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Relationship between abdominal adiposity, cardiovascular fitness, and biomarkers of cardiovascular risk in British adolescents

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Abstract

ObjectivePuberty is a critical time in the development of overweight and obesity. The aim of this study was to examine relationships between measures of adiposity, cardiovascular fitness, and biomarkers of cardiovascular disease risk in adolescents.

Methods

In a cross-sectional study design, 129 girls and 95 boys aged 12.9–14.4 years at various stages of puberty were included, along with their mothers (*n* = 217) and fathers (*n* = 207). Anthropometric assessments of adiposity were made, along with cardiovascular physical fitness, using the 20-m shuttle-run test, and biomarkers associated with cardiovascular risk, including glucose, insulin, triglyceride, fibrinogen, and C-reactive protein concentrations.

Results

Waist-to-height ratio (WHtR) values were similar in boys and girls and correlated positively with glucose, insulin, triglyceride, fibrinogen, and C-reactive protein concentrations, and inversely with cardiovascular fitness scores. Skinfold-thickness (SKF) measurements were higher in girls. high molecular weight (HMW)-adiponectin concentrations were lower in boys compared to girls, particularly in late puberty, and C-reactive protein levels were higher. Cardiovascular fitness, maternal body mass index (BMI), and paternal BMI contributed independently to the variance in waist measurements in girls and boys. Gender, triceps SKF, and WHtR, but not parental BMI, contributed independently to the variance in cardiovascular fitness.

Conclusion

There is a relationship between measures of adolescent adiposity and parental weight that involves factors other than cardiovascular fitness. Adolescent boys have relatively more abdominal fat than girls and a tendency to have a proinflammatory profile of biomarkers. These observations suggest that family and social environmental interventions are best undertaken earlier in childhood, particularly among boys.

Keywords: adiposity, cardiovascular fitness, cardiovascular risk, inflammation, puberty.

1. Introduction

Childhood obesity predicts obesity later in adulthood[1] and is associated with adult cardiovascular disease and related mortality.[2] Increasing prevalence of obesity in childhood is therefore likely to have pathophysiological consequences and translate to an increased incidence of cardiovascular events in adulthood.[3] It is now recognized that there is an interaction between overweight and fitness that contributes to cardiovascular risk and prognosis.[4] Adolescence is associated with increases in overweight and obesity[5] and a reduction in levels of physical activity.[6] Puberty is therefore a stage when promotion of healthy behaviors may be crucial. Family environment is likely to play an important role; for example, an overweight mother and a single-parent family are associated with an increased likelihood of a child being overweight or obese.[7]

In adolescence, calculation of body mass index (BMI) does not fully adjust for the effect of height.[8,9] The waist-to-height ratio (WHtR) is regarded as a better marker of adiposity[10] and may be a stronger predictor of cardiovascular disease risk factors.[11] Rising adiposity in childhood, however, is not necessarily accompanied by deteriorating metabolic profile.[12] It is likely that a combination of adiposity measures and proinflammatory markers is a better predictor than measurement of adiposity alone.[13] There is an effect of gender on these measures; for example it is reported that high-sensitivity C-reactive protein (hsCRP) concentrations are higher in girls.[14]

The aim of this study was to examine the effect of gender and puberty on the relationship between measures of adiposity and cardiovascular fitness and (i) inflammatory and metabolic biomarkers known to be associated with disease risk in adults, including serum CRP, insulin and lipid concentrations and (ii) parental BMI and waist circumference. We have

previously shown that aerobic fitness, estimated with a 20-metre shuttle-run test, is inversely related to measures of adiposity in adolescent boys and girls with no significant effect of pubertal status on the relationship.[8] It was hypothesised that in this group of adolescents those with greater adiposity and lower cardiovascular fitness are more likely to have a biomarker profile that is associated with increased cardiovascular risk. Furthermore, it was hypothesised that an increased cardiovascular risk profile is more likely in boys and in children whose parents are overweight or obese.

2. Methods

2.1 Participants

In a cross-sectional study of children in Year 8 (age range 12.9 –14.4 years) in 3 schools in Carmarthenshire, Wales,[15] information letters were sent to parents and guardians of potential participants. The response rate was 86%. The number of children registered as receiving free school meals was lower than the national average (1% *vs.* 16%),[15] and was similar in all 3 schools, indicating similar socioeconomic status.[16] Signed consent was obtained from parents or guardians and children. The study was conducted in accordance with the Declaration of Helsinki and approved by the local National Health Service Research Ethics Committee (Dyfed Powys; 07/WMW01/12).

Data on self-assessed pubertal status, along with measurements of weight, waist, height, and skinfold thickness (SKF) were available from 247 participants. The relationship between weight, waist, height, and cardiovascular fitness measurements in that group were presented in a previous publication.[8] For inclusion in the present study, those that had a measure of cardiovascular fitness and blood analysis that included insulin concentration, and

were selected. A total of 224 participants (129 girls and 95 boys) met the inclusion criteria. Weight, waist, height, and SKF measurements for this group were similar to the larger cohort (data not shown).

2.2 Physical measurements

All measurements were carried out by the same researcher, an experienced paediatric exercise physiologist. Children wore light clothing and were barefoot. Privacy was assured and two gender-specific adults were present at all times. Body height was measured using a portable stadiometer (Holtain Ltd, Crymych, Pembrokeshire, UK) and weight was measured using a calibrated Philips HP 5320 electronic scale (Philips N.V., Amsterdam, The Netherlands). Waist circumference was measured, directly over the skin, at the smallest circumference between the lower costal margin and iliac crest using anthropometric tape (Holtain Ltd). Waist is also reported as a percentage of height (WHtR%) when appropriate. SKF measurements were made at the triceps, biceps, subscapular, and suprailiac sites on the right side using Harpenden skin-fold callipers (Holtain Ltd, Brynberian, UK) and standard techniques. Anthropometric measurements were made in duplicate and if the values differed by greater than 1.0 mm or 0.1kg, a third measurement was taken. The intra-observer technical error of measurement (TEM)[16] was determined in a study of 20 measurements. The TEM was < 1.0 mm (coefficient of reliability [R] > 0.99) for height measurement. The TEM for waist was 0.98 mm (R = 0.973), and TEMs for SKF were 0.42 mm (R = 0.996), 0.42 mm (R = 0.989), 0.51 mm (R = 0.993), and 0.54 mm (R = 0.992), respectively, for triceps, biceps, subscapular, and suprailiac sites. Systolic and diastolic blood pressure was taken 3 times, after the child had been sitting quietly for 5 min (Dinamap IL, Critikron, Inc., Tampa, FL). The average of the second and third readings was recorded.

Parents were asked to complete a form reporting the child's birth-weight, as well as their own date of birth and adult height, weight, and waist measurements. Parental self-reported height and weight and/or waist measurements were available from parents of 218 of the 224 children: 217 mothers (mean age 41.7 years, 95% confidence interval (CI) [41.0-42.3 years]) and 207 fathers (mean age 44.0 years [95% CI 43.2–44.9 years]). Maternal BMI, calculated from self-reported weight and height, was 26.6 kg/m^2 (95% CI 25.9–27.3 kg/m^2, *n* = 209) and paternal BMI was 28.0 kg/m^2 (95% CI 27.5–28.6 kg/m^2, *n* = 196). Since self-reported height tends to be overestimated and weight underestimated, published equations were used to adjust parental BMI values.[17] The formula used for the mother's value was $BMI_{corrected} = 0.12 + 1.05XBMI_{self\text{-}reported}$, and for the father's it was $BMI_{corrected} = 0.12 + 1.05XBMI_{self\text{-}reported}$. Corrected maternal BMI was 27.8 kg/m^2 (95% CI 27.1–28.5 kg/m^2), and corrected paternal BMI was 29.2 kg/m^2 (95% CI 28.6-29.8 kg/m^2).

2.3 Pubertal assessment

Pubertal status was determined with self-assessment questionnaires using gender-specific line drawings of the stages[18] based on those described by Tanner.[19] Children completed the questionnaire alone and in private at home. Within each gender group, those reporting being in stages 1–3 (T1–3) and in stages 4–5 (T4–5), were combined for data analysis.[8] All children reporting being in stage 1(T1) for breast or genital development reported being in stages 2–4(T2–4) for pubic hair development (Table I). Status assessed by breast development or genital development was used for the correlations. Similar patterns were observed in correlations using pubic hair development. Since stages of pubertal development are not equivalent, analyses were performed separately for boys and girls.

2.4 Cardiovascular fitness

A 20-m shuttle-run test was used as an indirect measurement of maximal aerobic power.[20] Participants ran between 2 parallel lines 20 metres apart. A commercially available audio tape that emits a beep at the point where the runner should be pivoting at the next line was used. The pacer started at 8.5 km/h and increased by 8.5 km/h each minute and was timed for accuracy before each session. Boys and girls performed separately. Testing took place at the same time of day, supervised by a person trained in the method, who gave consistent verbal encouragement. All participants were fully familiarised with testing procedures prior to data collection. The test ended if running could not be maintained 2 laps in succession, or voluntarily if the participant was exhausted. The number of laps completed was used as the cardiovascular fitness score.

2.5 Biomarkers

Serum was prepared from blood samples collected between 9 am and 12 noon following an overnight fast and after the child had been sitting at least 30 min. Glucose was determined by the glucose oxidase method (Randox Laboratories LTD, Crumlin, CO Antrim, UK). Total cholesterol and triglyceride concentrations were determined by routine enzymatic techniques (Vitros 950 System, Ortho-Clinical Diagnostics, Amersham, Bucks, UK). Laboratory analytical variances were 1.5%, 1.6%, and 2%, respectively, for the above 3measurements. High density lipoprotein (HDL)-cholesterol was determined after precipitation of very-low-density and low-density lipoproteins (LDLs) with dextran sulphate and magnesium chloride (coefficient of variation (CV) 5.3%). LDL-C concentrations were estimated by the Friedwald formula. Insulin concentration was determined using an enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, NH; CV 7.5%). Interleukin 6 (IL-6) and high molecular weight (HMW)-adiponectin were measured using enzyme-linked immunosorbent assay kits from

R&D Systems (Abingdon, UK; CV 8.9% and 8.4%, respectively). Concentrations of hsCRP were measured using latex-enhanced immunoturbimetric assay (Randox Laboratories) on a Cobas FARA bioanalyser (Roche Products Ltd, Herts UK; lower detection limit 0.1 mg/l, CV 5.5%). Fibrinogen was determined using an automated coagulation analyzer (ACL Futura; Instrumentation Laboratory Company, Lexington, MA; CV 1.6%).

2.6 Statistics

Data were tested for normality by examining histograms of values and using the normality plot, Skewness value, and Shapiro-Wilk test. Waist, SKF, and biochemical variables were not normally distributed and were normalized by log-transformation before analysis and are presented as geometric means. The homeostatic model assessment (HOMA) index was calculated using insulinXglucose/22.5.[21]

Sex differences were analysed by independent (unpaired) Student's *t* tests. Effects of sex and puberty, or sex and waist measurement, were analysed by two-way analysis of variance (ANOVA). Log-log regression analysis was used to estimate the power with which to raise the height to correct SKF measurements.[8,23] Pearson correlation coefficients were calculated between pairs of variables. Linear and non-linear regression analyses were used, as appropriate, to determine the relationship between variables. Hierarchical multiple regression analysis was used to identify independent predictor variables, after ensuring no violation of the assumptions of normality, linearity, multicollinearity, and homoscedasticity. Since patterns differed by gender, the gender were also considered separately. Statistical analyses were performed using SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA) and Statistica Version 10 (StatSoft Inc., Tulsa, OK, USA). To take into account multiple measurements, the level of statistical significance was determined using the Bonferroni

correction. Uncorrected *p*-values are shown. A level of significance of $p < 0.05$ was set for multiple regression.

3. Results

3.1 Biomarkers associated with metabolic and cardiovascular risk in girls and boys

Biomarkers and cardiovascular fitness measurements were available from 129 girls and 95 boys (Table 2). In this cohort of 13-year-olds, boys had higher cardiovascular fitness scores than girls. They also tended to be taller and have higher birth weight. Height in girls correlated with birth weight ($r = 0.395$; $p < 0.01$, n = 129) but this was not significant in boys ($r = 0.146$; $p = 0.17$, $n = 92$), and there was no relationship between birth weight and any other measurement, among girls or boys or among their parents (data not shown). Girls had higher SKF measurements than boys; however, waist circumference expressed as a percentage of height (WHtR%) and the ratio of trunk-to-extremity SKF (T/E SKF) did not differ between genders. The small difference in age between T1–3 compared to T4–5 was statistically significant in girls and not in boys ($P = 0.016$, Table 3).

There was a relationship between height and the sum of the skinfold thicknesses (4SKF) in children in T1–3 ($r = 0.279$, $p = 0.017$, n = 73 for girls, and $r = 0.563$, $p < 0.001$, n = 42, for boys), but not in T4–5 ($r = 0.078$, $p = 0.570$, n = 56 for girls, and $r = -0.156$, $p = 0.265$, n = 53 for boys). Since 4SKF is dependent on height, the relationship between height and each SKF was also determined. Gradients obtained from log-log linear regression analysis of height and SKF, shown in Table3, indicate that most of the effect of puberty on the relationship between height and 4SKF was from the contribution of subscapular SKF and suprailiac SKF

measurements. This was markedly apparent in T1–3 boys. There was no significant relationship between triceps SKF and height in any group. There was a positive relationship between T/E SKF and height in girls in T1–3 and in boys in T1–3 and T4–5.

There was a similar relationship between WHtR and triceps SKF or T/E SKF irrespective of gender or pubertal status (Fig. 1). Non-linear regression lines and correlations did not differ significantly in slope; however, in boys with triceps SKF ≥ 10 mm, the mean deviation of the WHtR was 7.5% (6.3%–8.6%) above the girls' curvilinear regression line ($p < 0.001$). The regression lines in girls and boys crossed a WHtR of 44% at triceps SKF measurements of 23 mm and 16.5 mm, respectively (i.e., values were 42% higher in girls), while the regression lines crossed a WHtR of 44% at similar T/E SKF measurements in girls and boys.

Serum insulin concentrations were higher in girls than in boys (Table2) and correlated positively with WHtR in girls and boys (Fig. 2), regardless of pubertal status(data not shown). Glucose levels were lower in girls than in boys, and correlated positively with insulin ($r = 0.235$, $p = 0.008$, $n = 126$ for girls and $r = 0.398$, $p < 0.001$, $n = 93$ for boys). In boys, insulin concentrations correlated with height z scores ($r = 0.451$, $p < 0.001$, $n = 95$ for boys; $r = 0.119$, $p = 0.181$, $n = 129$ for girls). In hierarchical multiple regression analysis, glucose and WHtR were entered at Step 1 and explained 11% of the variance in insulin. After entry of gender and cardiovascular fitness at Step 2, the total variance in insulin explained by the model was 34%, $F(4,214) = 27.70$, $p < 0.001$. Gender and cardiovascular fitness explained an additional 23% of the variance in insulin after controlling for glucose and WHtR, F change (2, 214) = 38.15, $p < 0.001$. In the final model, the following were statistically significant: glucose ($\beta = 0.29$, $p < 0.001$), WHtR ($\beta = 0.26$, $p < 0.001$) and gender ($\beta = 0.45$, $p < 0.001$).

Serum concentrations of triglyceride and total cholesterol were higher in girls (Table2), regardless of pubertal status (data not shown). Fibrinogen and triglyceride concentrations, and diastolic blood pressure levels, each correlated positively with WHtR in boys and girls, while HDL cholesterol was inversely correlated (Fig. 2).

HMW-adiponectin concentrations were lower in boys (Table 2) and were lower in late puberty (ANOVA F(3,217)=8.201; gender $p < 0.001$ and puberty $p = 0.012$; interaction $p = 0.138$). Concentrations of HMW-adiponectin were 2.52 mg/l (95%CI 2.09-3.04 mg/l) in the 42 T1–3 boys and 1.82 mg/l (1.53–2.17 mg/l) in the 52 T4–5 boys. In girls, values were 2.80 mg/l (2.36-3.31 mg/l) in the 71 T13 girls and 3.04 mg/l (2.69-3.45 mg/l) in the 56 T4-5 girls(n=56). In hierarchical multiple regression analysis, gender and puberty were entered at Step 1 and explained 9% of the variance in HMW-adiponectin. After entry of WHtR, insulin and triceps SKF at Step 2, the total variance in HMW-adiponectin explained by the model was just 15%, $F(5,215) = 7.65$, $p < 0.001$. WHtR, insulin and T/E SKF explained an additional 6% of the variance in HMW-adiponectin after controlling for gender and puberty, F change (3, 215) = 4.94, $p = 0.002$. In the final model, the following were statistically significant: gender ($\beta = 0.30$, $p < 0.001$), puberty ($\beta = 0.14$, $p = 0.032$), and T/E SKF ($\beta = -0.15$, $p = 0.035$).

CRP concentrations were significantly lower in the girls compared to the boys (Table 2) and correlated with IL-6 ($r = 0.360$, $p < 0.001$ in 125 girls and $r = 0.466$, $p < 0.001$ in 94 boys). There was an association between CRP and WHtR in girls and boys (Fig. 2). In hierarchical multiple regression analysis, gender, IL-6 and WHtR were entered at Step 1 and explained 37% of the variance in CRP. After entry of puberty, insulin, T/E SKF and cardiovascular fitness at Step 2, the total variance in CRP explained by the model was 36% $F(4,211) = 18.58$, $p <$

0.001. In the final model the following were statistically significant: WHtR (β = 0.39, , $p < 0.001$), IL-6 (β = 0.29, , $p < 0.001$) and gender (β = 0.14, $p = 0.045$).

3.2 Characteristics of adolescents with higher WHtR

We compared children with the highest waist circumferences (> 44% of height; 35 girls and 26 boys) to the rest of the cohort (94 girls and 69 boys); these results are shown in Table 4. There were no differences in pubertal status among the 4 groups (data not shown). T/E SKF was higher in the group with WHtR >44%, in both girls and boys. Most children with WHtR >44% had a triceps SKF measurement of >23 mm in girls and > 16.5 mm in boys (Fig. 1A and 1C). These children also had higher diastolic blood pressure, insulin concentrations and HOMA measurements, and CRP and fibrinogen concentrations, and lower HDL-cholesterol concentrations, than the rest of the cohort. The cardiovascular fitness score was lower in girls and boys with the higher WHtR. In boys with a WHtR of >44%, there was an inverse correlation with insulin concentrations (lnInsulin = 6.251-1.050XlnFitnessScore; r = -0.727, $p < 0.001$), which was not seen in thinner boys or in girls (data not shown).

3.3 Parental weight and waist measurements

Parental weight measurements correlated with height (mothers: r = 0.315, $P < 0.001$; fathers: r = 0.381, $P < 0.001$) and correction of weight by height^2 (BMI) removed the effect of height. In men, the waist and WHtR correlated with height (r = 0.177, $P = 0.014$). When the formula waist^{2}X10/ht (W^2HtR)[23] was used, there was no relationship between waist and height in mothers or fathers. According to WHO criteria, and using corrected BMI values, 33% of mothers were normal weight and 29% were obese (BMI ≥ 30), while 11% of fathers were normal weight and 35% were obese.

3.4 Relationship between child and parental anthropometric variables

In the children with WHtR >44%, maternal BMI and W^2HtR were higher than for mothers of children with a WHtR ≤44% (Table 4). Paternal BMI was also significantly higher. Values from children for whom both maternal and paternal BMI were available were included in the following analyses (93 girls and 80 boys).

Correlations between anthropometric variables in children and their parents is shown in Table 5. In girls, WHtR correlated significantly with maternal BMI. There was no correlation between maternal and paternal BMI; however, there was a significant relationship between maternal and paternal W^2HtR (data not shown).

Hierarchical multiple regression analysis was used to determine the impact of parental BMI on children's WHtR, triceps SKF, and T/E SKF. Gender and cardiovascular fitness were entered at Step 1 and explained 25% of the variance in child WHtR. After entry of maternal and paternal BMI at Step 2, the total variance in child WHtR explained by the model was 33%, $F(4,184) = 22.22$, $p < 0.001$. Maternal and paternal BMI explained an additional 8% of the variance in child WHtR after controlling for gender and cardiovascular fitness, F change (2, 184) = 10.73, $p < 0.001$. In the final model, the following independent variables were statistically significant: gender (β = 0.31, $p < 0.001$), cardiovascular fitness (β = -0.49, $p < 0.001$), maternal BMI (beta = 0.22, $p < 0.001$) and paternal BMI (β = .17, $p = 0.007$). For child triceps SKF, gender and cardiovascular fitness, entered at Step 1, explained 32.5% of the variance. After entry of maternal and paternal BMI at Step 2, total variance in child triceps SKF explained by the model was 37.3%, $F(4,184) = 27.38$, $p < 0.001$. Maternal and paternal BMI explained an additional 4.8% of the variance, F change (2, 184) = 7.09, $p < 0.001$. In the final model, the following were statistically significant: cardiovascular fitness (β = -0.52, $p <$

0.001) and maternal BMI (β = 0.22, $p < 0.001$). For child T/E SKF, gender and cardiovascular fitness, entered at Step 1, explained 7% of the variance. After entry of maternal and paternal BMI at Step 2, total variance in child triceps SKF explained by the model was 14%, $F(4,184) = 7.47$, $p < 0.001$. Maternal and paternal BMI explained an additional 7% of the variance, F change (2, 184) = 7.38, $p = 0.001$. In the final model, the following were statistically significant: cardiovascular fitness (β = -0.25, $p = 0.001$) and paternal BMI (β = 0.22, $p = 0.002$).

3.5 Cardiovascular fitness

We have previously shown that the cardiovascular fitness score is inversely related to WHtR and to 4SKF in girls and boys in early/mid and late puberty.[8] Since 4SKF is dependent on height, we explored the relationship between cardiovascular fitness and triceps SKF. There is an inverse relationship, with no difference between those in T1-3 and T4-5 for each gender, and with similar combined slopes for girls (fitness score = 107.7-21.82xlnTricepsSKF, $r = 0.497$, $p < 0.001$, $n = 129$) and boys (lnWHtR = 3.842-0.287xlnTricepsSKF+0.095^{x}lnTricepsSKF^{2}, $r = 0.752$, $p < 0.001$, $n = 95$). However the combined regression line was significantly higher for boys compared to girls ($F(1,221) = 39.477$; $p < 0.01$). In hierarchical multiple regression analysis, gender and WHtR were entered at Step 1 and explained 39% of the variance in cardiovascular fitness. After entry of maternal and paternal BMI at Step 2, the total variance in cardiovascular fitness explained by the model was 39% $F(4,184) = 29.58$, $p < 0.001$. In the final model the following were statistically significant: gender ($\beta = 0.50$, , $p < 0.001$) and WHtR ($\beta = -0.45$, $p < 0.001$), with no independent contribution of maternal BMI or paternal BMI.

4. Discussion

In this study of adolescents, measures of adiposity correlated with biomarkers that are associated with cardiovascular disease risk in adults. Waist and SKF measurements correlate with height in children in early or mid-puberty; however, the influence of height is lost when waist measurement is expressed as a percentage of height (WHtR). Gender differences were observed. Although girls appeared to have more subcutaneous fat and reduced levels of cardiovascular fitness, boys had relatively greater central adiposity and higher levels of pro-inflammatory markers. Parental overweight and obesity were associated with greater adiposity in adolescent girls and boys, suggesting that strategies for reducing cardiovascular risk should be family focused.

The cohort was well controlled for age and socioeconomic status, and we were therefore able to estimate the independent effects of puberty and parental adiposity. There were, however, limitations. This was a cross-sectional design, and a comparison of children in T1-3 and T4-5 in longitudinal studies would be required to predict the effect of puberty in individual subjects. Furthermore, pubertal status and parental anthropometry (height, weight, and waist measurements) were self-reported by participants. Self-reported height tends to be overestimated and weight underestimated,[18] leading to an underestimation of obesity prevalence and an exaggeration of the relationship with cardiovascular disease risk.[25] We used the protocol developed by Taylor et al.[19] to estimated pubertal development. It should also be noted that children tend to underestimate pubertal development when using these line drawings.[19]

Sex hormones influence adipocyte deposition and function,[26] and adolescence has been considered a critical time in the development of obesity.[27,28] However, while there is a relationship between obesity and the early onset of puberty in girls, the data for boys are

inconclusive.[29] There is also insufficient evidence that puberty is causally linked to obesity development. In a large cross-sectional study, sexual dimorphism in fat patterning was present pre-puberty and increased across puberty, with girls having less waist fat.[28] The trend in British adolescents over recent decades for having increases in waist circumference exceed increases in BMI, particularly in girls, suggests that abdominal fatness is increasing at a greater rate than whole-body fatness.[30] In our study, despite having triceps SKF that was lower, boys had similar WHtR compared to girls. This observation could be explained by a relatively greater contribution of visceral fat to the abdominal measurement in boys.[31] Assessing the value of using WHtR and SKF measurements (triceps and trunk-to-extremity ratios) to track adiposity across puberty would require longitudinal studies with larger cohorts.

We identified gender differences in circulating concentrations of biomarkers that are associated with cardiovascular disease. Fasting insulin and triglyceride concentrations were higher in girls than in boys. In boys with higher WHtR, there was an inverse relationship between cardiovascular fitness and insulin levels. It is now recognized that cardiorespiratory fitness influences the relationship between adiposity and cardiovascular prognosis.[4,32] In boys, fat mass accrual during emerging adulthood is mitigated by physical activity.[33] It therefore could be speculated that encouraging physical fitness in this group might have a significant impact in reducing cardiometabolic risk. Higher hsCRP levels were observed in boys, which is consistent with a previous study of obese adolescents.[14] In contrast, in pre-pubertal children CRP is reported to be higher in girls[34] and adult women independent of exogenous oestrogen.[35] In adults, there is a strong inverse relationship between hsCRP levels and adiposity, and these independently predict cardiovascular disease events.[36] Although CRP levels in children predict values in adults[37] and predict adult obesity,[38] evidence that CRP in

children is a predictor of cardiovascular risk in adulthood is not strong.[39] In obese girls, high hsCRP concentrations are associated with insulin resistance.[33] In our study, although hsCRP correlated with WHtR in boys and girls, there was no relationship to fasting insulin. In linear regression analysis, there was an influence of WHtR on hsCRP concentrations that is independent of the ratio of trunk-to-extremity SKF. HMW-adiponectin, which has both anti-inflammatory and insulin-sensitising properties, is reported to be inversely related to adiposity and insulin resistance.[36] These relationships were not present in our study. However, we did observe that concentrations were lower in boys, particularly in the late puberty group, which may be explained by a direct effect of testosterone on adipocyte production.[40] Lower adiponectin concentrations, taken together with relatively greater waist circumference measurements and higher CRP levels, suggest that adolescent boys have more visceral adiposity and associated systemic inflammation.

It has been suggested that a WHtR above 0.5 in children should be used to predict health risk when those children become adults.[41] A study of Australian children aged 8-16 years showed that a WHtR of ≥0.46 for boys and ≥0.45 for girls identified those with percentage body fat ≥85th percentile.[42] In black South African adolescents a lower WHtR cutoff of 0.41 indicated metabolic risk.[43] In our study, adolescents with a WHtR above 0.44 had higher levels of cardiovascular biomarkers. It has been suggested that WHtR further specifies cardiometabolic risk within classifications that are based on BMI percentiles.[44] We observed that parental BMI measurements were also higher in those with a WHtR above 0.44. While this may indicate that childhood overweight and obesity is strongly related to the social environment, genetic factors may also play a role.[45] Birth weight is reported to predict later obesity.[46] In our study, the child's birth weight reported by the parents was associated with

height in girls; however, an association with markers of adiposity was not observed, suggesting that birth weight may not be a consistent predictor of later overweight and obesity.

In our group of 12-year olds, pubertal status had no impact on the level of physical fitness in girls or boys. In a recent longitudinal study of younger children, persistently low, or a decline in, cardiorespiratory fitness was associated with later cardiovascular disease risk.[47] Although cardiovascular fitness was not related to parental BMI in our study, the observation that parental overweight and obesity is associated with higher waist measurements in children suggests that strategies for reducing cardiovascular risk should be family focused.

5. Conclusion

In adolescents, there is a relationship between measures of adiposity and parental weight that involves factors other than cardiovascular fitness. Adolescent boys have relatively more abdominal fat than girls and tend to have a proinflammatory profile of biomarkers. These observations suggest that family and social environmental interventions to prevent obesity are best undertaken early in childhood, particularly in boys.

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Authors' Contributions

MSL carried out the statistical analyses and drafted the manuscript. JSB conceived of the study, helped collect and analyze the data, and helped draft the manuscript. Both authors have read and approved the final version of the manuscript and agree with the order of presentation of the authors.

Competing Interests

The authors declare that they have no competing interests.

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Table 1 Stage of puberty in girls (A) and boys (B) – children's estimates of pubertal stages using line drawings described in Methods. (Taylor et al., 2001)[18] based on Tanner (1962)[19]

	Girls (Breast)						Boys (Genitalia)					
Pubic hair	1	2	3	4	5	**Total**	1	2	3	4	5	**Total**
1	0	0	0	0	0	0	0	0	0	0	0	0
2	1	8	8	1	0	18	1	5	9	2	2	19
3	1	6	29	10	8	44	0	3	12	9	0	24
4	0	3	14	20	9	46	1	1	9	27	7	45
5	0	0	3	5	3	11	0	0	1	3	3	7
Total	2	17	54	36	20	129	2	9	31	41	12	95

Table 2Gender differences in markers of metabolic and cardiovascular risk in adolescent children (mean ± SD (95%CI)

	Girls mean (SD; 95% CI) (*n* = 129)	Boys mean (SD; 95% CI) (*n* = 95)	*p* value[a]
Age (years)	13.48 ± 0.30 (13.43-13.53)	13.53 ±0.33(13.46-13.60)	0.231
Anthropometry			
height (cm)	158.1 ±6.7 (156.9-159.3)	160.1 ±9.0(158.3-161.9)	0.056
waist (cm)[b]	65.2 ±1.1(63.9-66.6)	67.7 ±1.1(66.0-69.5)	0.023
weight (kg)	53.3 ±11.0(51.4-55.2)	53.2 ±13.1(50.5-55.8)	0.921
WHtR (%)[b]	41.3 ±1.1(40.5-42.1)	42.4 ±1.1(41.4-43.4)	0.088
birth weight (kg)	3.33 ±0.36(3.23-3.43)	3.52 ±0.59(3.40-3.64)[g]	0.017
subscapular SKF (mm)[b]	10.8 ±1.6(10.0-11.7)	8.8 ±1.7(7.9-9.8)	*<0.001*
suprailiac SKF (mm)[b]	12.2 ±1.6(11.3-13.3)	9.1 ±1.9(8.0-10.4)	*<0.001*
triceps SKF (mm)[b]	16.1 ±1.4(15.1-17.2)	12.7 ±1.6(11.6-13.9)	*<0.001*
biceps SKF (mm)[b]	10.2 ±1.5(9.5-11.0)	7.9 ±1.6(7.1-8.7)	*0.001*
4SKF (mm)[b,c]	50.2 ±1.5(47.1-53.6)	39.2 ±1.6(35.4-43.4)	*<0.001*
T/E SKF[b,d]	0.87 ±1.3(0.84-0.91)	0.87 ±1.36(0.82-0.93)	0.915
Blood pressure			
Systolic (mmHg)	115.6 ±10.1 (113.8-117.3)	116.8 ±12.6(114.2-119.3)	0.422
Diastolic (mmHg)	65.7 ±10.2(63.9-67.5)	64.7 ±11.0(62.4-66.9)	0.472
Cardiovascular fitness (laps)	44.5 ±1.4(41.9-47.2)	62.6 ±1.4(58.4-67.0)	*<0.001*
Biomarkers			
Total cholesterol (mmol/l)[b]	4.01 ±1.15(3.90-4.12)	3.76 ±1.18(3.63-3.89)	0.003
LDL-cholesterol (mmol/l)[b]	1.95 ±1.33(1.86-2.05)	1.81 ±1.35(1.70-1.92)	0.052
HDL-cholesterol (mmol/l)[b]	1.67 ±1.21(1.62-1.73)	1.60 ±1.24(1.53-1.67)	0.114
Triglyeride (mmol/l)[b]	0.71 ±1.43(0.66-0.75)	0.59 ±1.53(0.54-0.64)	*<0.001*
Glucose (mmol/l)[b]	4.80 ±0.27(4.75-4.85)[g]	4.92 ±0.35(4.85-4.99)[f]	0.006
Insulin (mIU/l)[b]	10.02 ±1.47(9.37-10.71)	6.96 ±1.58(6.34-7.63)	*<0.001*
HOMA (insulin*glucose/22.5)[b]	2.16 ±1.49(2.01-2.32)[g]	1.53 ±1.63(1.38-1.69)[f]	*<0.001*

Fibrinogen (g/l)[b]	2.62 ±1.19(2.54-2.70)[e]	2.63 ±1.18(2.54-2.73)[e]	0.807
hsCRP (mg/l)[b]	0.29 ±2.19(0.26-0.34)[f]	0.43 ±2.38(0.36-0.52)	*<0.001*
IL-6 (ng/l)[b]	0.57 ±2.09(0.50-0.64)[f]	0.69 ±2.14(0.59-0.81)[e]	0.047
HMW-Adiponectin (mg/l)[b]	2.93 ±1.77(2.65-3.24)[f]	2.11 ±1.89(1.85-2.40)[e]	*<0.001*

Notes:[a]Unpaired *t* test girls *vs.* boys Bonferroni-corrected α=0.002; [b]geometric mean ± SD; [c]sum of triceps, subscapular, biceps, and suprailiac SKF; [d]ratio of trunk (sum of subscapular and suprailiac)-to-extremity (sum of triceps and biceps) SKF; [e]data missing from 1 participant, [f]data missing from 2 participants, [g]data missing from 3 participants

Abbreviations: WHtR = Waist-to-height ratio; SKF = Skinfold-thickness; T/E SKF= the ratio of trunk-to-extremity skinfold thicknessesSKF; LDL = Low density lipoprotein; HDL = high-density lipoprotein; HOMA = homeostatic model assessment.

HsCRP = High-sensitivity C-reactive protein; HMW = High Molecular Weight; IL-6 = Interleukin 6.

Table 3 Gradient *b* (SE) derived from log-log relationship between height and SKF in children who reported being in stage 1-3 (T1-3) and in stage 4-5(T4-5) for breast development in girls and genital development in boys, using line drawings (Taylor et al., 2001)[18] based on Tanner (1962)[19]

	Girls		Boys	
Pubertal stage	T1-3, *n*=73	T4-5, *n*=56	T1-3, *n*=42	T4-5, *n*=53
Age, mean (SD, 95%CI)	13.4 ±0.30, 13.4-13.5)	13.6 ±0.29, 13.5-13.6)[#]	13.5 ±0.36, 13.4-13.6)	13.6 ±0.30, 13.5-13.7)
4SKF[a]	2.14 (0.93)	0.70 (1.34)	***4.66 (1.19)******	-1.28 (0.26)
Triceps	1.77 (0.92)	0.11 (1.28)	2.73 (1.08)	-2.23 (1.19)
Biceps	0.49 (0.99)	0.51 (1.59)	***3.53 (1.14)****	-2.26 (1.36)
Subscapular	***2.84 (1.08)****	0.27 (1.67)	***6.05 (1.27)******	0.18 (1.21)
Suprailiac	***3.51 (1.15)****	2.11 (1.61)	***6.66 (1.59)******	-0.43 (1.66)
T/E SKF[b]	***1.91 (0.58)****	1.10 (0.93)	***3.37 (0.64)******	***2.08 (0.75)****

[a], sum of four SKFs

[b], trunk-to-extremity SKF ratio ((subscapular+suprailiac)/triceps+biceps))

Bonferroni-corrected α=0.01; significant results in bold italics. *, $p \leq 0.01$; **, $p \leq 0.005$; ***, $p \leq 0.001$ significant relationship between height and 4SKF in children in T1-3 for girls and for boys; [#], $p=0.016$, compared to girls in T1-3 (independent *t* test)

Abbreviations: CI = confidence interval; SKF = Skinfold-thickness; T/E SKF= the ratio of trunk-to-extremity skinfold thicknessesSKF;

Table 4 Characteristics of children with waist measurements that were >44% height(mean(95%CI)).

WHtR (%)	> 44%	> 44%	≤ 44%	≤ 44%			
Sex	Girls	Boys	Girls	Boys		*p* value[a]	
n (% own gender group)	35 (27%)	26 (27%)	94 (73%)	69 (73%)	Waist	Sex	Interaction
Anthropometry							
height (cm)	159.3 (157.1-161.5)	160.4 (156.3-164.5)	157.7 (156.3-159.1)	160.0 (157.9-162.1)	0.393	0.145	0.598
WHtR (%)[b]	47.8 (46.8-49.0)	49.4 (47.9-50.9)	39.1 (38.7-39.5)	40.0 (39.4-40.6)	*<0.001*	0.003	0.584
4SKF[b,c]	78.5 (72.7-84.7)	71.4 (60.2-84.7)	42.6 (40.3-45.0)	31.3 (29.1-33.6)	*<0.001*	*<0.001*	0.017
T/E SKF[b,d]	1.06 (0.98-1.15)	1.02 (0.90-1.17)	0.81 (0.77-0.85)	0.82 (0.76-0.87)	*<0.001*	*0.741*	0.644
triceps SKF[b]	23.3 (21.1-25.7)	20.7 (19.0-23.7)	14.0 (13.3-14.9)	10.6 (9.7-11.4)	*<0.001*	*<0.001*	0.078
weight (kg)	66.4 (63.2-69.5)	65.7 (59.3-72.2)	48.5 (47.1-49.8)	48.4 (46.5-50.3)	*<0.001*	0.803	0.824
birth weight	3.37 (3.14-3.60)	3.46 (3.19-3.73)[e]	3.31 (3.20-3.43)	3.54 (3.40-3.68)[e]	0.613	0.044	0.976
Blood pressure							
Systolic (mmHg)	117.3 (114.7-120.0)	117.5 (111.2-123.9)	114.9 (112.7-117.1)	116.5 (113.8-119.2)	0.307	0.599	0.681
Diastolic (mmHg)	70.5 (67.4-73.6)	68.3 (63.0-73.7)	63.9 (61.9-66.0)	63.3 (61.0-65.6)	*<0.001*	0.375	0.629
Cardiovascular fitness (laps)	35.3 (31.3-39.2)	52.8 (44.6-61.0)	51.4 (48.4-54.5)	70.7 (66.5-74.9)	*<0.001*	*<0.001*	0.711
Biomarkers							
total cholesterol (mmol/l)[b]	3.97 (3.75-4.21)	3.69 (3.43-3.98)	4.02 (3.90-4.15)	3.78 (3.64-3.93)	0.468	0.006	0.809
LDL-cholesterol (mmol/l)[b]	2.05 (1.86-2.27)	1.81 (1.58-2.06)	1.92 (1.81-2.03)	1.81 (1.68-1.94)	0.449	0.039	0.454
HDL-cholesterol (mmol/l)[b]	1.52 (1.43-1.62)	1.49 (1.37-1.62)	1.73 (1.67-1.80)	1.64 (1.56-1.73)	*<0.001*	0.223	0.627
triglyeride (mmol/l)[b]	0.76 (0.66-0.87)	0.67 (0.56-0.81)	0.69 (0.64-0.73)	0.56 (0.50-0.61)	0.012	0.005	0.410
glucose (mmol/l)[b,f]	4.81 (4.72-4.90)	4.89 (4.76-5.01)	4.80 (4.74-4.85)	4.93 (4.84-5.01)	0.783	0.027	0.569
Insulin (mIU/l)[b]	11.4 (10.1-12.9)	8.7 (7.0-10.7)	9.5 (8.8-10.3)	6.4 (5.8-7.1)	*<0.001*	*<0.001*	0.314
HOMA (insulin*glucose/22.5) [b,f]	2.50 (2.20-2.84)	1.93 (1.53-2.42)	1.97 (1.78-2.20)	1.37 (1.21-1.55)	*<0.001*	*<0.001*	0.482
fibrinogen (g/l)[b,h]	3.04 (2.86-3.23)	2.96 (2.82-3.12)	2.48 (2.41-2.55,93)	2.52 (2.42-2.62,68)	*<0.001*	0.848	0.355
hsCRP (mg/l)[b]	0.62 (0.45-0.85,33)	0.74 (0.54-1.03)	0.23 (0.20-0.25)	0.35 (0.29-0.43)	*<0.001*	0.005	0.240
IL-6 (ng/l)[b,g]	0.78 (0.63-0.95)	0.76 (0.55-1.04,25)	0.50 (0.43-0.59,92)	0.67 (0.56-0.81)	0.016	0.241	0.157
HMW-Adiponectin(mg/l)[b,g]	2.75 (2.25-3.37)	1.88 (1.41-2.51,25)	3.00 (2.67-3.38,92)	2.19 (1.89-2.54)	0.188	*<0.001*	0.707
Parental data (CI; n)							
Maternal BMI (kg/m^2)	28.8 (26.7-30.9; 33)	28.3 (26.5-30.0; 26)	25.6 (24.6-26.5; 85)	26.1 (25.1-27.2; 65)	*0.001*	0.677	0.231
Maternal waist^2/htx10[b]	4.37 (3.91-4.89; 29)	4.31 (3.82-4.85; 24)	3.78 (3.55-4.03; 79)	3.75 (3.51-4.01; 63)	*0.001*	0.774	0.838
Paternal BMI (kg/m^2)	28.6 (26.9-30.3; 28)	30.6 (28.7-32.5; 22)	27.4 (26.5-28.2; 81)	27.8 (26.7-28.7; 66)	*0.004*	0.094	0.225
Paternal waist^2/htx10[b]	4.61 (4.34-4.90; 27)	5.02 (4.59-5.49; 21)	4.48 (4.33-4.65; 78)	4.63 (4.45-4.82; 66)	0.070	0.033	0.293

[a]2-way ANOVA, Bonferroni-corrected α=0.002; [b]geometric mean; [c]sum of triceps, subscapular, biceps and suprailiac SKF; [d]ratio of trunk (sum of subscapular and suprailiac)-to-extremity (sum of triceps and biceps) SKF; [e]data missing for 1 individual; [f]data missing for 3 girls ≤ 44% and 2 boys ≤ 44%; [g]data missing for 1 boy > 44%, 2 girls ≤ 44%; [h]data for 1 girl ≤ 44% and 1 boy ≤ 44%

Abbevations: SKF = Skinfold-thickness; LDL = Low density lipoprotein; HDL = high-density lipoprotein; HOMA = homeostatic model assessment

- hsCRP = High-sensitivity C-reactive protein; HMW = High Molecular Weight; BMI = Body Mass Index.

Table 5 Correlations between anthropometric variables in parents and children (Pearson's *r*)

	Girls (*n* = 93)					Boys (*n* = 80)				
	Height	BMI	WHtR (%)[a]	T/E SKF[a]	Triceps SKF[a]	Height	BMI	WHtR (%)[a]	T/E SKF[a]	Triceps SKF[a]
Child BMI	0.196	—	—	—	—	.0304**	—	—	—	—
Child WHtR (%)[a]	-0.023	***0.897****	—	—	—	-0.002	***0.881****	—	—	—
Child T/E SKF[a]	0.268**	***0.509****	***0.497****	—	—	***0.454****	***0.573****	***0.433****	—	—
Child Triceps SKF[a]	0.133	***0.748****	***0.700****	0.125	—	0.019	***0.764****	***0.797****	0.076	—
Maternal height	**0.359****	-0.029	-0.050	0.151	-0.075	***0.337****	0.132	0.056	-0.079	0.209
Paternal height	***0.297****	0.004	-0.042	-0.063	0.111	0.145	0.068	0.034	0.012	0.182
Midparental height[b]	***0.423****	-0.014	-0.060	0.044	0.035	***0.324****	0.134	0.060	-0.047	0.259*
Maternal BMI	0.094	***0.351****	***0.324****	0.172	***0.350****	0.181	0.286**	0.225*	0.129	0.279*
Paternal BMI	-0.160	0.215*	0.204*	0.291**	0.011	0.066	***0.315****	0.263*	0.254*	0.133
Maternal W^2HtR[a]	0.123	0.189	0.199	0.201	0.211*	0.271*	0.307**	0.261*	0.236*	0.301**
Paternal W^2HtR[a]	-0.087	0.068	0.108	0.198	-0.065	0.070	0.210	0.198	0.192	0.120

[a], log-transformed

[b], for girls = (father's height - 13cm. + mother's height)/2; for boys = (mother's height + 13cm. + father's height)/2

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; Bonferroni-corrected $\alpha = 0.004$, significant results in bold italicsAbbevations: BMI = Body Mass Index; SKF = Skinfold-thickness; T/E = trunk-to-extremity.

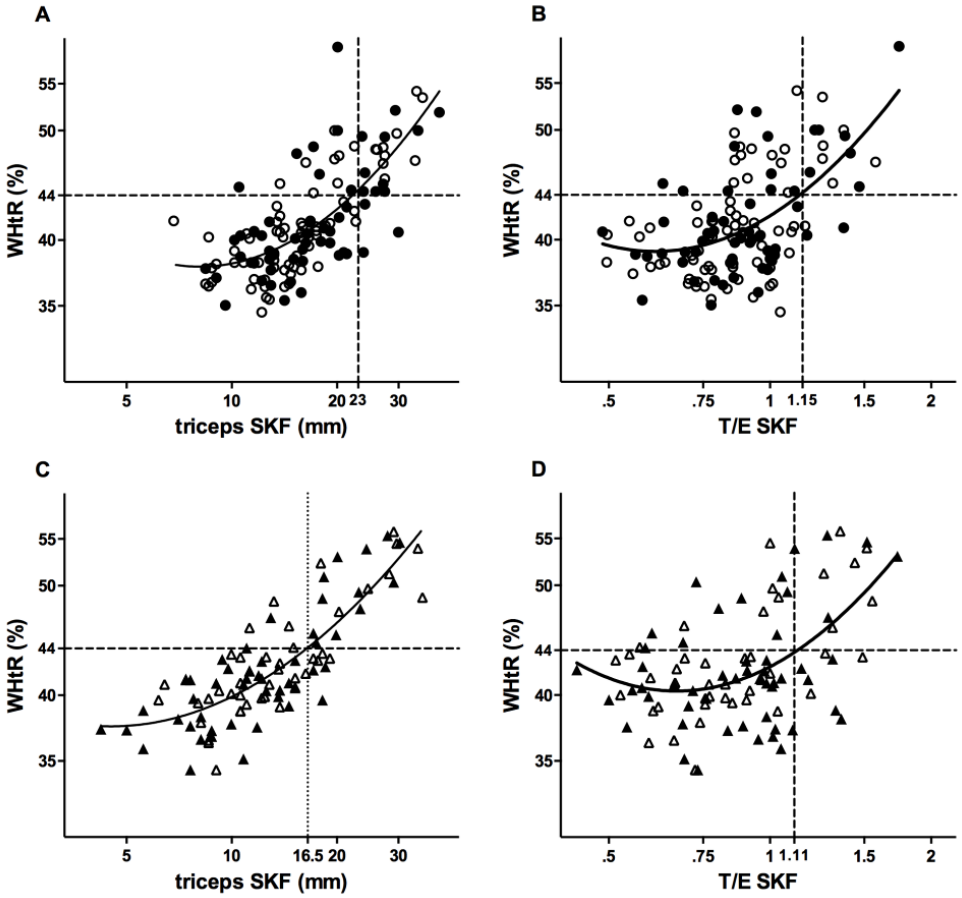

Fig. 1 Relationship between WHtR and triceps skinfold thickness (SKF; A, C) or the ratio of trunk-to-extremity (T/E) SKF (B, D), in girls (A, C) and boys (B, D). The open symbols represent those self-reporting being in Tanner stages 1-3 for breast development in girls and genital development in boys. The closed symbols represent Tanner stages 4-5.

Non-linear regression lines and correlations for the relationships indicated by the solid lines:

(A) lnWHtR=4.264-0.601*lnTricepsSKF+0.144*lnTricepsSKF^2, r^2=0.538, p<0.001 (129 girls)

(B) lnWHtR=3.738+0.294*lnT/E SKF+0.298*lnT/ESKF^2, r^2=0.308, p<0.001 (129 girls)

(C) lnWHtR=3.842-0.287*lnTricepsSKF+0.095*lnTricepsSKF^2, r^2=0.690, p<0.001 (95 boys)

(D) lnWHtR=3.746+0.247*lnT/ESKF+0.308*lnT/ESKF^2, r^2=0.271, p<0.001 (95 boys)

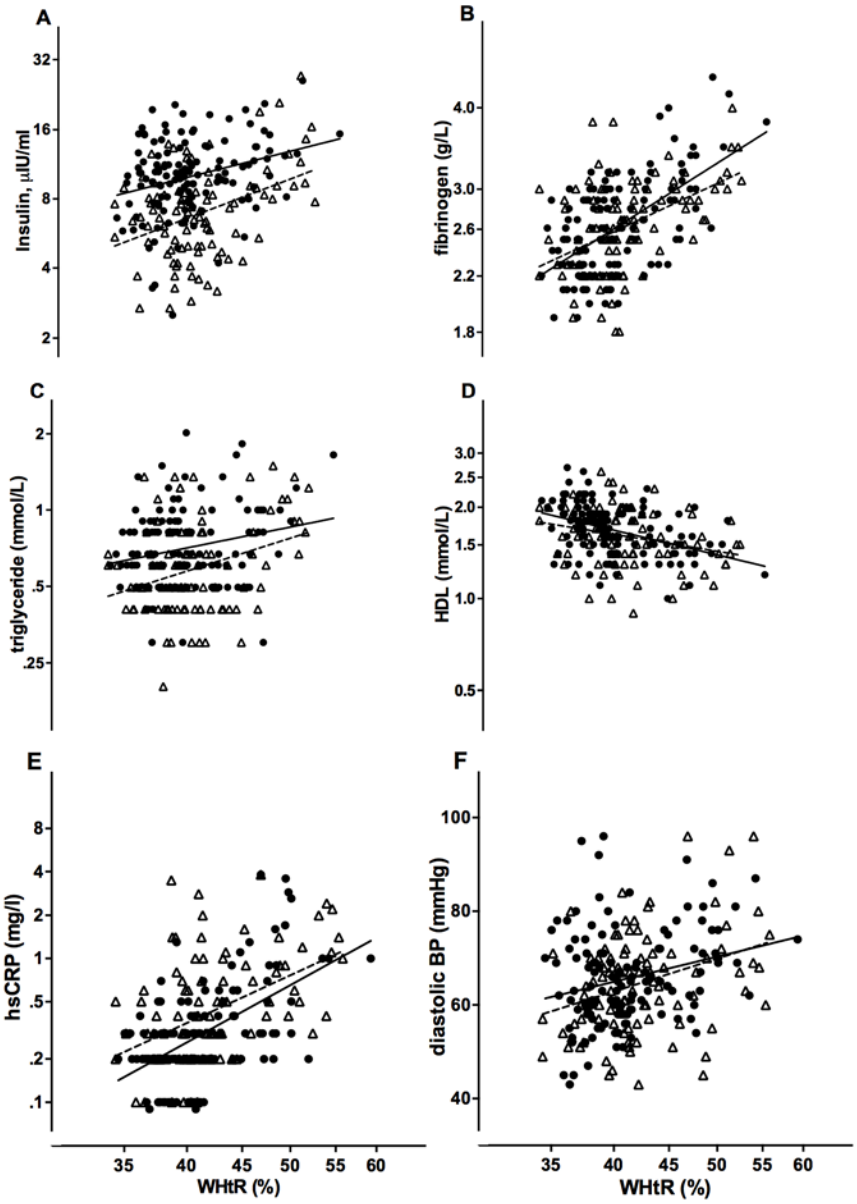

Figure 2

Relationship between WHtR (%) and other markers of metabolic and cardiovascular risk in adolescent girls (closed symbols, intact line) and boys (open symbols, broken line).

Linear regression lines and correlations for the relationships:

(a) lnInsulin = 1.041*lnWHtR-1.568; r= 0.288, p < 0.001 (129 girls) and lnInsulin = 1.572*lnWHtR-3.949; r = 0.394, p < 0.001 (95 boys)

(b) lnFibrinogen = 0.948*lnWHtR-2.565; r = 0.585, p < 0.001 (128 girls) and lnFibrinogen = 0.702*lnWHtR-1.662; r = 0.477, p < 0.001 (94 boys)

(c) lnTriglyceride = 0.771*lnWHtR-3.219; r = 0.226, p = 0.010 (129 girls) and lnTriglyceride = 1.186*lnWHtR-4.977; r= 0.322, p = 0.002 (95 boys)

(d) lnHDL = 3.309-0.751*lnWHtR; r= -0.411, p < 0.001 (129 girls) and lnHDL = 2.407-0.517*lnWHtR; r = -0.273, p = 0.008 (95 boys)

(e) lnCRP = 4.14*lnWHtR-16.62; $r = 0.553$, $p < 0.001$ (127 girls) and lnCRP = 3.46*lnWHtR-13.80; r=0.456, $p < 0.001$ (95 boys)

(f) diastolic BP=24.29*lnWHtR-24.66; r=0.252, $p = 0.004$ (129 girls) and diastolic BP = 31.80*lnWHtR-54.46; $r = 0.330$, $p < 0.001$ (95 boys)

DBP = diastolic blood pressure; HDL = high-density lipoprotein; hsCRP = High-sensitivity C-reactive protein

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