratio	0.015	8.941	4.762	**0.047**	<0.001	5.088	5.291	0.338
Body fat percentage	0.059	29.624	7.558	**<0.001**	0.060	15.668	4.388	**<0.001**
Fat mass index	0.065	0.226	0.059	**<0.001**	0.080	0.236	0.057	**<0.001**
				C-3 (n=514)				
Neck circumference	0.035	3.164	0.728	**<0.001**	0.026	1.630	0.557	**0.004**
Body mass index	0.088	2.364	0.413	**<0.001**	0.050	1.644	0.412	**<0.001**
Waist circumference	0.063	1.133	0.220	**<0.001**	0.039	0.648	0.191	**0.001**
Waist-to-height ratio	0.118	206.454	30.784	**<0.001**	0.130	193.054	30.910	**<0.001**
Body fat percentage	0.140	0.915	0.129	**<0.001**	0.089	1.024	0.192	**<0.001**
Fat mass index	0.118	1,884	0.285	**<0.001**	0.158	2.784	0.382	**<0.001**
				C-4 (n=514)				
Neck circumference	0.037	0.478	0.129	**<0.001**	0.024	0.539	0.189	**0.005**
Body mass index	0.061	0.671	0.141	**<0.001**	0.063	0.619	0.139	**0.002**
Waist circumference	0.041	0.241	0.055	**<0.001**	0.031	0.271	0.065	**<0.001**
Waist-to-height ratio	0.067	53.401	10.570	**<0.001**	0.99	56.768	10.655	**<0.001**
Body fat percentage	0.118	0.277	0.043	**<0.001**	0.99	0.36	0.065	**<0.001**
Fat mass index	0.098	0.574	0.094	**<0.001**	0.145	0.869	0.132	**<0.001**

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

Supplementary table 3. Multiple linear regression analyses of interleukin 6 (IL-6, pg/mJ) leptin (ng/mL), adiponectin (µg/mL), and visfatin (ng/mL) with anthropometric and body composition indices.

	Boys				Girls			
	Adjusted	β	SE	P	Adjusted	β	SE	P
Independent variables				**Il-6 (n=502)**				
Neck circumference	<0.001	-0.200	0.527	0.705	0.003	-1.552	1.139	0.174
Body mass index	<0.001	-0.106	0.590	0.857	<0.001	-0.753	1.001	0.453
Waist circumference	<0.001	-0.030	0.225	0.893	0.001	-0.425	0.391	0.279
Waist-to-height ratio	0.028	139.795	43.662	**0.002**	0.001	77.657	65.893	0.240
Body fat percentage	<0.001	0.255	0.183	0.164	<0.001	0.325	0.448	0.469
Fat mass index	0.012	0.739	0.384	**0.048**	0.007	1.402	0.831	0.093
				Leptin (n=499)				
Neck circumference	0.060	713.142	155.007	**<0.001**	0.179	1927.779	252.179	**<0.001**
Body mass index	0.165	1158.997	89.388	**<0.001**	0.374	1747.644	139.514	**<0.001**
Waist circumference	0.089	495.536	40.142	**<0.001**	0.269	554.476	54.713	**<0.001**
Waist-to-height ratio	0.286	66804.453	5902.475	**<0.001**	0.208	84803.472	9319.467	**<0.001**
Body fat percentage	0.242	300.358	26.911	**<0.001**	0.368	668.546	52.889	**<0.001**
Fat mass index	0.289	612.946	56.721	**<0.001**	0.261	1183.717	113.155	**<0.001**
				Adiponectin (n=506)				
Neck circumference	0.049	-0.357	0.084	**<0.001**	0.036	-0.463	0.138	**<0.001**
Body mass index	0.051	-0.282	0.110	**0.010**	0.042	-0.371	0.102	**<0.001**
Waist circumference	0.066	-0.175	0.036	**<0.001**	0.038	-0.162	0.047	**0.001**
Waist-to-height ratio	0.030	-23.791	7.015	**0.001**	0.036	-26.967	7.972	**0.001**
Body fat percentage	0.016	-0.045	0.032	**0.016**	0.047	-0.186	0.049	**<0.001**
Fat mass index	0.001	-0.100	0-0067	0.135	0.032	-0.319	0.101	**0.002**
				TNF- α (n=478)				
Neck circumference	<0.001	1.058	1.182	0.539	0.016	-2.052	3.976	0.413
Body mass index	0.001	2.915	1.199	0.628	0.001	-5.176	1.887	0.459
Waist circumference	0.003	0.366	0.526	0.541	0.003	-0,539	0.968	0.409
Waist-to-height ratio	<0.001	11.347	7.602	0.881	<0.001	8.959	6.398	0.926
Body fat percentage	<0.001	-0.104	0.101	0.743	0.002	-0.466	4.619	0.460
Fat mass index	0.002	-0.290	0.632	0.662	0.001	0.114	1.840	0.924
				Visfatin (n=324)				
Neck circumference	0.107	-0.100	0.025	**<0.001**	0.012	-0.046	0.030	0.124
Body mass index	0.026	-0.005	0.033	0.876	<0.001	-0.019	0.024	0.421
Waist circumference	0.093	-0.017	0.015	0.264	<0.001	-0.005	0.011	0.627
Waist-to-height ratio	0.010	1.263	2.136	0.555	0.012	2.689	1.736	0.124
Body fat percentage	<0.001	0.006	0,011	0.606	<0.001	-0.004	0.011	0.722
Fat mass index	0.057	0.025	0.022	0.273	0.002	0.022	0.024	0.278

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

Supplementary table 4. Multiple linear regression analyses of cardiovascular risk score with anthropometric and body composition indices.

	Boys				Girls			
	Adjusted	β	SE	P	Adjusted	β	SE	P
Independent variables	**Clustered CVD risk factor (n=315)**							
Neck circumference	0.130	0.287	0.103	**<0.001**	0.207	0.834	0.248	**<0.001**
Body mass index	0.095	0.201	0.104	**<0.001**	0.111	0.432	0.095	**<0.001**
Waist circumference	0.125	0.175	0.027	**<0.001**	0.073	0.141	0.028	**<0.001**
Waist-to-height ratio	0.175	34.634	7.176	**<0.001**	0.174	21.415	6.394	**<0.001**
Body fat percentage	0.113	0.119	0.033	**<0.001**	0.143	0.194	0.042	**<0.001**
Fat mass index	0.109	0.268	0.076	**<0.001**	0.290	0.419	0.088	**<0.001**

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

Supplementary table 1. Multiple linear regression analyses of maximal oxygen consumption (VO_{2max}, ml/kg/min), systolic blood pressure (SBP, mm Hg), diastolic blood pressure (DBP, mm Hg), total cholesterol/ high-density lipoprotein cholesterol (TC/HDL, mg/dL) and low-density lipoprotein cholesterol (LDL, mg/dL) with anthropometric and body composition indices.

	Boys				Girls			
	Adjusted R^2	β	SE	P	Adjusted R^2	β	SE	P
Independent variables				**$VO_{2\ max}$ (n=2146)**				
Neck circumference	0.231	-1.173	0.062	**<0.001**	0.018	-0.467	0.081	**<0.001**
Body mass index	0.253	-0.910	0.047	**<0.001**	0.044	-0.497	0.044	**<0.001**
Waist circumference	0.231	-0.379	0.022	**<0.001**	0.037	-0.197	0.019	**<0.001**
Waist-to-height ratio	0.113	-61.143	3.823	**<0.001**	0.039	-31.074	3.138	**<0.001**
Body fat percentage	0.285	-0.339	0.016	**<0.001**	0.066	-0.266	0.018	**<0.001**
Fat mass index	0.111	-0.684	0.036	**<0.001**	0.058	-0.492	0.039	**<0.001**
				SBP (n=2191)				
Neck circumference	0.353	2.526	0.155	**<0.001**	0.353	2.526	0.155	**<0.001**
Body mass index	0.239	1.237	0.100	**<0.001**	0.168	1.059	0.096	**<0.001**
Waist circumference	0.281	0.552	0.042	**<0.001**	0.200	0.515	0.042	**<0.001**
Waist-to-height ratio	0.053	66.232	7.317	**<0.001**	0.042	57.874	6.994	**<0.001**
Body fat percentage	0.047	0.279	0.033	**<0.001**	0.127	0.436	0.042	**<0.001**
Fat mass index	0.033	0.531	0.073	**<0.001**	0.026	0.603	0.090	**<0.001**
				DBP (n=2191)				
Neck circumference	0.113	1.195	0.111	**<0.001**	0.089	1.216	0.133	**<0.001**
Body mass index	0.132	0.793	0.061	**<0.001**	0.095	0.741	0.071	**<0.001**
Waist circumference	0.126	0.293	0.023	**<0.001**	0.105	0.328	0.030	**<0.001**
Waist-to-height ratio	0.058	46.204	4.984	**<0.001**	0.035	47.497	5.554	**<0.001**
Body fat percentage	0.065	0.198	0.022	**<0.001**	0.092	0.323	0.035	**<0.001**
Fat mass index	0.061	0.434	0.049	**<0.001**	0.047	0.588	0.072	**<0.001**
				Total cholesterol/HDL cholesterol (n=514)				
Neck circumference	0.098	0.098	0.015	**<0.001**	0.048	0.073	0.019	**<0.001**
Body mass index	0.148	1.715	0.183	**<0.001**	0.045	0.003	0.010	**<0.001**
Waist circumference	0.106	0.022	0.003	**<0.001**	0.030	0.027	0.005	**<0.001**
Waist-to-height ratio	0.222	5.999	0.617	**0.001**	0.126	4.562	0.688	**<0.001**
Body fat percentage	0.226	0.025	0.003	**<0.001**	0.081	0.032	0.005	**<0.001**
Fat mass index	0.143	0.062	0.009	**<0.001**	0.143	0.062	0.009	**<0.001**
				LDL cholesterol (n=513)				
Neck circumference	0.013	1.377	0.663	**0.039**	0.007	-0.844	0.516	0.103
Body mass index	0.001	0.445	0.407	0.275	<0.001	-0.400	0.390	0.306
Waist circumference	<0.001	0.094	0.152	0.535	0.002	-0.205	0.169	0.225
Waist-to-height ratio	0.002	39.593	30.87	0.201	<0.001	25.630	31.298	0.414
Body fat percentage	0.002	0.186	0.147	0.209	<0.001	-0.046	0.187	0.806
Fat mass index	0.002	0.354	0.286	0.216	0.004	0.560	0.400	0.162

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

Supplementary table 2. Multiple linear regression analyses of triglycerides (mg/dL), of insulin resistance (HOMA score), C-reactive protein (CRP, mg/dL), C3-protein (C-3, mg/dL) and C4-protein (C-4, mg/dL) with anthropometric and body composition indices.

	Boys				Girls			
	Adjusted	β	SE	P	Adjusted R^2	β	SE	P
Independent variables				**Triglycerides (n=514)**				
Neck circumference	0.038	1.669	0.490	**<0.001**	0.042	1.799	0.553	**<0.001**
Body mass index	0.033	1.669	0.524	**<0.001**	0.037	1.262	0.416	**<0.001**
Waist circumference	0.053	0.748	0.193	**<0.001**	0.042	0.590	0.181	**0.001**
Waist-to-height ratio	0.019	97.477	41.998	**0.003**	<0.001	0.108	1.690	0.108
Body fat percentage	0.015	0.382	0.190	**0.045**	0.036	0.637	0.200	**0.002**
Fat mass index	<0.001	0.802	0.418	0.056	0.01	1.037	0.047	**0.021**
				HOMA (n=342)				
Neck circumference	0.169	0.256	0.043	**<0.001**	0.073	0.604	0.160	**<0.001**
Body mass index	0.117	0.151	0.043	**0.001**	0.029	0.124	0.050	**0.015**
Waist circumference	0.162	0.066	0.019	**<0.001**	0.009	0.037	0.024	0.119
Waist-to-height ratio	0.032	8.029	3.110	**0.011**	0.003	-4.321	3.612	0.233
Body fat percentage	0.044	0.043	0.014	**0.003**	0.015	0.045	0.024	0.060
Fat mass index	0.021	0.069	0.033	**0.039**	<0.001	0.009	0.047	0.847
				CRP (n=315)				
Neck circumference	0.055	0.351	0.094	**<0.001**	<0.001	-0.092	0.094	0.328
Body mass index	0.028	0.291	0.106	**0.007**	0.025	0.221	0.069	**0.002**
Waist circumference	0.033	0.120	0.040	**0.003**	0.002	-0.034	0.030	0.257
Waist-to-height ratio	0.015	8.941	4.762	**0.047**	<0.001	5.088	5.291	0.338
Body fat percentage	0.059	29.624	7.558	**<0.001**	0.060	15.668	4.388	**<0.001**
Fat mass index	0.065	0.226	0.059	**<0.001**	0.080	0.236	0.057	**<0.001**
				C-3 (n=514)				
Neck circumference	0.035	3.164	0.728	**<0.001**	0.026	1.630	0.557	**0.004**
Body mass index	0.088	2.364	0.413	**<0.001**	0.050	1.644	0.412	**<0.001**
Waist circumference	0.063	1.133	0.220	**<0.001**	0.039	0.648	0.191	**0.001**
Waist-to-height ratio	0.118	206.454	30.784	**<0.001**	0.130	193.054	30.910	**<0.001**
Body fat percentage	0.140	0.915	0.129	**<0.001**	0.089	1.024	0.192	**<0.001**
Fat mass index	0.118	1,884	0.285	**<0.001**	0.158	2.784	0.382	**<0.001**
				C-4 (n=514)				
Neck circumference	0.037	0.478	0.129	**<0.001**	0.024	0.539	0.189	**0.005**
Body mass index	0.061	0.671	0.141	**<0.001**	0.063	0.619	0.139	**0.002**
Waist circumference	0.041	0.241	0.055	**<0.001**	0.031	0.271	0.065	**<0.001**
Waist-to-height ratio	0.067	53.401	10.570	**<0.001**	0.99	56.768	10.655	**<0.001**
Body fat percentage	0.118	0.277	0.043	**<0.001**	0.99	0.36	0.065	**<0.001**
Fat mass index	0.098	0.574	0.094	**<0.001**	0.145	0.869	0.132	**<0.001**

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

Supplementary table 3. Multiple linear regression analyses of interleukin 6 (IL-6, pg/mJ) leptin (ng/mL), adiponectin (µg/mL), and visfatin (ng/mL) with anthropometric and body composition indices.

	Boys				Girls			
	Adjusted	β	SE	P	Adjusted	β	SE	P
Independent variables				**Il-6 (n=502)**				
Neck circumference	<0.001	-0.200	0.527	0.705	0.003	-1.552	1.139	0.174
Body mass index	<0.001	-0.106	0.590	0.857	<0.001	-0.753	1.001	0.453
Waist circumference	<0.001	-0.030	0.225	0.893	0.001	-0.425	0.391	0.279
Waist-to-height ratio	0.028	139.795	43.662	**0.002**	0.001	77.657	65.893	0.240
Body fat percentage	<0.001	0.255	0.183	0.164	<0.001	0.325	0.448	0.469
Fat mass index	0.012	0.739	0.384	**0.048**	0.007	1.402	0.831	0.093
				Leptin (n=499)				
Neck circumference	0.060	713.142	155.007	**<0.001**	0.179	1927.779	252.179	**<0.001**
Body mass index	0.165	1158.997	89.388	**<0.001**	0.374	1747.644	139.514	**<0.001**
Waist circumference	0.089	495.536	40.142	**<0.001**	0.269	554.476	54.713	**<0.001**
Waist-to-height ratio	0.286	66804.453	5902.475	**<0.001**	0.208	84803.472	9319.467	**<0.001**
Body fat percentage	0.242	300.358	26.911	**<0.001**	0.368	668.546	52.889	**<0.001**
Fat mass index	0.289	612.946	56.721	**<0.001**	0.261	1183.717	113.155	**<0.001**
				Adiponectin (n=506)				
Neck circumference	0.049	-0.357	0.084	**<0.001**	0.036	-0.463	0.138	**<0.001**
Body mass index	0.051	-0.282	0.110	**0.010**	0.042	-0.371	0.102	**<0.001**
Waist circumference	0.066	-0.175	0.036	**<0.001**	0.038	-0.162	0.047	**0.001**
Waist-to-height ratio	0.030	-23.791	7.015	**0.001**	0.036	-26.967	7.972	**0.001**
Body fat percentage	0.016	-0.045	0.032	**0.016**	0.047	-0.186	0.049	**<0.001**
Fat mass index	0.001	-0.100	0-0067	0.135	0.032	-0.319	0.101	**0.002**
				TNF- α (n=478)				
Neck circumference	<0.001	1.058	1.182	0.539	0.016	-2.052	3.976	0.413
Body mass index	0.001	2.915	1.199	0.628	0.001	-5.176	1.887	0.459
Waist circumference	0.003	0.366	0.526	0.541	0.003	-0,539	0.968	0.409
Waist-to-height ratio	<0.001	11.347	7.602	0.881	<0.001	8.959	6.398	0.926
Body fat percentage	<0.001	-0.104	0.101	0.743	0.002	-0.466	4.619	0.460
Fat mass index	0.002	-0.290	0.632	0.662	0.001	0.114	1.840	0.924
				Visfatin (n=324)				
Neck circumference	0.107	-0.100	0.025	**<0.001**	0.012	-0.046	0.030	0.124
Body mass index	0.026	-0.005	0.033	0.876	<0.001	-0.019	0.024	0.421
Waist circumference	0.093	-0.017	0.015	0.264	<0.001	-0.005	0.011	0.627
Waist-to-height ratio	0.010	1.263	2.136	0.555	0.012	2.689	1.736	0.124
Body fat percentage	<0.001	0.006	0,011	0.606	<0.001	-0.004	0.011	0.722
Fat mass index	0.057	0.025	0.022	0.273	0.002	0.022	0.024	0.278

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

Supplementary table 4. Multiple linear regression analyses of cardiovascular risk score with anthropometric and body composition indices.

	Boys				Girls			
	Adjusted	β	SE	P	Adjusted	β	SE	P
Independent variables	**Clustered CVD risk factor (n=315)**							
Neck circumference	0.130	0.287	0.103	**<0.001**	0.207	0.834	0.248	**<0.001**
Body mass index	0.095	0.201	0.104	**<0.001**	0.111	0.432	0.095	**<0.001**
Waist circumference	0.125	0.175	0.027	**<0.001**	0.073	0.141	0.028	**<0.001**
Waist-to-height ratio	0.175	34.634	7.176	**<0.001**	0.174	21.415	6.394	**<0.001**
Body fat percentage	0.113	0.119	0.033	**<0.001**	0.143	0.194	0.042	**<0.001**
Fat mass index	0.109	0.268	0.076	**<0.001**	0.290	0.419	0.088	**<0.001**

Significant results are in **bold** ($p < 0.05$).

β indicates estimated unstandardized regression coefficient; SE, standard error.

All analyses were controlled for age and Tanner stage.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any pre-specified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7-10*
Bias	9	Describe any efforts to address potential sources of bias	11
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10-11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	11

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-14
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	4
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-19
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	20

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Correction: *Neck circumference and clustered cardiovascular risk factors in children and adolescents: cross-sectional study*

Castro-Piñero J, Delgado-Alfonso A, Gracia-Marco L The UP&DOWN Study Group, *et al*. Neck circumference and clustered cardiovascular risk factors in children and adolescents: cross-sectional study. *BMJ Open* 2017;7:e016048. doi:10.1136/bmjopen-2017-016048

An error in translating the results from the tables to the text has been included in this study. Statistical analysis was correct as well as the results shown in tables and figure. The correction of this error does not change the results or conclusions of the study, but for clarification, the following corrections are noted:

1. In Results section of the Abstract, 'NC was negatively associated with maximum oxygen consumption (R2=0.231, P<0.001 for boys; R2=0.018, P<0.001 for girls) and positively associated with SBP, DBP, TC/HDL-c, TG, HOMA, complement factors C-3 and C-4, leptin, adiponectin and clustered CVD risk factor in both sexes (R2 from 0.035 to 0.353, P<0.01 for boys; R2 from 0.024 to 0.215, P<0.001 for girls). Moreover, NC was positively associated with serum C reactive protein, LDL-c and visfatin only in boys (R2 from 0.013 to 0.107, P<0.05).' should read 'NC was negatively associated with maximum oxygen consumption (R2=0.231, P<0.001 for boys; R2=0.018, P<0.001 for girls) and adiponectin (R2=0.049, P<0.001 for boys; R2=0.036, P<0.001 for girls); and positively associated with SBP, DBP, TC/HDL-c, TG, HOMA, complement factors C-3 and C-4, leptin and clustered CVD risk factor in both sexes (R2 from 0.035 to 0.353, P<0.01 for boys; R2 from 0.024 to 0.215, P<0.001 for girls). Moreover, NC was positively associated with serum C reactive protein and LDL-c only in boys (R2 from 0.013 to 0.055, P<0.05)'.
2. In Clustered CVD risk factors measurement section of the Method, 'The VO2max z-score was inverted, because higher cardiorespiratory fitness is associated with lower fatness' should read 'The VO2max and adiponectin z-scores were inverted, because higher values of both are associated with lower fatness'.
3. Related to Table 3 of the Results, 'More specifically, NC was negatively correlated with VO2max (r=−0.481, P<0.001 for boys; r=−0.672, P<0.001 for girls), positively correlated with SBP, DBP, TG, HOMA, C-3, C-4, leptin and adiponectin in both sexes (r from 0.167 to 0.419; all, P<0.01), and with TC/HDL-c, LDL-c, CRP and visfatin only in boys (r from 0.243 to 0.388; all, P<0.001).' should read 'More specifically, NC was negatively correlated with VO2max (r=−0.481, P<0.001 for boys; r=−0.672, P<0.001 for girls), adiponectin (r=−0.228, P<0.001 for boys; r=−0.198, P<0.001 for girls) and visfatin only in boys (r=−0.338, P<0.001), and positively correlated with SBP, DBP, TG, HOMA, C-3, C-4 and leptin in both sexes (r from 0.167 to 0.419; all, P<0.01), and with TC/HDL-c, LDL-c and CRP only in boys (r from 0.243 to 0.388; all, P<0.001)'.
4. Related to Table 4 of the Results, 'NC was negatively associated with VO2max (R2=0.231, P<0.001 for boys; R2=0.018, P<0.001 for girls), and positively associated with SBP, DBP, TC/HDL-c, TG, HOMA, C-3, C-4, leptin, adiponectin and clustered CVD risk factor in both sexes (R2 from 0.035 to 0.353, P<0.01 for boys; R2 from 0.024 to 0.215, P<0.001 for girls). Moreover, NC was positively associated with CRP, LDL-c and visfatin only in boys (R2 from 0.013 to 0.107, P<0.05).' should read 'NC was negatively associated with maximum oxygen consumption (R2=0.231, P<0.001 for boys; R2=0.018, P<0.001 for girls), adiponectin (R2=0.049, P<0.001 for boys; R2=0.036, P<0.001 for girls) and visfatin only in boys (R2=0.107, P<0.001); and positively associated with SBP, DBP, TC/HDL-c, TG, HOMA, complement factors C-3 and C-4, leptin and clustered CVD risk factor in both sexes (R2 from 0.035 to 0.353, P<0.01 for boys; R2 from 0.024 to 0.215, P<0.001 for girls). Moreover, NC was positively associated with CRP and LDL-c only in boys (R2 from 0.013 to 0.055, P<0.05)'
5. In NC and single CVD risk factors section of Discussion 'In the present study, IL-6 and visfatin were associated with WHtR and FMI in both sexes and only with NC in

boys' should read 'In the present study, IL-6 was associated with WHtR and FMI in boys only, whilst visfatin was associated with NC in boys only'

BMJ Open 2018;**8**:e016048corr1. doi:10.1136/bmjopen-2017-016048corr1