Online-Only Supplemental Material

Effect of Arterial Stiffness and Carotid Intima-Media Thickness Progression on the Risk of Dysglycemia, Insulin Resistance, and Dyslipidaemia: A Temporal Causal Longitudinal Study

Andrew O. Agbaje MD, MPH[1], Alan R. Barker PhD[2], Gary F. Mitchell MD,[3] Tomi-Pekka Tuomainen MD, PhD[1]

[1]Institute of Public Health and Clinical Nutrition, School of Medicine, University of Eastern Finland, Kuopio, Finland; [2]Children's Health and Exercise Research Centre, Sport and Health Sciences, University of Exeter, Exeter, United Kingdom; [3]Cardiovascular Engineering, Inc, Norwood, MA, United States.

Andrew O. Agbaje, MD, MPH, Cert. Clinical Research (Harvard)
Institute of Public Health and Clinical Nutrition, School of Medicine, Faculty of Health Sciences, University of Eastern Finland, Kuopio Campus.
Address: Yliopistonranta 1, P.O. Box 1627, 70211 Kuopio, Finland
E-mail: andrew.agbaje@uef.fi

Content list:

Expanded Research Design and Methods

Data Availability Statement

The informed consent obtained from the 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/.

Study cohort

Data were from the ALSPAC birth cohort, which investigates factors that influence childhood development and growth. Altogether, 14,541 pregnancies from women residing in Avon, southwestern England, UK, who had a total of 14,676 fetuses, were enrolled between April 1, 1991, and December 31, 1992. When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join the study originally resulting in 913 additional pregnancies. The total sample size for analyses using any data collected after 7 years of age is 15,454 pregnancies, resulting in 15,589 foetuses. Of these 14,901 were alive at 1 year of age. Regular clinic visits of the children commenced at 7 years of age and are still ongoing. Study data at 24.5 years were collected and managed using REDCap electronic data capture tools.[1] For our analysis, we included participants who had both cfPWV and cIMT measurements at age 17.7 years (Figure 1). The demographic characteristics of excluded participants were similar to those included in this study as described in the supplementary appendix. 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[2-4] 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).

Anthropometry and body composition

Anthropometry (height and weight) at ages 17.7 and 24.5 years was assessed using standard protocols.[5] At ages 17.7 and 24.5 years, body composition (total fat mass, trunk fat mass, and lean mass) was assessed using a dual-energy Xray absorptiometry (DEXA) scanner as earlier described.[5-7] We calculated body mass index by dividing weight by squared height. Participants having >24.9 kg/m^2 of body mass index were classified as overweight and obese while those below this cut points were classified as normal weight.[7,8]

Vascular phenotype

At age 17.7 years clinic visit, cfPWV arterial measure was recorded three times. For the cfPWV, a cuff was placed over the right carotid artery in the participant's neck, while another was located over the femoral artery in their upper right thigh. The distance between the participant's suprasternal notch and the top of the thigh cuff was measured, as was the distance between their suprasternal notch and the bottom of the neck cuff on the right side. cfPWV and transit time to the nearest 0.01 ms were automatically computed from measurements of pulse transit time and distance travelled by the pulse between two recording sites using Vicorder (Skidmore Medical, Bristol, UK) portable physiologic vascular testing equipment. All measurements were taken independently by one of two trained vascular technicians (inter-observer mean difference 0·2 m/sec, SD 0·1).[5-7] At 24.5 years, cfPWV was measured, five minutes after resting in a semi-prone position, using a Vicorder instrument (Skidmore Medical, Bristol, UK) with two blood pressure measurement channels and two Velcro pressure sensor cuffs applied over each of the carotid and femoral arteries. The cfPWV measurement was repeated until three readings that were within 0.5 m/sec of each other had been recorded.

cIMT was assessed by ultrasound using a linear 12-MHz transducer (Vivid7, GE Medical, Chicago, Illinois) as earlier reported.[5,7] The average of cIMT at 17.7 years serially measured at 3 different cardiac cycles was computed. Interobserver variability for cIMT was assessed in a separate sample of 25 young adults (coefficient of variation: 4.4 ± 2.2%).[5,7] The right and left common carotid arteries at age 24.5 years were imaged using an ultrasound machine (CardioHealth Panasonic and a 13.5 MHz linear array broadband transducer (probe; centre frequency 9.0 MHz).[7] Participants were placed in a supine position with the head rotated by 45 degrees from the midpoint. An automated guide line was placed at the bulb (a longitudinal scan that included the common carotid artery and the carotid bifurcation) with the region-of-interest box and IMT trace lines automatically positioned 1 cm away from the guide line. The scanner automatically saved an image when the region-of-interest box turned green, indicating good image quality. An automated cIMT measurement, recorded from the posterior wall of the artery, was saved after three consecutive cardiac cycles. When interrogating the common carotid, the CardioHealth system calculated and displayed the cIMT that is updated at each detected R-wave of the cardiac cycle. Once the measurement achieved a predefined quality threshold, scanning automatically stopped and a report was generated. Raw data were checked for outliers and cIMT value >1.0 mm was reviewed by a trained research scientist to assess validity. Abnormal values due to measurement error were removed. Participants had between 1 to 3 cIMT measures for each of the right and left carotid arteries. For our analysis, we computed the mean of the average measurement of the right and left common carotid arteries as cIMT.[7]

Cardiometabolic and lifestyle factors

Heart rate and blood pressure were measured at ages 17.7 years as previously detailed.[5-7] Blood pressure readings at the 24-year clinic visit were taken using an Omron M6 upper arm blood pressure/pulse monitor. Participants were asked to sit and rest for two minutes prior to taking the first seated blood pressure reading. Using standard protocols, fasting blood samples at ages 17.7 and 24.5 years were collected, spun, and frozen at –80 °C and a detailed assessment of glucose, insulin, high sensitivity C-reactive protein, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides, has been reported (coefficient of variation was <5%).[7] Specifically, fasting insulin was measured using an ultrasensitive automated microparticle enzyme immunoassay (Mercodia), which does not cross-react with proinsulin. Sensitivity of the immunoassay was 0.07 mU/L. Participants with fasting HDL-C <1.0 mmol/L, LDL-C >3.0 mmol/L, triglyceride >2.0 mmol/L, were categorised at risk of dyslipidaemia[9] and glucose >6.1 mmol/L, and insulin >11.78 mU/L, were categorised at risk of type 2 diabetes.[10,11] We calculated homeostatic model assessment of insulin resistance (HOMA-IR) and HOMA-percent pancreatic beta-cell function (HOMA-%β) from (fasting plasma insulin x fasting plasma glucose / 22.5) and ((20 x fasting plasma insulin) / (fasting plasma glucose − 3.5)), respectively.[12] Participants had young-onset type 2 diabetes when clinic fasting glucose was ≥ 7 mmol/L or reported physician diagnosis at 17.7 or 24.5 years clinic visit.

Questionnaire to assess smoking behavior were administered at the 17.7-year[6,7] and 24.5-year clinic visits. The participants were asked whether they smoked in the last 30 days, smoked a whole cigarette, smoked every day, their frequency of use, etc. At the 17.7-year clinic visit, participants were briefly asked about their personal and family (mother, father, and siblings) medical history such as, a history of hypertension, diabetes, high cholesterol, and vascular disease. Physical activity at age 15.5 years was assessed with ActiGraphTM accelerometer worn for 7 days. Moderate to vigorous physical activity cut point was >2296 count per minute.[5,7] At 24.5 years physical activity was assessed using ActiGraph GT3X+ accelerometer device worn for four consecutive days, ideally starting the day after the clinic visit. Moderate to vigorous physical activity reported in minutes per day was based on a previously established cutoff of >2020 count per minute.[7] Valid days were

considered as wear time of at least 500 minutes, after excluding intervals of >60 minutes of zero counts.

Missing data and multiple imputations

Eligible sample size varied by predictor and outcome measure, as presented in Supplemental Tables S2 and S3. Exclusions via listwise deletion of missing values ranged from 15.6 to 63.9 percent for covariates. We restricted study participants to those who had complete outcome variables at age 17 years follow-up (n=3862) and complete predictors and outcome variables at 24 years of age follow-up (n=1799). We conducted a Little's missing completely at random (MCAR) test to ascertain data missingness.[7] The observed minimum and maximum values were constraints for the imputation process and 20 cycles of imputation with 10 iterations resulted in 20 imputed data sets. The multiple imputation module in SPSS pooled the results from these imputed data. In line with previous evidence,[3,7] the percentage of missing values would be sufficiently addressed with 20 imputations[7] Imputed results of cross-sectional analyses are presented in Supplemental Tables S4.

Statistical Analysis

Participant's descriptive characteristics were summarized as means and standard deviation, medians, and interquartile ranges, or frequencies and percentages. We explored sex differences using Independent t-tests, Mann Whitney-U tests, or Chi-square tests for normally distributed, skewed or dichotomous variables, respectively. We assessed the normality of variables by histogram curve, quantile-quantile plot, and Kolmogorov-Smirnov tests. We conducted a logarithmic and reciprocal transformation of skewed variables and confirmed normality prior to further analysis.

We investigated the separate cross-sectional associations of cfPWV and cIMT with each of fasting LDL, HDL, triglyceride, insulin, and glucose at 24.5 years using linear regression models at both 17.7 and 24.5 years. All analyses were adjusted for age, sex, low-density lipoprotein cholesterol, insulin, triglyceride, high-sensitivity C-reactive protein, high-density lipoprotein cholesterol, heart rate, glucose, systolic blood pressure, and fat mass and lean mass depending on the outcome, moderate to vigorous physical activity, smoking status, and family history of hypertension/diabetes/high cholesterol/vascular disease. All covariates were specific to the age analysed i.e covariates at age 17.7 years for analysis at 17.7 years and covariates at 24.5 years for cross-sectional analysis at age 24.5 years.

We investigated the separate longitudinal associations of cfPWV and cIMT at 17.7 years with each of fasting LDL, HDL, triglyceride, insulin, and glucose categories at 24.5 years using binary logistic regression. The logistic regression outcome variables were defined as participants with fasting HDL-C <1.0 mmol/L, LDL-C >3.0 mmol/L, triglyceride >2.0 mmol/L, were categorised at risk of dyslipidaemia[9] and glucose >6.1 mmol/L, and insulin >11.78 mU/L, were categorised at risk of type 2 diabetes.[10,11] Univariable analysis was adjusted for sex, while multivariable analysis was adjusted for baseline covariates such as sex, age, low-density lipoprotein cholesterol, insulin, triglyceride, high-sensitivity C-reactive protein, high-density lipoprotein cholesterol, glucose, heart rate, systolic blood pressure, and fat mass and/or lean mass depending on the outcome, moderate to vigorous physical activity at 15.5 years, smoking status and family history of hypertension/diabetes/high cholesterol/vascular disease.

We examined the separate prospective associations of the 7-year progression in cfPWV and cIMT with the longitudinal progression in each of fasting LDL, HDL, triglyceride, insulin, glucose, HOMA-IR and HOMA-%β serially measured at ages 17.7 and 24.5 years using linear mixed-effect models for repeated measures with restricted maximum likelihood estimation. The estimates quantify the effect of the longitudinal progression in the predictors on the longitudinal progression in the outcome variables. We decided a priori to select the model with the least Bayesian information criterion (BIC). The least BIC resulted in a model with gender as a factor and a random

intercept modeled on the subject level. We selected a variance component covariance type and determined the effect of the progression in predictors on progression in outcome variables. All analyses were adjusted for sex, age at 17.7 years, and covariates measured at 17.7 and 24.5 years such as low-density lipoprotein cholesterol, insulin, glucose, triglyceride, high-sensitivity C-reactive protein, and/or high-density lipoprotein cholesterol, depending on the outcome, heart rate, systolic blood pressure, fat mass, lean mass, smoking status, family history of hypertension/diabetes/high cholesterol/vascular disease and moderate to vigorous physical activity at 15.5 and 24.5 years.

Lastly, we used structural equation modeling with autoregressive cross-lagged design to examine the separate temporal associations of cfPWV and cIMT with each of fasting LDL, HDL, triglyceride, insulin, glucose, HOMA-IR and HOMA-%β. The cross-lagged models first tested the separate associations of cfPWV and cIMT at 17.7 years with each of fasting LDL, HDL, triglyceride, insulin, glucose, HOMA-IR, and HOMA-%β at 24.5 years; and secondly tested the separate associations of fasting LDL, HDL, triglyceride, insulin, glucose, HOMA-IR and HOMA-%β at 17.7 years with cfPWV and cIMT at 24.5 years. These models were adjusted for all the baseline covariates listed above, including the time in years between 17.7 and 24.5 years. In the cross-lagged design, the potential association could be: cfPWV and cIMT leading to metabolic risks, metabolic risks leading to cfPWV and cIMT or bidirectional associations of cfPWV and cIMT with metabolic risks. If a path from cfPWV and cIMT at time t-1 (17.7 years) to each of fasting LDL, HDL, triglyceride, insulin, glucose, HOMA-IR and HOMA-%β at time t-2 (24.5 years) reach significant (p-value<0.05), changes in the earlier variables are considered to lead to changes in the later one, and vice versa. A stronger predictive effect is determined by a larger standardized regression coefficient. We concluded that the cross-lagged models had good fit with the following indices: the root-mean-square error of approximation (<0.008, the value <0.05 is considered to indicate a good model-data fit), the normed fit index (>1.000), the relative fit index (>0.981), the incremental fit index (>1.000), the Tucker–Lewis Fit Index (>0.996), the comparative fit index (>1.000), which are considered good fit if values are >0.90.[7] We included error terms in the model.

All covariates were selected based on previous studies.[5-8,13] We excluded pubertal status/somatic maturation from the model because all participants had reached adult-like maturity status by 17.7 years of age and >95% had reached maturity by 15.5 years. We performed collinearity diagnoses and accepted results with a variance inflation factor <5. There were a few statistically significant sex interactions however, we presented both the combined results adjusted for sex and sex-stratified results. We considered differences and associations with a 2-sided p-value <0.05 as statistically significant and made conclusions based on effect estimates and their confidence intervals (CI). In our recent work, supplemental tables 1-3 has been published as supplementary materials.[7] We presented partial correlational analyses adjusted for age and sex in Supplemental Tables S6 and 7 to buttress our findings, especially the paradoxical associations. Analyses involving 40% of a sample of 10,000 ALSPAC children at 0.8 statistical power, 0.05 alpha, and 2-sided p-value would show a minimum detectable effect size of 0.049 standard deviations if they had relevant exposure for a normally distributed quantitative variable.[14] All statistical analyses were performed using SPSS statistics software, Version 27.0 (IBM Corp, Armonk, NY, USA) and structural equation modeling was conducted using IBM AMOS version 27.0.

References

1. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381.

doi:10.1016/j.jbi.2008.08.010

2. Boyd A, Golding J, Macleod J, et al. Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol.* 2013;42(1):111-27. doi: 10.1093/ije/dys064.

3. Fraser A, Macdonald-Wallis C, Tilling K, et al. Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. *Int J Epidemiol.* 2013;42(1):97-110. doi: 10.1093/ije/dys066.

4. Northstone K, Lewcock M, Groom A, Boyd A, Macleod J, Timpson N, Wells N. The Avon Longitudinal Study of Parents and Children (ALSPAC): an update on the enrolled sample of index children in 2019. *Wellcome Open Res.* 2019;4:51. doi: 10.12688/wellcomeopenres.15132.1.

5. Chiesa ST, Charakida M, Georgiopoulos G, et al. Determinants of Intima-Media Thickness in the Young: The ALSPAC Study. *JACC Cardiovasc Imaging*. 2021;14(2):468-478. doi:10.1016/j.jcmg.2019.08.026

6. Dangardt F, Charakida M, Georgiopoulos G, et al. Association between fat mass through adolescence and arterial stiffness: a population-based study from The Avon Longitudinal Study of Parents and Children. *Lancet Child Adolesc Health*. 2019;3(7):474-481. doi:10.1016/S2352-4642(19)30105-1

7. Agbaje AO, Barker AR, Tuomainen T-P. Effects of Arterial Stiffness and Carotid Intima-Media Thickness Progression on the Risk of Overweight/Obesity and Elevated Blood Pressure/ Hypertension: a Cross-Lagged Cohort Study. *Hypertension*. 2022;79(1):159-169. doi:DOI: 10.1161/HYPERTENSIONAHA.121.18449

8. Ness AR, Leary SD, Mattocks C, et al. Objectively measured physical activity and fat mass in a large cohort of children. *PLoS Med*. 2007;4(3):e97. doi:10.1371/journal.pmed.0040097

9. Mach F, Baigent C, Catapano AL, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk [published correction appears in Eur Heart J. 2020;41(44):4255]. *Eur Heart J*. 2020;41(1):111-188. doi:10.1093/eurheartj/ehz455

10. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2011;34 Suppl 1(Suppl 1):S62-S69. doi:10.2337/dc11-S062

11. Ascaso JF, Pardo S, Real JT, Lorente RI, Priego A, Carmena R. Diagnosing insulin resistance by simple quantitative methods in subjects with normal glucose metabolism. *Diabetes Care*. 2003;26(12):3320-3325. doi:10.2337/diacare.26.12.3320

12. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care*. 2004;27(6):1487-1495. doi:10.2337/diacare.27.6.1487

13. Agbaje AO, Barker AR, Tuomainen TP. Cardiorespiratory Fitness, Fat Mass, and Cardiometabolic Health with Endothelial Function, Arterial Elasticity, and Stiffness. Med Sci Sports Exerc. 2022;54(1):141-152. doi: 10.1249/MSS.0000000000002757

14. Golding J, Pembrey M, Jones R; ALSPAC Study Team. ALSPAC--the Avon Longitudinal Study of Parents and Children. I. Study methodology. *Paediatr Perinat Epidemiol*. 2001;15(1):74-87. doi:10.1046/j.1365-3016.2001.00325.x

Supplemental Results

Supplemental Table S1 Characteristics of participants excluded from the study

Variables	**Included participants**		**Excluded participants**			
	Mean (SD)	**n**	**Mean/SD**	**n**	***P* for difference**	**Cohen's D**
Age (years)	17.72 (0.32)	3862	18.12 (0.61)	1349	**<0.0001**	0.82
Anthropometry and body composition						
Body height (m)	1.71 (0.09)	3806	1.71 (0.10)	1259	**0.007**	0
*Weight (kg)	64.40 (142.6)	3811	66.30 (119.3)	1253	**<0.0001**	0.25
*Body mass index (kg/m^2)	21.78 (52.1)	3806	22.69 (46.9)	1253	**<0.0001**	0.32
Lean mass (kg)	45.53 (9.88)	3757	45.72 (10.47)	1090	0.584	NA
*Total fat mass (kg)	16.01 (80.8)	3757	17.70 (71.3)	1090	**<0.0001**	0.29
*Trunk fat mass (kg)	7.88 (45.5)	3757	8.87 (40.6)	1090	**<0.0001**	0.29
Metabolic indices						
Total cholesterol (mmol/L)	3.75 (0.68)	2586	3.77 (0.70)	699	0.422	NA
HDL (mmol/L)	1.27 (0.30)	2586	1.27 (0.30)	699	0.911	NA
LDL (mmol/L)	2.10 (0.61)	2586	2.11 (0.61)	699	0.740	NA
*Triglyceride (mmol/L)	0.75 (3.47)	2586	0.77 (3.89)	699	0.065	0.10
*C-reactive protein (mg/L)	0.54 (1.01)	2586	0.61 (0.40)	699	**0.039**	0.06
*Insulin (mU/L)	6.67 (89.1)	2543	6.94 (193.6)	688	**0.001**	0.19
Glucose (mmol/L)	5.04 (0.62)	2586	5.04 (0.46)	699	0.770	NA
Vascular measure						
Pulse rate (beats/mins)	65 (10)	3854	66 (10)	809	0.197	NA
Systolic BP (mm Hg)	114 (10)	3854	114 (11)	809	0.694	NA
Diastolic BP (mm Hg)	64 (6)	3854	66 (7)	809	**<0.0001**	0.31
*Carotid IMT (mm)	0.47 (0.35)	3861	0.48 (0.27)	815	0.807	NA
*Carotid-femoral PWV (m/s)	5.70 (7.68)	3857	5.62 (3.54)	26	0.389	NA
Lifestyle factors						
Smoking status (n, %)	911 (27.2)	3344	268 (31.4)	853	**0.017**	NA
Family history of HDCV (n, %)	1162 (30.1)	3857	289 (31.2)	927	0.533	NA

The values are means (standard deviations) and *median (range/interquartile range) except for maturation status and social economic status in percentage. Differences between participants were tested using Student's t-test for normally distributed continuous variables, Mann–Whitney U test for skewed continuous variables, and Chi-square test for dichotomous variable. A 2-sided P-value <0.05 is considered statistically significant and is bolded. Cohen's D effect size was calculated for statistically significant differences: 0.2 = low, 0.5 = moderate, 0.8 = large effect.[7]

HDCV, hypertension/diabetes/high cholesterol/vascular disease; IMT, intima-media thickness; NA, not applicable; PWV, pulse wave velocity; Smoking status, participants had smoked cigarette in the past 30 days.

Supplemental Table S2 Missing data at 24.5 years of age

Variable	n (valid sample size)	Eligible sample size	% Missing
Age	1799	1799	0
Sex	1799	1799	0
Anthropometry and body composition			
Height (m)	1799	1799	0
Weight (kg)	1799	1799	0
Body mass index	1799	1799	0
Lean mass (kg)	1799	1799	0
Total fat mass (kg)	1799	1799	0
Trunk fat mass (kg)	1799	1799	0
Metabolic indices			
Low-density lipoprotein (mmol/L)	1490	1799	17.2
Glucose (mmol/L)	1491	1799	17.1
C-reactive protein (mg/L)	1352	1799	24.8
Vascular measure			
Systolic blood pressure (mm Hg)	1799	1799	0
Diastolic blood pressure (mm Hg)	1799	1799	0
Carotid-femoral pulse wave velocity (m/s)	1799	1799	0
Carotid intima-media thickness (mm)	1799	1799	0
Lifestyle factors			
Moderate to vigorous physical activity (mins/day)	649	1799	63.9
Smoking status	1779	1799	1.1
Family history of HCDV	1519	1799	15.6

H-D-C-V, hypertension/diabetes/high cholesterol/vascular disease[7]

Supplemental Table S3 Missing data at 17.7 years of age

Variable	n (valid sample size)	Eligible sample size	% Missing
Age	3862	3862	0
Sex	3862	3862	0
Anthropometry and body composition			
Height (m)	3806	3862	1.5
Weight (kg)	3811	3862	1.3
Body mass index	3806	3862	1.5
Lean mass (kg)	3756	3862	2.8
Total fat mass (kg)	3756	3862	2.8
Trunk fat mass (kg)	3756	3862	2.8
Metabolic indices			
Low-density lipoprotein (mmol/L)	2587	3862	33.0
Glucose (mmol/L)	2587	3862	33.0
C-reactive protein (mg/L)	2587	3862	33.0
Vascular measure			
Systolic blood pressure (mm Hg)	3856	3862	0.2
Diastolic blood pressure (mm Hg)	3856	3862	0.2
Carotid-femoral pulse wave velocity (m/s)	3862	3862	0
Carotid intima-media thickness (mm)	3862	3862	0
Lifestyle factors			
Smoking status	3344	3862	27.2
Family history of H-C-D-V	3859	3862	0.1

H-D-C-V, hypertension/diabetes/high cholesterol/vascular disease[7]

Supplemental Table S4 Cross-sectional associations of carotid-femoral pulse wave velocity and carotid intima-media thickness with fasting metabolic indices at 17.7 and 24.5 years of age

3862 participants					***17.7 years of age***					
	Low density lipoprotein (mmol/L)		**High density lipoprotein (mmol/L)**		**Triglyceride (mmol/L)**		**Insulin (mU/L)**		**Glucose (mmol/L)**	
	β (95% CI)	p-value	*β* (95% CI)	p-value	*β* (95% CI)	p-value	*β* (95% CI)	p-value	*β* (95% CI)	p-value
Carotid-femoral PWV	0.196 (-0.241 – 0.632)	0.379	0.022 (-0.209 – 0.253)	0.851	-0.026 (-0.139 – 0.087)	0.649	0.137 (-0.031 – 0.306)	0.110	0.144 (-0.343 – 0.630)	0.561
Carotid intima-media thickness	0.294 (-0.233 – 0.821)	0.273	0.150 (-0.103 – 0.404)	0.245	-0.003 (-0.133 – 0.127)	0.961	0.156 (-0.023 – 0.335)	**0.088**	-0.009 (-0.542 – 0.524)	0.974
1799 participants					***24.5 years of age***					
Carotid-femoral PWV	-0.042 (-0.565 – 0.481)	0.875	0.130 (-0.157 – 0.416)	0.375	0.060 (-0.058 – 0.177)	0.321	0.202 (0.045 – 0.360)	**0.012**	0.656 (0.116 – 1.196)	**0.017**
Carotid intima-media thickness	-0.178 (-0.688 – 1.044)	0.686	0.020 (-0.441 – 0.480)	0.933	0.011 (-0.181 – 0.203)	0.910	-0.102 (-0.399 – 0.135)	0.399	-0.019 (-0.802 – 0.765)	0.963

Multivariable linear regression analysis was adjusted for sex, age, low-density lipoprotein cholesterol, insulin, triglyceride, high-sensitivity C-reactive protein, high-density lipoprotein cholesterol, and/or fasting plasma glucose depending on outcomes, heart rate, systolic blood pressure, fat mass, lean mass, moderate to vigorous physical activity, smoking status and family history of hypertension/diabetes/high cholesterol/vascular disease. Skewed variables were logarithmically transformed before linear regression analyses. *β,* unstandardized regression which indicates the effect of a 1-SD change in a predictor variable on a given outcome variable. Multiple imputations were used to account for missing cases. PWV, pulse wave velocity

Supplemental Table S5: Longitudinal progression in arterial stiffness and carotid intima-media thickness in relation to progression in fasting metabolic and lipid indices from age 17.7 through 24.5 years based on body mass index categories at 17.7 years

	Low-density lipoprotein (mmol/L)		**High-density lipoprotein (mmol/L)**		**Triglyceride (mmol/L)**		**Insulin (mU/L)**		**Glucose (mmol/L)**		**HOMA-IR**		**HOMA-%β**	
	Effect estimate (95% CI)	p-value	*Effect estimate* (95% CI)	p-value	*Effect estimate* (95% CI)	p-value	*Effect estimate* (95% CI)	p-value	*Effect estimate* (95% CI)	p-value	*Effect estimate* (95% CI)	p-value	*Effect estimate* (95% CI)	p-value
3038 normal weight participants (<24.99/kg/m^2)														
Carotid-femoral PWV	0.463 (-0.167 – 1.094)	0.149	0.479 (0.140 – 0.818)	**0.006**	0.107 (-0.040 – 0.254)	0.154	-0.013 (-0.216 – 0.190)	0.899	0.463 (-0.069 – 0.995)	0.088	0.048 (-0.170 – 0.267)	0.664	-0.106 (-0.339 – 0.127)	0.373
Carotid intima-media thickness	0.142 (0.061 – 0.222)	**0.001**	0.280 (0.238 – 0.322)	**<0.0001**	0.050 (0.031 – 0.068)	**<0.0001**	-0.021 (-0.047 – 0.005)	0.120	0.237 (0.174 – 0.300)	**<0.0001**	0.014 (-0.014 – 0.042)	0.328	-0.085 (-0.114 – -0.057)	**<0.0001**
767 overweight/obese participants (>24.99/kg/m^2)														
Carotid-femoral PWV	0.407 (-1.156 – 1.970)	0.608	0.913 (0.199 – 1.627)	**0.012**	0.338 (-0.035 – 0.712)	0.075	0.388 (-0.032 – 0.087)	0.070	-0.462 (-1.349 – 0.424)	0.305	0.404 (-0.073 – 0.881)	0.097	0.533 (0.061 – 1.005)	**0.027**
Carotid intima-media thickness	0.266 (0.102 – 0.431)	**0.002**	0.320 (0.231 – 0.409)	**<0.0001**	0.017 (-0.027 – 0.061)	0.441	-0.001 (-0.052 – 0.050)	0.969	0.225 (0.112 – 0.339)	**<0.0001**	0.056 (-0.001 – 0.113)	0.052	-0.023 (-0.077 – 0.032)	0.415

Multivariable analysis was adjusted for sex, age at 17.7 years, and covariates at 17.7 and 24.5 years such as low-density lipoprotein cholesterol, insulin, triglyceride, high-sensitivity C-reactive protein, high-density lipoprotein cholesterol, heart rate, fasting plasma glucose, systolic blood pressure, total fat mass or lean mass depending on the outcome, moderate to vigorous physical activity at 15.5 years, smoking status, and family history of hypertension/diabetes/high cholesterol/vascular disease. Skewed variables were logarithmically transformed before analyses. Effect estimate was from linear mixed-effect model analyses for repeated measures; CI, confidence interval; PWV, pulse wave velocity. HOMA-IR, homeostatic model assessment of insulin resistance was computed from (fasting insulin x fasting glucose /22.5); HOMA-%β, homeostatic model assessment of beta-cell function computed as ((20 x fasting plasma insulin) / (fasting plasma glucose − 3.5)). P-value <0.05 was considered statistically significant.

Supplemental Table S6: Age- and sex-adjusted correlation of arterial stiffness and carotid intima-media thickness with fasting metabolic and lipid indices at 17.7 and 24.5 years

Variables at 17.7 years	***cfPWV at 17.7 years**		***cIMT at 17.7 years**		***Variables at 24.5 years***	***cfPWV at 24.5 years**		**cIMT at 24.5 years**	
	Correlation	*p-value*	*Correlation*	*p-value*		*Correlation*	*p-value*	*Correlation*	*p-value*
High-density lipoprotein	-0.018	0.350	0.028	0.161	High-density lipoprotein	0.003	0.901	0.020	0.490
Low-density lipoprotein	0.018	0.365	0.008	0.683	Low-density lipoprotein	0.039	0.153	-0.024	0.402
*Triglyceride	0.035	0.072	-0.009	0.657	*Triglyceride	0.049	0.070	-0.025	0.384
Glucose	0.034	0.082	0.004	0.843	Glucose	0.151	**<0.0001**	0.011	0.710
*Insulin	0.054	**0.007**	0.015	0.436	*Insulin	0.086	**0.001**	-0.067	**0.022**

p-value was from partial correlation analyses adjusted for age at 17.7 years or 24.5 years and sex, p-value <0.05 was considered statistically significant, *skewed variables were log-transformed.

Supplemental Table S7: Age- and sex-adjusted correlation of arterial stiffness and carotid intima-media thickness with fasting metabolic and lipid indices alternated at ages 17.7 and 24.5 years

Variables at 24.5 years	***cfPWV at 17.7 years**		***cIMT at 17.7 years**		***Variables at 17.7 years***	***cfPWV at 24.5 years**		**cIMT at 24.5 years**	
	Correlation	*p-value*	*Correlation*	*p-value*		*Correlation*	*p-value*	*Correlation*	*p-value*
High-density lipoprotein	-0.057	**0.010**	0.041	0.063	High-density lipoprotein	-0.013	0.668	-0.024	0.453
Low-density lipoprotein	0.039	0.077	-0.032	0.146	Low-density lipoprotein	0.002	0.936	0.016	0.628
*Triglyceride	0.045	**0.046**	-0.021	0.347	*Triglyceride	-0.016	0.587	0.015	0.649
Glucose	0.034	0.132	0.002	0.940	Glucose	0.087	**0.003**	0.058	0.069
*Insulin	0.080	**<0.0001**	-0.055	**0.014**	*Insulin	-0.007	0.801	<0.0001	0.988

P-value was from partial correlation analyses adjusted for age at 17.7 years and sex, p-value <0.05 was considered statistically significant, *skewed variables were log-transformed.