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

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SUPPLEMENTAL DIGITAL CONTENT 1

1. SUPPLEMENTAL METHODS

Data availability

The informed consent obtained from Avon Longitudinal Study of Parents and Children (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/).

Study sample

The cohort and study design are described in detail elsewhere (http:// www.alspac.bris.ac.uk). This website also contains details of all data that is available through a fully searchable data dictionary and online variables search tool (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary). 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 18th January 2001 through 11th January 2003. Whereas arterial outcomes were measured at age 10 years clinic visit from 19th February 2002 through 15th March 2003. 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. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004).

Anthropometric and social measures

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. Based on questionnaire responses participant's mother's social economic status was grouped using the 1991 British Office of Population and Census Statistics classification. Time (years) to age at peak height velocity (aPHV), an objective measure of pubertal or maturation status without having to rely on physical examination or self-report was derived using Superimposition by Translation And Rotation mixed-effects growth curve analysis (1).

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 dual energy Xray absorptiometry 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 high FMI and LMI if values are above 75th percentile and low if less than 25th percentile of the values. Whereas moderate FMI and LMI are 25th to 75th percentile of the values. Systolic and diastolic blood pressure were measured with an automated monitor (Dinamap 9301 Vital Signs Monitor). The child was first given a simple

explanation of what would happen in the session using the analogy of an inflating balloon to explain the action of the cuff. Two cuffs were used depending on the size of the child's upper arm circumference (ideally the right arm was used): If < 23cm a small adult size cuff (blue in colour) was used and if ≥ 23cm an adult cuff (dark blue in colour) was used. A piece of cotton tubing was slid onto the child's arm to cushion it before the cuff was attached. The initial inflation was set to 130 mmHg. The child was asked to press 'start' on the machine. While it took the measurements, the tester asked the parent whether the child had recently or presently had an infection. The parents were also asked about any medications the child was currently on and when they had last been taken. Two readings of systolic and diastolic blood pressure and pulse rates were recorded and the mean of each was calculated. Non-fasting blood samples were collected via standard procedures at 9 years clinic visit with samples spun immediately and frozen at −80°C (2). Plasma lipids (total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol) were assayed by modifying the standard Lipid Research Clinics Protocol using enzymatic reagents for lipid determination. All assay coefficients of variation were <5% (3). Total plasma adiponectin concentrations were assessed using ELISA (R&D Systems, Abingdon, UK), and the interassay coefficient of variation (CV) was 7% (2). Participants' plasma adiponectin level was classified in tertile (3 equal distribution of low, moderate and high categories).

Determining cardiometabolic health

We categorized participants as having good or poor cardiometabolic health if they have or do 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 >75th percentile, HDL <25th percentile, triglycerides >75th percentile, and or BMI >75th percentile meets criteria for diagnosing metabolic syndrome (4). We classified children as having poor cardiometabolic health if their systolic BP, triglycerides and or FMI lies above the 75th percentile and HDL falls below the

25th percentile of the studied population values. Children with good cardiometabolic health had systolic BP, triglycerides and or FMI below 75th percentile and HDL values above 25th percentile. We used total FMI in place of BMI since it provided a more precise measure of adiposity in this age group (5).

Assessment of cardiorespiratory fitness

We assessed CRF at a heart rate of 170 beats per minute (bpm) (PWC_{170}) via physical work capacity (measured in watts) (6) using an electronically braked cycle ergometer (Lode Rechor P) with child-adjusted ergometer saddle and handlebar height. After a 1 min warm-up period children pedaled 55–65 revolutions per minute for 3 min at each workload (20, 40 and 60 W), interspersed with a 2 min rest. During the exercise test, a heart rate monitor (Polar S180). strapped across the chest recorded heart rate every 5 s. 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. To consider a test successful completed, each child was required to achieve at the first stage- a heart rate of ≥80 bpm in the first stage, indicating a response to the workload, and ≥150 beat/mins in the last stage of the test, which approximates to a workload >70% of maximum heart rate. At a heart rate of ≥150 beat/mins in the final stage, children were considered to exercise at a capacity close to the predicted value of 170 beat/mins. PWC_{170} is moderately correlated ($r = 0.49\text{-}0.54$, $p \leq 0.01$) with absolute peak oxygen uptake assessed on a cycle ergometer or on a treadmill among 11-16-year-olds after controlling for maturity offset and sex (7).

Scaling of predicted work capacity by body size

Scaling CRF by body mass is inappropriate in evaluating aerobic fitness in children and a recommended allometric scaling (8,9), was performed from a log-linear regression model with sex and body mass or lean mass as independent variables and PWC_{170} as the dependent variable. The scaling exponent (b) for CRF scaled by body mass or lean mass was (0.21, 95% confidence interval

[CI] = 0.18 to 0.23) and (0.54, [0.51 to 0.58]) respectively. The resultant diminished significant association with body mass (r = -0.05, P = 0.002) or lean mass (r = 0.04, P = 0.02) provided a better CRF measure than ratio-scale. CRF was therefore expressed as absolute CRF (Watts[W]), CRF allometrically scaled for body mass ($W \cdot kg\ BM^{-0.21}$) and CRF allometrically scaled for lean mass ($W \cdot kg\ LM^{-0.54}$). We also categorized participants' CRF scaled by body mass or lean mass in tertile (3 equal distribution of low, moderate and high groups).

Vascular study protocol (year 10 follow-up)

Pulse wave velocity

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 pulse wave velocity (PWV) was first assessed then brachial artery distensibility, and lastly, brachial endothelial function testing, using high-resolution ultrasound techniques (10,13). As described in detailed earlier (10,13), all measurements were taken independently by trained vascular technicians. A high-fidelity micromanometer (SPC-301, Millar Instruments, Houston, TX, USA) was utilized in the transcutaneous measure of pressure-pulse waveforms from the radial and carotid pulse synchronous with the electrocardiogram signal. We calculated the PWV as ratio of the arterial path length between the two recording points to the meantime difference for the pulse waveform to travel between the two recording points (SphygmoCorversion 7.1, Scanmed, UK).

Arterial elasticity

Ultrasound images of the right brachial artery were recorded onto SVHS video using an ALOKA 5500 high-resolution ultrasound system with a 5 – 10 MHz linear array probe (Keymed, UK). We assessed brachial artery elasticity on the artery that was imaged for flow-mediated dilatation (FMD) measurement. 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.

Brachial artery flow-mediated dilatation

Using high-resolution ultrasound (ALOKA 5500) at 5 – 10 cm above the antecubital fossa, the right brachial artery was scanned. As previously described (10), the induction of the brachial artery FMD occurred after a 5 min inflation of a pneumatic cuff to 200 mmHg. The cuff was position around the forearm just below the medial epicondyle and deflated after the inflation using an automatic air regulator (Logan Research, UK). The edge-detection software was used in measuring the brachial artery diameter (Brachial Tools, MIA, IA, USA). We expressed FMD as the maximum percentage change in vessel diameter from baseline. We continuously recorded the magnitude of the flow stimulus by pulse wave Doppler. We did not allometrically scale the FMD variable for baseline diameter because the scaling exponent was close to 1, hence the results relate to unscaled FMD. FMD is influenced by vessel size through blood flow-related shear stress on the vessel wall and by its component in the calculation of %FMD as usually reported (10). However, in our study population the absolute change in FMD was unrelated to resting vessel size. (10). Moreover, previous studies on relationship between shear stress and %FMD remains inconsistent (10,11). Therefore, we did not present a measure of shear stress stimulus in relation to FMD in our study population.

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 (10). 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 (10). 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 (10). 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 (R2: 0.02 for FMD, 0.03 for carotid-radial PWV and 0.006 for DC).

Covariables

Based on previous evidence (3,10–15), we accounted for the following potential confounding factors in the analyses: age in years, sex, systolic blood pressure, total fat mass or CRF allometrically scaled for lean mass, lean mass, mother's social-economic status by occupation; systolic blood pressure; somatic maturation status; time in years between the measurement of exposures at 9 years and measurement of arterial outcomes at age 10 years; brachial artery diameter, and LDL cholesterol.

Dealing With Missing Data

There were diverse missing data for the predictor and outcome variables. We studied only 5566 children with complete data on all arterial outcomes, 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 imputed the missing values of predictors and covariates using the multiple imputation procedure in SPSS version 25 (IBM SPSS Statistics, IBM Corporation, Armonk, NY) to minimize selection bias. We generated 20 imputation data sets at 10 iterations using linear regression type imputation (3).

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 ANOVA 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, PWV via multivariable linear regression, first exploring adjustment for age and sex (model 1); adjusted for age, sex, total fat mass or CRF scaled by lean mass (model 2); adjusted for age, sex, and lean mass or CRF scaled by 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. Covariates were retained in the model because of biological plausibility and previous evidence (3,10–15). A logarithm transformed total and trunk fat mass, adiponectin and DC variables were used in regression analyses, but logged DC variable was back-transformed by exponentiation, yielding geometric means. We investigated the associations of CRF, adiponectin, total and trunk FMI, and LMI categories with FMD, DC, PWV using multivariate regression models with Sidak correction adjusted for the above-detailed covariates. We examined the mediating effects of CRF on the

associations of cardiometabolic health with FMD, DC and PWV through bootstrapped (10,000 samples) (16–18) 3-model linear regression analyses that were 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 PWV (Equation 2), linear regression on the association of cardiometabolic health with FMD, DC and 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 (16,17). 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 association, we performed a similar multivariable analysis but modeled FMD, DC, and PWV as exposures, and CRF allometrically scaled for body mass, CRF allometrically scaled for lean mass, adiponectin, total and trunk fat mass, lean mass and as outcomes. Model 1 was unadjusted while Model 2 was adjusted for all covariates in the uni-directional analyses. All analyses were repeated for observed (non-imputed) data set (Tables S5,7 and 8, Supplemental Digital Content 6,8 and 9). Collinearity diagnoses were performed for all analyses and variance inflation factor <5 was considered acceptable. Interactions between sex and the main exposures were statistically non-significant, both by running the fully adjusted and unadjusted analyses. Therefore, we presented combined results and adjusted for sex. Differences and associations with p-values <0.05 from 2-sided tests were considered statistically significant. Conclusions are drawn

based on effect estimates and their confidence intervals (CI) rather than arbitrary p-value cut-point from statistical tests. 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 standard deviation (SD) if they had relevant exposure for a normally distributed quantitative variable (19). 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. 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. All statistical analyses were performed using SPSS statistics software, Version 25.0 (IBM Corp, Armonk, NY, USA), while mediation analyses were performed using PROCESS SPSS script Version 3.5 (18).

2. MISSING DATA AND MULTIPLE IMPUTATION

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 complete arterial variables (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 slightly different adiposity with those that had complete cases (see Table S1, Supplemental Digital Content 2). 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). Of 5566 children with complete arterial 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. We conducted a Little's missing completely at random (MCAR) test to ascertain data missingness (20). 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 multiple imputations using SPSS version 25 (see Table S3 Supplemental Digital Content 4, presents the 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 imputations 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 imputations (computed using Rubin's formula) (21).

We examined 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). 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). Where multiple imputations have been conducted, presenting imputed results is preferred to presenting non-imputed results (listwise deletion) (22).

Supplemental Digital Content 1 References

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