

**Cardiorespiratory fitness, fat mass and cardiometabolic health with endothelial function, arterial elasticity, and stiffness**

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**Running head:** Aerobic fitness and fat mass with arteries

## Abstract

**Purpose:** To determine whether estimated cardiorespiratory fitness (CRF), fat mass (FM), lean mass (LM) and adiponectin bi-directionally associate with arterial function and structure and if CRF mediates the relationship between cardiometabolic health and arterial outcomes in 9-11-year-old children using the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort, UK.

**Methods:** Brachial artery flow-mediated dilation (FMD), distensibility coefficient (DC) and carotid-radial pulse wave velocity (PWV) was measured by ultrasonography; CRF was measured during submaximal ergometer test; total and trunk FM and LM by dual-energy Xray absorptiometry; plasma adiponectin by enzyme assay; and cardiometabolic health was computed using International Diabetes Federation criteria. We tested bi-directionality by including CRF, FM, LM, and adiponectin as exposures and FMD, DC, and PWV as outcomes, alternatively.

**Results:** Among 5566 participants (2816 [51%] girls, median age 9.75 years), CRF/body mass<sup>-0.21</sup> was directly related to DC ( $\beta$  [95% CI] = 0.004 [ $<0.0001$  to 0.008];  $P = 0.046$ ) while CRF/LM<sup>-0.54</sup> was inversely associated with PWV (-0.034 [-0.063 to -0.003]; 0.032) after adjusting for covariates. These associations remained in bi-directional analyses. Total and trunk FM and LM were bi-directionally and positively associated with FMD and DC. Total and trunk FM but not LM had bi-directional and inverse associations with PWV. Adiponectin was not related to FMD, DC or PWV. CRF partially mediated the associations of cardiometabolic health with FMD (1.5% mediation); DC (12.1% mediation); and PWV (3.5% mediation).

**Conclusions:** Associations of poor cardiometabolic health with adverse arterial structure and function in childhood may be mitigated by increasing CRF. CRF and body composition were directly associated with arterial function. In the reverse analysis, better arterial function was associated with higher CRF and total FM.

**Keywords:** Aerobic fitness, children, mediation, metabolic syndrome. obesity

## **1. Introduction**

Atherosclerotic cardiovascular diseases progress from altered arterial function (endothelial function) through an altered arterial structure (arterial elasticity and arterial stiffness), beginning in childhood (1-5). The development and progression of atherosclerosis are directly related to modifiable risk factor status among youth and young adults (5,6). Atherosclerotic progression primarily regulated by endothelial changes (7,8) often peaks with cardiovascular morbidity and mortality (9). Hence, identifying factors that could mitigate arterial disease progression from childhood is warranted.

Cardiorespiratory fitness (CRF) predicts cardiovascular health and poor CRF is related to an elevated risk of cardiometabolic disease in children and adolescents (10–12). CRF was positively associated with arterial elasticity, measured with finger plethysmography, in 329 children after exercise (13). Other studies showed no relationship between CRF and arterial structure, assessed using carotid-radial and carotid-ankle pulse wave velocity (PWV), in 53 boys after exercise (14) or endothelial function, measured as flow-mediated dilatation (FMD) in 129 children (15). However, high CRF was associated with higher PWV estimated from brachial waveform in 646 children (16). These conflicting reports among small to moderately-sized populations require further investigation in a larger cohort whilst controlling for important covariates like somatic maturation and body composition. Moreover, the associations of CRF with arterial measures in a single pathway (uni-directional) may not inform whether a potential temporal relationship exists (12,17). A multi-dimensional approach investigating whether the arterial function and structure also relate to CRF (bi-directionality, which is a reverse association) would improve understanding of these associations and inform future intervention programs on target options (CRF, arterial outcomes, or both) to prevent atherosclerosis from early life and to evaluate intervention effects (18).

Low adiponectin levels, a cytokine secreted by adipose tissue, has been directly associated with altered arterial structure and function in adults, and other atherosclerotic risk factors such as insulin

resistance and metabolic syndrome in children and adolescents (19–25). Adiponectin measured during childhood predicted adult preclinical carotid atherosclerosis better than other conventional risk factors such as body mass index and systolic blood pressure (26). Adiponectin has been inversely associated with CRF in children, suggesting that higher lean mass-induced CRF in tandem with lower-fat mass-enhanced adiponectin may occur concurrently in healthy children (22). Fitter children tend to have higher lean mass and lower fat mass, which may increase adiponectin level leading to improved endothelial function and arterial compliance (22). It is still unclear whether fitness independently influences the associations of adiponectin with arterial structure and function (22). Moreover, whether a temporal relationship exists between adiponectin and arterial function and structure in childhood remains unknown. The association of fat mass with arterial structure (PWV) and function (FMD) in children remains controversial although this association is likely dependent on lean mass and cardiometabolic health status (27–29). It is still unexamined whether these arterial measures, in turn, associates with lower fat mass, and higher lean mass in a temporal fashion.

Low CRF associates with poor cardiometabolic health (10,12) while altered arterial structure (PWV) is directly associated with poor cardiometabolic health in adolescence (30). A mediation analysis study reported that the association of increased fat mass with poor cardiometabolic health in children could be decreased by increasing CRF since CRF had a ten percent indirect or mediating role (31). Besides, the association of increased fat mass with altered arterial structure (PWV) is influenced by cardiometabolic health (29). It remains unclear whether a higher CRF, reflecting increased muscle metabolism and vasodilation via exercise-induced hyperaemia, improves arterial structure and function thereby attenuating the arterial effects of poor cardiometabolic health i.e, the mediating role of CRF in the relationship between cardiometabolic and arterial health. The Avon Longitudinal Study of Parents and Children (ALSPAC), provides extensive and valid measures on the variables of interest (32). Among a prospective cohort of 5566 children aged 9 – 11 years, we

investigated the associations of CRF, fat mass, lean mass, and adiponectin with separate measures of arterial function (FMD) and structure (PWV). Secondly, we investigated the reverse associations of FMD and PWV with CRF, fat mass, lean mass, and adiponectin. Lastly, we examined, the mediating role of CRF on the associations of cardiometabolic health with FMD and PWV. It was hypothesized that 1) CRF, fat mass, lean mass, and adiponectin would have bi-directional relationships with arterial measures, and 2) increased CRF would attenuate the associations of poor cardiometabolic health with altered arterial function and structure.

## **2. Methods**

For an unabridged methodology (see Methods, Supplemental Digital Content 1, which details measurement of variables and statistical analysis).

### *2.1. Study design and participants*

ALSPAC is a comprehensive and longitudinal birth cohort study that investigates factors influencing childhood development and growth. ALSPAC enrolled 15 541 pregnant women with an expected delivery date from April 1991 until the end of December 1992, representing 85% of the eligible general population in three health authorities in Bristol, UK. From birth through childhood, a cohort of 14 062 liveborn children was followed up, via questionnaires, thereafter regular annual clinic follow-up began at age 7 and continues to date. Exposures variables were measured during the age 9 years clinic visit from 18<sup>th</sup> January 2001 through 11<sup>th</sup> January 2003. Whereas arterial outcomes were measured at age 10 years clinic visit from 19<sup>th</sup> February 2002 through 15<sup>th</sup> March 2003. A detailed description of the cohort and study design is available elsewhere (32,33). Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

### *2.2. Anthropometric and social measures*

The infant's sex was abstracted from obstetric records and/or birth notifications. The exact age of each child was determined from their birth date and examination date. Height and body weight were measured using standard protocols. The participant's mother's social-economic status was grouped according to the 1991 British Office of Population and Census Statistics classification. An objective measure of pubertal (somatic) status (time in years to age at peak height velocity) was derived using Superimposition by Translation And Rotation mixed-effects growth curve analysis (34).

### *2.3. Assessments of body composition and other cardiometabolic factors*

Total and trunk fat mass and lean mass at age 9 years were assessed using a Lunar Prodigy DEXA scanner (GE Medical Systems, Madison, Wisconsin). Total and trunk fat mass and lean mass indices (FMI and LMI) were computed by dividing fat mass and lean mass by squared height. We categorized participants into age- and population-specific distribution of high total and trunk FMI, and LMI if values are above 75<sup>th</sup> percentile and low if less than 25<sup>th</sup> percentile of the study group values. Whereas moderate FMI and LMI are 25<sup>th</sup> to 75<sup>th</sup> percentile of the values. Systolic and diastolic blood pressure was measured with an automated monitor (Dinamap 9301 Vital Signs Monitor). Plasma lipids (total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol) were determined by enzymatic reagents (35). All assay coefficients of variation were <5% (29). Total plasma adiponectin was assayed using ELISA (R&D Systems, Abingdon, UK) and the interassay coefficient of variation (CV) was 7% (35). Participants' plasma adiponectin level was classified in tertile (3 equal distribution of low, moderate and high categories).

### *2.4. Determining cardiometabolic health*

We categorized participants as having good or poor cardiometabolic health if they had or did not have three or more cardiometabolic risk factors according to the modified National Cholesterol Education Program and International Diabetes Federation. These guidelines stipulate that children and adolescents who have at least three of systolic or diastolic blood pressure >75<sup>th</sup> percentile, HDL <25<sup>th</sup> percentile, triglycerides >75<sup>th</sup> percentile, and or body mass index (BMI) >75<sup>th</sup> percentile meets criteria for diagnosing metabolic syndrome (36). We classified children as having poor cardiometabolic health if their systolic blood pressure, triglycerides, and or FMI lies above the 75<sup>th</sup> percentile and HDL falls below the 25<sup>th</sup> percentile of the studied population values. Children with good cardiometabolic health had systolic blood pressure, triglycerides, and or FMI below the

75th percentile and HDL above the 25th percentile. We used total FMI in place of BMI since it provided a more precise measure of adiposity in this age group (37).

### *2.5. Assessment of cardiorespiratory fitness*

We assessed CRF at a heart rate of 170 beats per minute (bpm) via physical work capacity [PWC<sub>170</sub>], measured in watts (38), on an electronically braked cycle ergometer (Lode Rechor P). PWC<sub>170</sub> is moderately correlated ( $r = 0.49-0.54$ ,  $p \leq 0.01$ ) with absolute peak oxygen uptake (39). After a 1-min warm-up period, children pedaled at 55 – 65 revolutions per minute for 3 min at each workload (20, 40 and 60 W), interspersed with a 2-min rest. The workload required to elicit a heart rate of 170 beat/mins was predicted from the mean heart rate at the end of each stage by regression models. At a heart rate of  $\geq 150$  beat/mins in the final stage, children were considered to exercise at about the predicted value of 170 beat/mins. We computed a log-linear regression model with sex and body mass or lean mass as independent variables and CRF as the dependent variable (40,41) and expressed CRF as absolute CRF (Watts[W]), allometrically scaled CRF/body mass ( $W \cdot kg \text{ BM}^{-0.21}$ ) and allometrically scaled CRF/lean mass ( $W \cdot kg \text{ LM}^{-0.54}$ ) (Supplemental Digital Content 1). We also categorized participants' CRF allometrically scaled by body mass or lean mass in tertile categories of low, moderate, and high groups.

### *2.6. Assessment of arterial stiffness, elasticity, and endothelial function*

During a regular clinic visit which lasted over 3 hours, arterial measurements were acquired in approximately 40 minutes by six trained research technicians. Of these arterial measures, carotid-radial PWV was first assessed then brachial artery distensibility, and lastly, brachial endothelial function testing, using high-resolution ultrasound techniques (8,42). A high-fidelity micromanometer (SPC-301, Millar Instruments, Houston, TX, USA) measured pressure-pulse waveforms from the radial and carotid pulse. The electrocardiogram signal provided an R-timing reference. Integrated software analyzed the mean time difference between R-wave and pressure

wave on a beat-to-beat basis over 10 seconds. We calculated carotid-radial PWV as a ratio of the arterial path length between the two recording points to the meantime difference for the pulse waveform (SphygmoCor version 7.1, Colson, UK). We assessed brachial artery elasticity on the arterial segment using an ALOKA 5500 high-resolution ultrasound system with a 5 – 10 MHz linear array probe (Keymed, UK). A pediatric cuff was used for arm circumference <25 cm and an adult cuff for arm circumference >25 cm when blood pressure was measured at the time of image acquisition. Real-time B-mode images were recorded and saved on SVHS video for 20 seconds which were later analyzed offline at the Vascular Physiology Laboratory Unit. The luminal diameter excursion from diastole to systole was computed as the elasticity of the artery. We calculated the distensibility coefficient (DC), from the distension and the pulse pressure was expressed as a mean percent change in cross-sectional area per unit change in blood pressure. Using high-resolution ultrasound (ALOKA 5500) at 5 – 10 cm above the antecubital fossa, the right brachial artery was scanned after a 5 min inflation of a pneumatic cuff to 200 mmHg (8). The edge-detection software was used in measuring the brachial artery diameter (Brachial Tools, MIA, IA, USA) from electrocardiogram-triggered ultrasound images captured at 3-second intervals throughout the 11-minute recording protocol. We expressed FMD as the maximum percentage change in vessel diameter from baseline. We did not allometrically scale FMD for baseline diameter because the scaling exponent was close to 1.0, hence our results relate to unscaled FMD adjusted for baseline diameter.

### *2.7. Inter-rater and intra-participant reproducibility of arterial measures*

Over a 2-year study period, the within- and between-technician reproducibility for the acquisition of arterial measures was assessed at three different stages (8). During this study period, healthy staff volunteers (i.e. adults who were not ALSPAC participants), aged 18 – 30 years, were studied at the beginning (n = 23), middle (n = 25), and end (n = 10). All the participants underwent assessment of vascular measures on at least two occasions. The coefficients of variation between technicians for

FMD, carotid-radial PWV, and DC measures were 10.5, 4.6, and 6.6 at the beginning of the study, 7.7, 3.4 and 6.6 at the middle of the study, and 7.7, 4.1, and 10% at the end respectively (8).

Technician performance explained < 5% of the variation in the individual vascular measures. The intra-participants variability of the measures was assessed by collection of repeat vascular measures among randomly selected 231 children (3% of the cohort) within 6 weeks of their initial visit (8).

The coefficients of intra-participant variation for FMD, carotid-radial PWV, and DC were 10.9, 8.7, and 18 respectively. This intra-participant variability outlined the influence of temperature, time of day, position of the ultrasound probe, tonometer, and physiological haemodynamic such as blood flow, vessel size, and reactive hyperaemia, although these parameters accounted for minimal variation in vascular measures ( $R^2$ : 0.02 for FMD, 0.03 for carotid-radial PWV and 0.006 for DC).

### *2.8. Dealing with missing data and multiple imputations*

Altogether 7706 children attended the clinic visit at 9 years of age and 6972 children were present at the age 10 years clinic visit. We restricted study participants to those who had all arterial outcomes (FMD, DC, and carotid-radial PWV) at age 10 years (n=5566). Children who were excluded for incomplete arterial outcomes had similar CRF, arterial, and metabolic measures, but trivially different body composition compared to complete cases (see Table S1, Supplemental Digital Content 2, Characteristics of participants excluded from the study). However, eligible sample size for the present study varied by vascular measures that was conducted at the age 10 years clinic visit (see Table S2, Supplemental Digital Content 3, Listwise exclusion of missing data among study participants). Among 5566 children with complete vascular variables, exclusions via listwise deletion of missing values ranged from 4.7 to 51.8 percent for predictors and 0.2 to 54.9 percent for covariates. The complete case sample sizes for predictors were 2813 (51%) for CRF, 5304 (95%) for total and trunk fat mass and lean mass, and 3767 (68%) for adiponectin. We conducted a Little's missing completely at random (MCAR) test to ascertain data missingness (42). Little's MCAR test: Chi-Square = 724.274, Degree of freedom = 346, P-value <0.0001. Since the p-

value is statistically significant we conclude that the variables are not missing completely at random. We then conducted linear regression type multiple imputations (29) using SPSS version 25 (IBM SPSS Statistics, IBM Corporation, Armonk, NY) to minimize selection bias (see Table S3, Supplemental Digital Content 4, presents imputed variables). Constraints for the imputation process were the observed minimum and maximum values; for skewed variables, the logarithmic transformation was used in the imputation model. 20 cycles of imputation via regression model produced 20 imputed data sets. Outcomes from these imputed data were pooled using the multiple imputation module in SPSS. Given the percentage of missing values we estimated that 20 imputation cycles would be sufficient: the variable with the highest missing value (54.9%, mother's social-economic status), had an estimate that was 99% efficient after 20 cycles of imputations (computed using Rubin's formula) (43). We examined the histogram's normality curves for imputed variables ensuring that the distributions of predictors and covariates had the same pattern as in the observed data. Variable distributions after imputation (mean, standard deviation (SD), and percentages) were congruent with the observed data (see Table S4, Supplemental Digital Content 5, Characteristics of cohort participants with imputed data). We presented results in the main article as tables and figures from the pooled outcomes of the imputation, and the same analyses using the observed (non-imputed) are also presented (see Table S5–8, Supplemental Digital Content 6–9, complete case analyses of associations between predictors and outcomes). Where multiple imputations have been conducted, presenting imputed results is preferred to presenting non-imputed results (44).

## *2.9. Statistical analyses*

We summarized descriptive characteristics as means with SD or medians with interquartile ranges, frequencies in percentages (%), and sex differences were explored using Independent t-tests or Mann Whitney-U tests for normally distributed or skewed variables, Chi-square test for dichotomous variable, and one-way analysis of variance for multi-categorical variable. The normal

distribution of variables was assessed by histogram and Kolmogorov-Smirnov tests. We separately examined the associations of absolute CRF, CRF allometrically scaled for body mass, CRF allometrically scaled for lean mass, adiponectin, total fat mass, trunk fat mass, or lean mass with FMD, DC, carotid-radial PWV via multivariable linear regression, first exploring adjustment for age and sex (model 1); adjusted for age, sex, total fat mass or CRF allometrically scaled for lean mass (model 2); adjusted for age, sex, and lean mass or CRF allometrically scaled for lean mass (model 3); additional adjustment of model 2 for puberty and diameter of brachial artery (model 4); adjusted model 4 for maternal socioeconomic status, low-density lipoprotein cholesterol, systolic blood pressure and time (years) difference between measurement of exposure and outcome variable (model 5). We presented 5 models to provide extensive details regarding how different covariates influenced the associations. All covariates were selected based on previous studies and biological plausibility (2,8,10,29,45). A logarithm transformed total and trunk fat mass, adiponectin and DC variables were used in the analysis but logged DC variable was back-transformed by exponentiation. To correct for multiple testing, we investigated the associations of categories of CRF allometrically scaled for body mass, CRF allometrically scaled for lean mass, adiponectin, LMI, total, and trunk FMI with arterial outcomes using generalized linear models. All analyses were corrected for multiple testing using Sidak correction method. The results are presented as estimated marginal means. The Sidak corrected multivariate analyses yields similar results as the uni-directional analysis thus also serving as sensitivity analysis. We also examined the mediating effects of CRF allometrically scaled for lean mass on the associations of cardiometabolic health with FMD, DC and carotid-radial PWV through bootstrapped (10,000 samples) (46-48) linear regression analysis that was adjusted for age, sex, brachial artery diameter, puberty, and maternal socio-economic status. There were 3 equations per regression model; linear regression on the association of CRF allometrically scaled for lean mass with cardiometabolic health (Equation 1), linear regression on the association of CRF allometrically scaled for lean mass with FMD, DC, and

carotid-radial PWV (Equation 2), linear regression on the association of cardiometabolic health with FMD, DC and carotid-radial PWV (Equation 3), and Equation 3' accounted for the mediating role of CRF allometrically scaled for lean mass. The proportion of mediating roles was estimated as the ratio of the difference between Equation 3 and Equation 3' divided by Equation 3 and expressed in percentage. A mediating or indirect role is confirmed when there are statistically significant associations between (a) the predictor and mediator, (b) the predictor and outcome, (c) the mediator and outcome, and (d) the association between the predictor and outcome variable was attenuated upon inclusion of the mediator (46,47). We tested the hypothesis of a significant mediating effect using the Sobel test and reported the z-scores and p-values. We also reported the point estimates and 95% CI for the mediating effect of CRF allometrically scaled for lean mass. To test for bi-directional associations (where the relationship between the independent and dependent variable is analyzed in a reversed pattern), we performed multivariable regression analyses but modeled arterial outcomes (FMD, DC, and carotid-radial PWV) as predictor, and absolute CRF, CRF allometrically scaled for body mass, CRF allometrically scaled for lean mass, adiponectin, total and trunk fat mass, and lean mass as outcomes, separately. Model 1 was unadjusted while Model 2 was adjusted for all covariates detailed in uni-directional analyses. Differences and associations with p-values <0.05 from 2-sided tests were considered statistically significant. Analyses involving 50% of a sample of 10,000 ALSPAC children at 80% statistical power, 0.05 alpha, and 2-sided p-value would show a minimum detectable effect size of 0.048 SD if they had relevant exposure for a normally distributed quantitative variable (49). For a primary outcome, our sensitivity analysis revealed that a fixed sample size of 5566 participants yielded Cohen's  $f^2$  effect size of 0.003 at 90% power and a two-sided alpha threshold of 0.05. We performed all statistical analyses using SPSS statistics software, Version 25.0 (IBM Corp, Armonk, NY, USA) and mediation analyses by PROCESS SPSS script Version 3.5 (48).

### 3. Results

Of the 7706 children who participated in the ALSPAC study ‘focus @ 9’ clinic examination, 5566 (2816 [51%] girls, median age 9.75 years) children had complete data for outcome (arterial) variables and were included in the analysis. We presented our participant's demography in Table 1, and there are sex differences in nearly all variables except for age, maternal social-economic status, and DC.

#### *3.1. Associations of cardiorespiratory fitness and adiponectin with arterial function and structure*

CRF/body mass<sup>-0.21</sup> was directly associated with DC ( $\beta$  [95% CI] = 0.004 [ $<0.0001$  to 0.008];  $P = 0.046$ ) while CRF/lean mass<sup>-0.54</sup> was inversely related to carotid-radial PWV (-0.034 [-0.063 to -0.003]; 0.032) after full model adjustment (Table 2, Model 5). However, CRF was not associated with FMD. There was a statistically significant difference between categories (low, moderate, and high) of either CRF/body mass<sup>-0.21</sup> or CRF/lean mass<sup>-0.54</sup> in relation to DC (Figure 1 and Table S6, Supplemental Digital Content 7). Plasma adiponectin was not related to FMD, DC, and carotid-radial PWV, just as there was no difference in the association of adiponectin categories with FMD, DC, and carotid-radial PWV.

#### *3.2. Associations of total and trunk fat mass and lean mass with arterial function and structure*

Total fat mass, trunk fat mass and lean mass were directly associated with FMD and DC after full model adjustments (Table 2, Model 5). Total fat mass (-0.324 [-0.393 to -0.256];  $<0.0001$ ) and trunk fat mass (-0.265 [-0.323 to -0.206];  $<0.0001$ ) but not lean mass were inversely associated with carotid-radial PWV following similar adjustment strategy. Participants with high levels of total FMI, trunk FMI and LMI had statistically significant higher FMD and lower carotid-radial PWV (Figure 1 and Table S6, Supplemental Digital Content 7).

#### *3.3. Mediating effects of cardiorespiratory fitness on the associations of cardiometabolic health and arterial function and structure*

CRF/lean mass<sup>-0.54</sup> mediated the relationships of cardiometabolic health with FMD, DC and carotid-radial PWV by 1.5%, 12.1% and 3.5% respectively after adjusting for age, sex, diameter of the brachial artery, puberty, and mother's socio-economic status (Figure 2).

#### *3.4. Associations of arterial function and structure with cardiorespiratory fitness and adiponectin*

DC was directly associated with CRF/body mass<sup>-0.21</sup> (0.242 [0.033 to 0.451]; 0.023) while carotid-radial PWV was inversely associated with CRF/lean mass<sup>-0.54</sup> (-0.030 [-0.060 to -0.001]; 0.043) after full model adjustments (Table 3, Model 2). FMD had no relationship with CRF measures. FMD, DC, and carotid-radial PWV had no association with adiponectin.

#### *3.5. Associations of arterial function and structure with total and trunk fat mass and lean mass*

FMD and DC were directly associated with total fat mass, trunk fat mass, and lean mass after adjusting for all uni-directional covariates (Table 3, Model 2). However, carotid-radial PWV was inversely associated with total fat mass (-0.050 [-0.060 to -0.039]; <0.0001) and trunk fat mass (-0.057[-0.070 to -0.045]; <0.0001) but not lean mass, after covariate adjustment.

#### **4. Discussion**

We showed the bi-directional associations of CRF allometrically scaled for lean mass, CRF allometrically scaled for body mass, total fat mass, trunk fat mass, and lean mass with arterial function (FMD) and structure (DC and carotid-radial PWV) in a large cohort of 9-11-year olds, after controlling for cardiometabolic and lifestyle factors (Tables 2 and 3). Another novel finding was that CRF allometrically scaled for lean mass partly mediated the relationships of cardiometabolic health with FMD, DC, and carotid-radial PWV (Figure 2). We also reported that poor cardiometabolic health was related to low CRF allometrically scaled for lean mass in addition to other studies that have reported an inverse association between CRF and cardiometabolic health (10,12), suggesting temporality. Since fat mass mediates the association of cardiometabolic health with arterial stiffness in adolescence (29), improving CRF in childhood may be clinically important to preventing metabolic syndrome, cardiovascular diseases, and preclinical atherosclerosis (5,12,13). Together, these findings suggest a protective effect of CRF and the physiologic adaptation of arterial function and structure to body composition and cardiometabolic health in childhood.

Recent studies in children and adolescents have reported no relationships between CRF and carotid-radial PWV (14), and between CRF and FMD (15), an association of CRF with DC but not with arterial stiffness (13), an association between CRF and carotid artery elasticity (6), and a higher CRF in relation to high PWV (16). Some of these studies (13-16), with small to moderate sample sizes, did not account for important covariates such as sex (8), brachial artery diameter (8), blood pressure (28), lean mass (28) or total fat mass (29) and puberty. In our study, controlling for these covariates revealed that allometrically scaled CRF relates to arterial structure independent of body composition and other risk factors and that arterial health is associated with CRF although with some inconsistencies. We also report that these associations are bi-directional suggesting that healthy arterial structure may enhance CRF plausibly via increased vital capacity and tidal volume, improved oxygenation for muscle contractility, better oxidative phosphorylation, and cardiac

compliance (50). Evidence suggests that altered arterial structure may lead to diminished lung function since the loss of small arteries elasticity results in decreased elastic recoil of the lung tissue (50,51). Endothelial dysfunction may result in the proliferation of pulmonary vascular endothelial cells and type II pneumocytes leading to a cascade of increased pro-inflammatory cytokines, arterial stiffness, and diminished lung function (50,51). On the other hand, an optimal CRF may improve endothelial function, vasculogenesis, aortic compliance, nitric oxide production and decrease arterial stiffness via sympathetic control, endothelium-mediated flow-induced vasodilatation, metabolic vasodilatation, and myogenic control (50). Small conduit and resistance arteries respond more to endothelium-dependent vasodilatation while smaller arterioles have a more myogenic response to increased perfusion pressure and metabolic control (50). The oxygen/metabolic sensor coupled to vascular smooth muscle cell enhances the metabolic control of resistance arteries by increasing the production of the vasodilatory prostaglandins in response to decreased oxygen and tissue acidosis from increased carbon dioxide, leading to an erythrocyte ATP-induced vasodilatation (50).

A systematic review and meta-analysis concluded that obesity was associated with higher arterial stiffness in children, but studies that address confounders, such as age, sex, and puberty are needed (2). A recent study reported an association between high total fat mass but not trunk fat mass at 9 years and high carotid-femoral PWV at 17 years (29). On the contrary, we found that high total and trunk fat mass were associated with lower carotid-radial PWV at 9-11 years in both directions. Our result suggests that the pattern of fat distribution and the quantity of fat mass are both important determinants of arterial health in 9-11-year-olds. Although it may be argued that the carotid-radial PWV assesses muscular arteries rather than elastic arteries, evidence suggests that atherosclerotic changes occur in the former during early life (1,8,45). Besides, additional analyses showed that a one-unit increase in carotid-radial PWV (arterial stiffness) resulted in a three-unit decrease in arterial elasticity or compliance (DC), suggesting the importance of carotid-radial PWV measure in

early childhood, independent of sex and brachial wall diameter. Our findings in comparison with previous studies revealed that the influence of fat mass on arterial stiffness in children vary across elastic and muscular arteries (27,29).

While examining the varied associations of fat mass and lean mass with arterial function and structure, a study found that controlling for lean mass yielded a negative but non-significant relationship between fat mass and carotid-radial PWV (27). They opined that lean mass was the main reason for the association between fat mass and arterial indices (27). The authors also reported a positive association between FMI and FMD in 8-9-year old suggesting an adaptive response of endothelial-dependent dilation to increased fat mass (27), although only about 4% of our study participants are obese (42). Interestingly, we found that controlling for lean mass had minimal influence on the positive associations of fat mass with FMD and DC or the inverse relationship of fat mass with carotid-radial PWV. We observed a bi-directional relationship between fat mass and late childhood/early adolescents' arterial health. Taken together, our findings suggest that better arterial function and structure may influence the accumulation of fat mass just as excess fat mass may alter arterial function and structure (2,27,29,45). An efficient arterial network may enhance adipogenesis and white adipose tissue browning, lipid droplet formation, low macrophage activation and effective fat deposit, which can be described as an 'arterial paradox' (52). We also noted that high lean mass independent of total fat mass was associated with high FMD and DC in either direction, but no relations with carotid-radial PWV. However, additional analysis revealed that high LMI was significantly related to lower carotid-radial PWV suggesting that an increase in lean mass may be protective of arterial structure (27).

A key finding was that CRF allometrically scaled for lean mass partially mediated the association of cardiometabolic health with FMD, DC and carotid-radial PWV in this population. There is a mediating or indirect role when there are statistically significant associations between (a) an independent variable and a mediator, (b) the independent and the dependent variable, (c) the

mediator and dependent variable, and (d) the diminished association between the independent and dependent variable when the mediator is included in the model (46,47). We defined cardiometabolic health as poor if children had three or more cardiometabolic risk factors such as systolic or diastolic blood pressure >75th percentile, HDL <25th percentile, triglycerides >75th percentile, and or FMI >75th percentile. Although CRF allometrically scaled for lean mass appears to associate with FMD in this mediation analysis it must be noted that due to the components of cardiometabolic health, we avoided dual adjustments for the same variables. Plausible explanations may be that an increased blood pressure stimulates higher collagen deposits which alters the arterial wall elastin-collagen ratio. Similarly, dyslipidemia and increasing fat deposits result in disarrayed endothelium, friable elastin molecules, lower levels of adiponectin, and elevated inflammatory markers such as cytokines, macrophages, growth factors that ultimately stiffen the arteries (50,53,54). However, elevated CRF may increase vessel compliance via higher pulsatile perfusion, decreased late pulse pressure augmentation, higher ventricular ejection during exercise that enhances nitric oxide production, and activation of calcium-sensitive K<sup>+</sup> channels linked to endothelial-derived hyperpolarizing factor (13,50,53,54). Thus, poor cardiometabolic health in children may indirectly result in arterial stiffness through diminished CRF. It should be noted that the mediation role of CRF is circa 4% and 12% for carotid-radial PWV and DC, measures of arterial structure, respectively. This CRF mediation role may have a large public health and clinical significance since our population is young, 9-11 years old, and with most having good cardiometabolic health and arterial structure and function. Our finding reinforces the need for primordial prevention of cardiometabolic-induced arterial diseases from childhood by improving CRF.

Although low childhood adiponectin has been described as a novel pediatric risk factor in better predicting early signs of atherosclerosis in adulthood in comparison to other risk factors such as body mass index and systolic blood pressure (26), we found no association between adiponectin and arterial function and structure in childhood. This result is in consonance with a normal-weight

adolescents' study, where adiponectin was not related to FMD or DC (25). Perhaps, the influence of childhood adiponectin on altered arterial function and structure in adulthood (26), may remain undetected in childhood and adolescence, particularly in normal-weight individuals, since there is an ongoing vascular adaptation to growth and development. Moreover, less than ten percent of our cohort participants had poor cardiometabolic health which might be protective of severe atherosclerosis. Therefore, adiponectin may not predict altered arterial structure and function in these apparently healthy children. Future studies investigating this question may compare a similarly healthy population with those having higher cardiometabolic risks.

This study had several strengths such as a comprehensive measure of cardiometabolic, fat mass, lean mass, and vascular variables in a large well-characterized cohort (ALSPAC) and an objective measure of pubertal status (34). With sufficient statistical power (49), we provided novel evidence on the mediating effect of CRF allometrically scaled for lean mass on cardiometabolic health and arterial outcomes and the bi-directional associations of CRF and body composition with arterial measures in children. From a clinical perspective, we computed cardiometabolic health using diagnostic guidelines (36) rather than compute a cardiometabolic risk score as often used in epidemiological studies (10). This approach improves the clinical relevance of our findings and makes for easier interpretation, particularly among pediatric health care providers. The sub-maximal measure of CRF, although moderately correlated with maximal aerobic capacity (39), might have decreased its predictive ability. Nonetheless, we adequately controlled this CRF measure for body composition by allometric scaling (40). Almost half of our participants had missing CRF data, and a complete case analysis involving these participants only would be bias to selection and decrease the precision and power of the study (43,44,55). We addressed this limitation by conducting 20 cycles of multiple imputations in line with established practices in epidemiological studies and presented both imputed and complete case results which were similar (29,43,44,55). The mechanistic basis for our findings would require longitudinal studies to

distinguish between early life preclinical atherosclerosis and adaptive effects in relation to growth and development. We may not extrapolate our results to other ethnic groups since we studied mostly Caucasian children. Lastly, our observational study precludes us from inferring causality.

## **5. Conclusion**

Our findings suggest that CRF, fat mass and lean mass associates with arterial function and structure in 9-11-year old and may have temporal or bi-directional relationships. We found no evidence associating adiponectin with arterial outcomes in our apparently healthy children. The effective prevention of preclinical markers of atherosclerosis and cardiometabolic diseases from childhood may focus on improving CRF since we have demonstrated that allometrically scaled CRF for lean mass mediates the associations of cardiometabolic health with arterial function and structure.

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## **Data availability**

The informed consent obtained from ALSPAC participants does not allow the data to be made freely available through any third party maintained public repository. However, data used for this

submission can be made available on request to the ALSPAC Executive. The ALSPAC data management plan describes in detail the policy regarding data sharing, which is through a system of managed open access. Full instructions for applying for data access can be found here: <http://www.bristol.ac.uk/alspac/researchers/access/>. The ALSPAC study website contains details of all the data that are available (<http://www.bristol.ac.uk/alspac/researchers/our-data/>).

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**Table 1** Characteristics of cohort participants.

Variable	All participants		Boys		Girls		P for sex difference
	Mean (SD)	n	Mean/SD	n	Mean/SD	n	
* Age (years)	9.75 (0.33)	5566	9.7(0.33)	2750	9.75 (0.33)	2816	0.967
Somatic maturation (puberty) by time to aPHV (n, %)	22 (0.5)	4203	0	2003	22 (1)	2200	<0.0001
<b>Social-economic status based on Mother's occupation</b>							
I – Professional (%)	5.1	127	5.9	75	4.2	52	0.857
II - Managerial and Technical (%)	36.3	910	35.6	453	37.0	457	
IIIa - Skilled non-manual (%)	39.1	981	38.6	491	39.6	490	
IIIb - Skilled manual (%)	1.7	43	1.5	19	1.9	24	
IV - Partly skilled (%)	14.7	370	15.8	201	13.7	169	
V - Unskilled (%)	3.1	78	2.7	34	3.6	44	
<b>Anthropometry and Fat measure</b>							
Body height (m)	1.40 (0.06)	5510	1.40 (0.06)	2729	1.39 (0.06)	2781	0.003
*Weight (kg)	33.0 (8.6)	5555	32.8 (8.0)	2743	33.40 (9.20)	2812	0.022
*Body mass index (kg/m <sup>2</sup> )	16.95 (3.32)	5506	16.74 (3.09)	2727	17.22 (3.54)	2779	<0.0001
Lean mass (kg)	24.52 (3.19)	5304	25.49 (2.93)	2622	23.57 (3.14)	2682	<0.0001
Lean mass indexed for squared height (kg/m <sup>2</sup> )	12.56 (0.99)	5255	13.00 (0.86)	2606	12.12 (0.91)	2649	<0.0001
*Total fat mass (kg)	7.14 (6.06)	5304	5.67 (5.16)	2622	8.41 (6.04)	2682	<0.0001
*Total fat mass indexed for squared height (kg/m <sup>2</sup> )	3.67 (2.94)	5255	2.94 (2.55)	2606	4.37 (2.92)	2649	<0.0001
*Trunk fat mass (kg)	2.65 (2.68)	5304	2.04 (2.17)	2622	3.28 (2.81)	2682	<0.0001
*Trunk fat mass indexed for squared height (kg/m <sup>2</sup> )	1.37 (1.34)	5255	1.04 (1.07)	2606	1.70 (1.39)	2649	<0.0001
<b>Metabolic profile</b>							
High-density lipoprotein (mmol/L)	1.40 (0.31)	3769	1.43 (0.31)	1902	1.37 (0.30)	1867	<0.0001
Low-density lipoprotein (mmol/L)	2.35 (0.60)	3769	2.26 (0.58)	1902	2.45 (0.61)	1867	<0.0001
*Triglyceride (mmol/L)	1.00 (0.60)	3769	0.97 (0.60)	1902	1.04 (0.59)	1867	<0.0001
*Adiponectin (µg/ml)	12.35 (7.02)	3767	11.88 (6.38)	1900	13.04 (7.37)	1867	<0.0001
<b>Fitness measure</b>							
CRF (W)	63.92 (9.07)	2813	67.13 (8.25)	1179	61.61 (8.94)	1634	<0.0001
CRF/BM (W·kg BM <sup>-1</sup> )	1.92 (0.38)	2810	2.07 (0.38)	1178	1.82 (0.34)	1632	<0.0001
CRF/BM <sup>0.21</sup> (W·kg BM <sup>-0.21</sup> )	31.31 (4.31)	2810	33.04 (3.93)	1178	30.06 (4.14)	1632	<0.0001
CRF/LM <sup>0.54</sup> (W·kg LM <sup>-0.54</sup> )	11.51 (1.46)	2683	11.90 (1.36)	1122	11.21 (1.46)	1561	<0.0001
<b>Vascular measure</b>							
Pulse rate (beats/min)	79 (11)	5512	77 (10)	2722	81 (11)	2790	<0.0001
Systolic blood pressure (mmHg)	103 (9)	5511	102 (9)	2722	103 (10)	2790	0.049
Diastolic blood pressure (mmHg)	57 (6)	5512	57 (6)	2722	58 (6)	2790	0.002
Brachial artery diameter (mm)	2.67 (0.30)	5566	2.76 (0.29)	2750	2.59 (0.29)	2816	<0.0001
Flow-mediated dilation (%)	8.07 (3.37)	5566	7.72 (3.25)	2750	8.41 (3.44)	2816	<0.0001
Flow-mediated dilation absolute (mm)	0.21 (0.08)	5566	0.21 (0.09)	2750	0.22 (0.09)	2816	0.029
PWV carotid-radial (m/s)	7.54 (1.22)	5566	7.63 (1.24)	2750	7.46 (1.20)	2816	<0.0001
*Distensibility coefficient (% per mmHg)	11.51 (6.91)	5566	11.66 (6.88)	2750	11.35 (6.96)	2816	0.310

The values are means (standard deviations) and \*median (interquartile range) except for maturation status and social economic status in percentage. Differences between girls and boys were tested using Student's t-test for normally distributed continuous variables, Mann-Whitney U test for skewed continuous variables, Chi-square test for dichotomous variable, and one-way analysis of variance for multi-categorical variable. A 2-sided P-value <0.05 is considered statistically significant. aPHV, age at peak height velocity; BM, body mass; CRF, cardiorespiratory fitness; LM, lean mass; PWV, pulse wave velocity; W, watt.

**Table 2** Multivariable adjusted association of cardiorespiratory fitness, fat mass, lean mass and adiponectin with endothelial function, arterial elasticity and arterial stiffness

n = 5566	Model 1		Model 2		Model 3		Model 4		Model 5	
	$\beta$ (95% CI)	P-value	$\beta$ (95% CI)	P-value	$\beta$ (95% CI)	P value	$\beta$ (95% CI)	P-value	$\beta$ (95% CI)	P-value
<b>Flow-mediated dilation (%)</b>										
CRF (W)	-0.008 (-0.021 to 0.005)	0.216	-0.008 (-0.021 to 0.005)	0.232	-0.001 (-0.015 to 0.012)	0.827	0.002 (-0.011 to 0.014)	0.791	0.002 (-0.011 to 0.014)	0.806
CRF/BM <sup>-0.21</sup> (W·kg BM <sup>-0.21</sup> )	-0.011 (-0.039 to 0.017)	0.427	-0.012 (-0.040 to 0.016)	0.408	-0.008 (-0.036 to 0.020)	0.579	<0.0001(-0.027 to 0.027)	0.999	-0.001 (-0.028 to 0.026)	0.953
CRF/LM <sup>-0.54</sup> (W·kg LM <sup>-0.54</sup> )	0.010 (-0.071 to 0.091)	0.813	0.009 (-0.072 to 0.090)	0.826	-0.005 (-0.086 to 0.076)	0.912	-0.006 (-0.084 to 0.072)	0.888	-0.010 (-0.088 to 0.069)	0.811
*Adiponectin (µg/ml)	-0.045 (-0.298 to 0.208)	0.726	-0.048 (-0.303 to 0.206)	0.708	-0.113 (-0.370 to 0.143)	0.384	-0.188 (-0.439 to 0.062)	0.139	-0.183 (-0.431 to 0.064)	0.146
*Total fat mass (kg)	-0.032 (-0.197 to 0.134)	0.708	-0.030 (-0.196 to 0.136)	0.719	0.178 (-0.011 to 0.368)	0.065	0.535 (0.366 to 0.704)	<0.0001	0.585 (0.401 to 0.769)	<0.0001
*Trunk fat mass (kg)	0.013 (-0.128 to 0.154)	0.859	0.014 (-0.128 to 0.155)	0.848	0.200 (0.039 to 0.361)	<b>0.015</b>	0.489 (0.344 to 0.634)	<0.0001	0.535 (0.377 to 0.693)	<0.0001
Lean mass (kg)	-0.064 (-0.094 to -0.034)	<0.0001	-0.080 (-0.114 to -0.045)	<0.0001	-0.064 (-0.094 to -0.034)	<0.0001	0.041 (0.006 to 0.077)	<b>0.022</b>	0.047 (0.011 to 0.084)	<b>0.011</b>
<b>*Distensibility co-efficient (% per mmHg)</b>										
CRF (W)	0.002 (<0.0001 to 0.004)	0.085	0.002 (<0.0001 to 0.004)	0.109	0.001 (-0.001 to 0.003)	0.160	0.002 (<0.0001 to 0.004)	0.053	0.002 (<0.0001 to 0.004)	0.057
CRF/BM <sup>-0.21</sup> (W·kg BM <sup>-0.21</sup> )	0.003 (-0.001 to 0.007)	0.093	0.004 (<0.0001 to 0.008)	0.076	0.003 (-0.001 to 0.007)	0.111	0.004 (<0.0001 to 0.008)	<b>0.044</b>	0.004 (<0.0001 to 0.008)	<b>0.046</b>
CRF/LM <sup>-0.54</sup> (W·kg LM <sup>-0.54</sup> )	0.009 (-0.003 to 0.021)	0.144	0.009 (-0.003 to 0.021)	0.129	0.010 (-0.002 to 0.022)	0.109	0.009 (-0.003 to 0.020)	0.156	0.009 (-0.003 to 0.021)	0.151
*Adiponectin (µg/ml)	-0.006 (-0.039 to 0.026)	0.697	-0.005 (-0.038 to 0.027)	0.759	-0.003 (-0.036 to 0.030)	0.868	-0.010 (-0.043 to 0.022)	0.528	-0.009 (-0.042 to 0.024)	0.589
*Total fat mass (kg)	0.014 (-0.009 to 0.037)	0.222	0.015 (-0.008 to 0.038)	0.192	0.006 (-0.020 to 0.032)	0.649	0.037 (0.013 to 0.061)	<b>0.003</b>	0.043 (0.017 to 0.070)	<b>0.001</b>
*Trunk fat mass (kg)	0.013 (-0.006 to 0.033)	0.170	0.014 (-0.005 to 0.033)	0.145	0.007 (-0.015 to 0.029)	0.528	0.033 (0.012 to 0.053)	<b>0.002</b>	0.038 (0.016 to 0.060)	<b>0.001</b>
Lean mass (kg)	0.004 (<0.0001 to 0.008)	0.084	0.003 (-0.002 to 0.008)	0.201	0.004 (<0.0001 to 0.008)	0.060	0.009 (0.004 to 0.014)	<b>0.001</b>	0.009 (0.004 to 0.014)	<b>0.001</b>
<b>Pulse wave velocity carotid-radial (m/s)</b>										
CRF (W)	-0.009 (-0.015 to -0.004)	<b>0.002</b>	-0.005 (-0.010 to <0.0001)	<b>0.031</b>	-0.005 (-0.011 to <0.0001)	<b>0.040</b>	-0.004 (-0.009 to 0.001)	0.096	-0.004 (-0.009 to 0.001)	0.096
CRF/BM <sup>-0.21</sup> (W·kg BM <sup>-0.21</sup> )	-0.006 (-0.016 to 0.004)	0.248	-0.011 (-0.021 to -0.001)	<b>0.031</b>	-0.004 (-0.014 to 0.006)	0.455	-0.010 (-0.020 to -0.001)	0.064	-0.009 (-0.019 to 0.001)	0.073
CRF/LM <sup>-0.54</sup> (W·kg LM <sup>-0.54</sup> )	-0.024 (-0.054 to 0.006)	0.120	-0.032 (-0.062 to -0.003)	<b>0.033</b>	-0.033 (-0.064 to -0.003)	<b>0.032</b>	-0.034 (-0.064 to -0.005)	<b>0.024</b>	-0.033 (-0.063 to -0.003)	<b>0.032</b>
*Adiponectin (µg/ml)	0.10 (0.012 to 0.189)	<b>0.026</b>	0.065 (-0.021 to 0.151)	0.138	0.060 (-0.028 to 0.149)	0.179	0.047 (-0.038 to 0.132)	0.279	0.049 (-0.036 to 0.135)	0.254
*Total fat mass (kg)	-0.369 (-0.429 to -0.310)	<0.0001	-0.373 (-0.433 to -0.313)	<0.0001	-0.341 (-0.410 to -0.272)	<0.0001	-0.299 (-0.362 to -0.236)	<0.0001	-0.324 (-0.393 to -0.256)	<0.0001
*Trunk fat mass (kg)	-0.305 (-0.356 to -0.254)	<0.0001	-0.308 (-0.359 to -0.257)	<0.0001	-0.279 (-0.337 to -0.221)	<0.0001	-0.245 (-0.299 to -0.191)	<0.0001	-0.265 (-0.323 to -0.206)	<0.0001
Lean mass (kg)	-0.040 (-0.051 to -0.030)	<0.0001	-0.011 (-0.023 to 0.002)	0.085	-0.042 (-0.052 to 0.031)	<0.0001	0.005 (-0.008 to 0.018)	0.445	0.002 (-0.011 to 0.016)	0.765

BM, body mass; CI, confidence interval; CRF, cardiorespiratory fitness; LM, lean mass; W, watt. The regression coefficient ( $\beta$ ) quantifies the association between dependent and independent variables. A 2-sided P-value <0.05 was considered statistically significant and is bolded.

\*Logarithm transformation of skewed variables were used in analyses.

Model 1: Adjusted for age and sex.

Model 2: Adjusted for age, sex and total fat mass or cardiorespiratory fitness per lean mass, when predictor variable is total fat mass.

Model 3: Adjusted for age, sex, lean mass or cardiorespiratory fitness per lean mass when predictor variable is lean mass.

Model 4: Additional adjustment of Model 2 for puberty and brachial artery diameter

Model 5: Additional adjustment of Model 4 for low-density lipoprotein, systolic blood pressure, maternal socio-economic status and time (years) between fat mass, lean mass, adiponectin, cardiorespiratory fitness data collection and measurement of arterial function and structure.

**Table 3** Association of endothelial function, arterial elasticity, and arterial stiffness with cardiorespiratory fitness, fat mass, lean mass and adiponectin

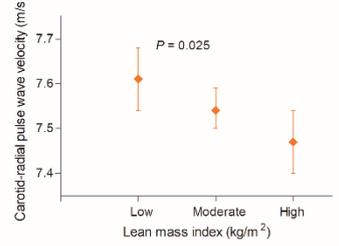
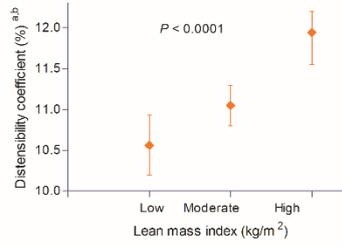
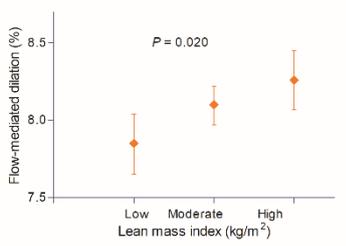
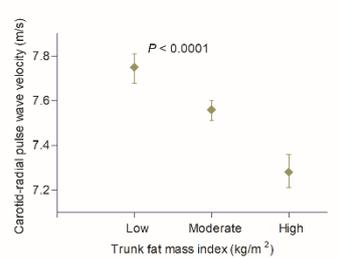
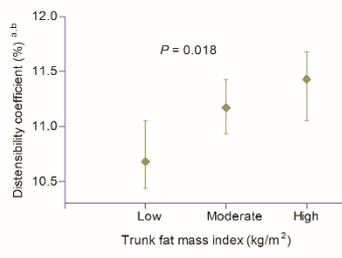
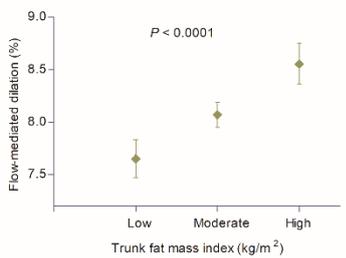
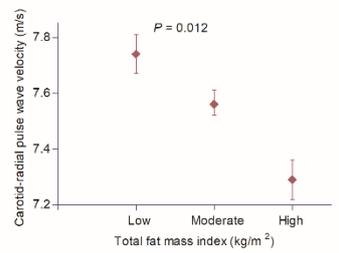
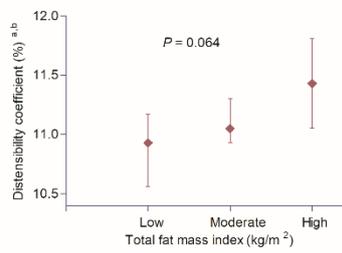
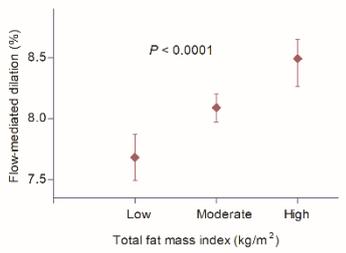
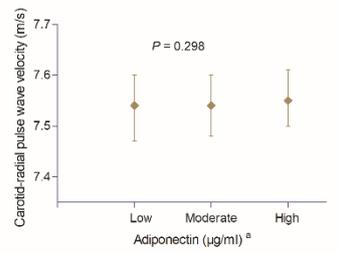
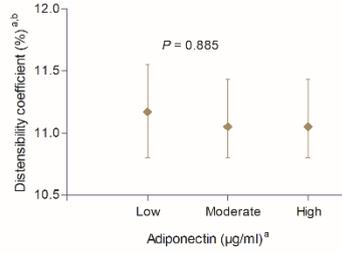
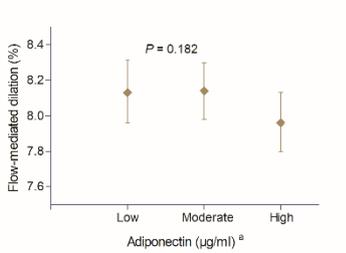
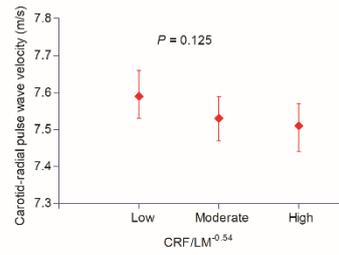
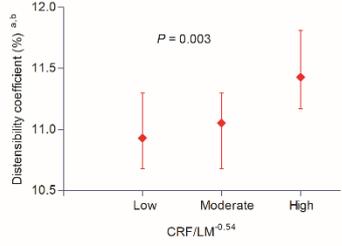
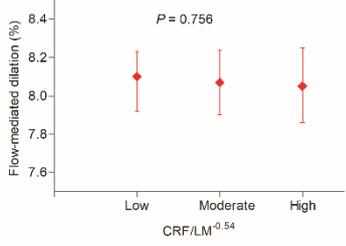
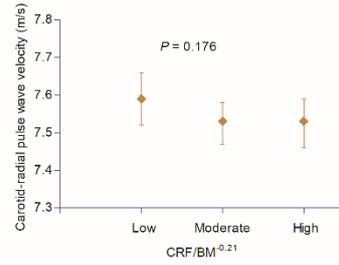
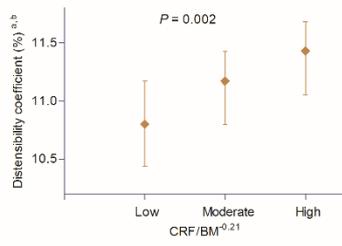
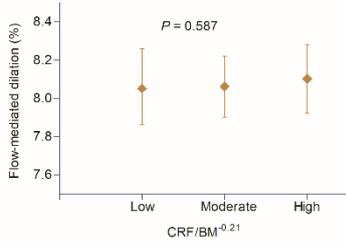
Variable	n= 5566													
	CRF (W)		CRF/BM <sup>-0.21</sup> (W·kg BM <sup>-0.21</sup> )		CRF/LM <sup>-0.54</sup> (W·kg LM <sup>-0.54</sup> )		*Adiponectin (µg/ml)		*Total fat mass (kg)		*Trunk fat mass (kg)		Lean mass (kg)	
	<i>β</i> (95% CI)	<i>P</i> -value	<i>β</i> (95% CI)	<i>P</i> -value	<i>β</i> (95% CI)	<i>P</i> -value	<i>β</i> (95% CI)	<i>P</i> -value	<i>β</i> (95% CI)	<i>P</i> -value	<i>β</i> (95% CI)	<i>P</i> -value	<i>β</i> (95% CI)	<i>P</i> -value
Flow-mediated dilation (%)	-0.099 (-0.161 to -0.037)	<b>0.002</b>	-0.038 (-0.066 to -0.009)	<b>0.010</b>	0.003 (-0.008 to 0.013)	0.615	<0.001 (-0.004 to 0.004)	0.979	0.003 (-0.001 to 0.008)	0.187	0.005 (<0.001 to 0.010)	0.059	-0.081 (-0.106 to -0.056)	<0.0001
*Distensibility co-efficient (% per mmHg)	0.570 (0.109 to 1.031)	<b>0.015</b>	0.214 (-0.001 to 0.429)	0.051	0.058 (-0.019 to 0.135)	0.137	-0.006 (-0.036 to 0.024)	0.695	0.018 (-0.015 to 0.051)	0.283	0.025 (-0.014 to 0.063)	0.211	0.178 (-0.007 to 0.362)	0.059
Carotid-radial pulse wave velocity(m/s)	-0.362 (-0.534 to -0.189)	<b>&lt;0.0001</b>	-0.017 (-0.097 to 0.062)	0.668	-0.016 (-0.045 to 0.013)	0.267	0.010 (<0.001 to 0.020)	<b>0.044</b>	-0.081 (-0.093 to -0.069)	<b>&lt;0.0001</b>	-0.095 (-0.109 to -0.080)	<b>&lt;0.0001</b>	-0.192 (-0.262 to -0.123)	<b>&lt;0.0001</b>
	<b>Model 2</b>													
Flow-mediated dilation (%)	0.019 (-0.043 to 0.081)	0.549	0.003 (-0.027 to 0.032)	0.862	-0.003 (-0.014 to 0.008)	0.558	-0.004 (-0.008 to <0.0001)	0.056	0.012 (0.008 to 0.016)	<b>&lt;0.0001</b>	0.016 (0.011 to 0.020)	<b>&lt;0.0001</b>	0.027 (0.007 to 0.047)	<b>0.009</b>
*Distensibility co-efficient (% per mmHg)	0.594 (0.156 to 1.031)	<b>0.008</b>	0.242 (0.033 to 0.451)	<b>0.023</b>	0.050 (-0.025 to 0.125)	0.193	-0.008 (-0.039 to 0.022)	0.585	0.004 (0.002 to 0.006)	<b>&lt;0.0001</b>	0.005 (0.002 to 0.007)	<b>&lt;0.0001</b>	0.229 (0.088 to 0.369)	<b>0.001</b>
Carotid-radial pulse wave velocity (m/s)	-0.148 (-0.316 to 0.020)	0.083	-0.076 (-0.155 to 0.004)	0.063	-0.030 (-0.060 to -0.001)	<b>0.043</b>	0.005 (-0.005 to 0.015)	0.350	-0.050 (-0.060 to -0.039)	<b>&lt;0.0001</b>	-0.057 (-0.070 to -0.045)	<b>&lt;0.0001</b>	0.013 (-0.040 to 0.067)	0.628

BM, body mass; CI, confidence interval; CRF, cardiorespiratory fitness; LM, lean mass; W, watt. The regression coefficient ( $\beta$ ) quantifies the association between dependent and independent variables. A 2-sided P-value <0.05 was considered statistically significant and is bolded.

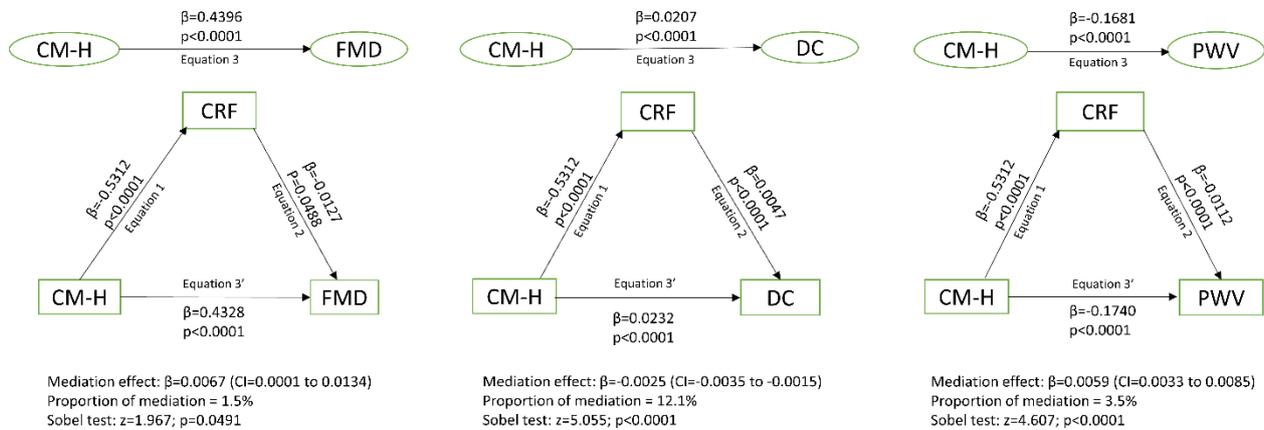
\*Logarithm transformation of skewed variables were used in analyses.

Model 1: Unadjusted associations of arterial function and structure with cardiorespiratory fitness, fat mass, lean mass and adiponectin.

Model 2: Associations were adjusted for age, sex, puberty, systolic blood pressure, mother's socio-economic status, total fat mass or cardiorespiratory fitness/lean mass, low-density lipoprotein, diameter of the brachial artery and between measures of exposure and outcome variable.



**Figure 1** Associations of cardiorespiratory fitness, fat mass, lean mass and adiponectin categories with endothelial function, arterial elasticity and arterial stiffness in 5566 children aged 9-11 years. Estimated marginal means were computed from general linear models of exposure categories in relation to outcome variables with Sidak correction adjusted for age, sex, puberty, systolic blood pressure, mother's socio-economic status, total fat mass or cardiorespiratory fitness scaled by lean mass, low-density lipoprotein, diameter of the brachial artery and time between measures of exposure and outcome variable. <sup>a</sup>These variables were log-transformed by using natural log. <sup>b</sup>This logged variable was back-transformed by exponentiation and values are geometric mean. Associations with p-value <0.05 were considered statistically significant. A significant p-value indicates that at least one category significantly differs from other tertile groups. Categories of total fat mass, trunk fat mass, and lean mass are high if values are above 75<sup>th</sup> percentile and low if less than 25<sup>th</sup> percentile. Whereas moderate category lies between the 25<sup>th</sup> to 75<sup>th</sup> percentile of the values. Cardiorespiratory fitness and adiponectin were classified in tertile (3 equal distribution of low, moderate and high groups). BM, body mass; CRF, cardiorespiratory fitness; LM, lean mass.



**Figure 2** Mediating effect of cardiorespiratory fitness on the associations of cardiometabolic health with endothelial function, arterial elasticity and arterial stiffness in 5566 children, 9-11 years of age. Analyses were adjusted for age, sex, diameter of the brachial artery, puberty and mother's socio-economic status. Associations with p-value  $< 0.05$  were considered statistically significant.  $\beta$ , unstandardized regression coefficients, CI, confidence interval; CRF, cardiorespiratory fitness allometrically scaled by lean mass; CM-H, cardiometabolic health; DC, distensibility co-efficient which measures arterial structure; FMD, flow-mediated dilation which measures endothelial function; PWV, pulse wave velocity which measures arterial structure.