

1 ***Association of cardiovascular risk factors with MRI indices of cerebrovascular***
2 ***structure and function and white matter hyperintensities in young adults***

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38 **Key Points**

39 **Questions:** Are cardiovascular risk factors associated with early changes in
40 brain blood vessel density, size and curvature, brain blood flow, and brain white
41 matter integrity in young adults?

42 **Results:** In individuals with average age of 25, vascular risk factors, including
43 higher blood pressures and body mass index, were correlated with reduced
44 blood vessel density, and, reduced brain blood vessel density was associated
45 with reduced cerebral blood flow and early injury to brain cell connections.

46 **Meaning:** In young adults, the structure of brain blood vessels, as well as
47 cerebral blood flow and lesions of brain white matter, were correlated with risk
48 factors for vascular disease, suggesting the young adult period may be a target
49 for primordial prevention of cerebrovascular disease.

50

51 **Abstract**

52 **Importance:** Risk of stroke and brain atrophy in later life relate to levels of
53 cardiovascular risk in early adulthood. However, it is unknown whether
54 cerebrovascular changes are already present in young adults.

55 **Objective:** To examine relationships between modifiable cardiovascular risk
56 factors and cerebrovascular structure, function and white matter integrity in
57 young adults.

58 **Design, Setting, and Participants:** A cross-sectional observational study
59 completed between August 2014 and May 2016 at the University of Oxford,
60 United Kingdom. Participants recruited through active and passive recruitment
61 from the local community, including invitation from the Oxford University
62 Hospitals Hypertension Service.

63 **Exposures:** Clinic and ambulatory blood pressure (mmHg), body mass index
64 (kg/m^2), objective physical activity (hours/week), alcohol intake (drinks/week),
65 smoking (pack years), peak oxygen uptake ($\text{ml}/\text{kg}/\text{min}$), peak exercise blood
66 pressure (mmHg), lipid profile (mg/dL), insulin resistance and use of anti-
67 hypertension medication.

68 **Main Outcomes and Measures:** Cerebral vessel density ($\text{vessels}/\text{cm}^3$), caliber
69 (μm) and tortuosity, brain white matter hyperintensity lesion count (number),
70 and in a subgroup ($n=52$) brain blood arrival time (seconds) and cerebral blood
71 flow ($\text{ml}/100\text{g}/\text{min}$) assessed by brain magnetic resonance.

72 **Results** 125 participants (mean age 25 ± 5 years, 49% female) were recruited.
73 Cerebrovascular morphology and white matter hyperintensity count correlated with
74 cardiovascular risk factors in univariable and multivariable models. In a risk score, for
75 each healthier modifiable risk factor, characterised as: ambulatory blood pressure

76 <130/80mmHg; BMI < 25kg/m²; top tertile of cardiovascular fitness; non-smoker; <8
77 alcoholic drinks/week; normotensive exercise blood pressure response; cholesterol
78 <200mg/dL; and fasting glucose <100mg/dL, vessel density increased by 0.3
79 vessels/cm³ (95%CI 0.1 to 0.5, p=0.003), vessel caliber by 8μm (95%CI 3 to 13,
80 p=0.01) and white matter hyperintensity lesions reduced by 1.6 lesions (95%CI 0.6 to
81 2.8, p=0.006). In subgroup analysis, cerebral blood flow varied with vessel density
82 and increased by 2.5ml/min/100g per risk score (95%CI 0.05 to 4.98, p=0.05).

83 **Conclusions and Relevance** In this preliminary study, involving young adults
84 without clinical evidence of cerebrovascular disease, modifiable cardiovascular
85 risk factors were associated with MR indices of cerebral vessel structure and
86 function, and white matter hyperintensities. Further research is needed to
87 determine the clinical importance of these findings for the primordial prevention
88 of cerebrovascular disease.

89 **Key words:** brain health, cardiovascular risk factors, young adults,

90

91

92 **Introduction**

93 A life-course approach to understand risk of cardiovascular disease is well
94 established^{1, 2} and it is accepted that changes in cardiac and vascular structure that
95 underlie this risk emerge very early in life^{3, 4}. Whether modifiable cardiovascular risk
96 factors, and novel early life exposures such as birth complications, influence the
97 early cerebrovasculature is less well studied.

98

99 Cardiovascular risk closely relates to cerebrovascular injury and cognitive decline in
100 older adults^{5, 6}. Markers of cerebral injury in mid-life, including white matter
101 hyperintensity lesions, predict future stroke, dementia and all-cause mortality^{7, 8}.
102 Progression of white matter hyperintensity lesions is faster in association with
103 metabolic dysfunction and hypertension⁹. Experimental studies demonstrate
104 cardiovascular risk factors result in remodelling of the brain vasculature, including
105 vessel rarefaction, reduced vessel caliber and cerebral blood flow¹⁰. Elevated blood
106 pressure, dyslipidemia and low fitness in early adulthood are known to predict brain
107 health in older adult life^{2, 11, 12}. Whether cerebrovascular morphological changes are
108 already evident in young adults, and correlate with white matter hyperintensity
109 lesions and risk factors at this age, is unclear.

110

111 Advances in brain MRI allow automated segmentation and analysis of vessel
112 morphology, white matter hyperintensity lesions^{13, 14} and blood flow¹⁵; thus making it
113 possible to build a robust and sensitive quantification of brain health for an
114 individual^{13, 14}. Therefore, the objective of the current study was to use multi-modality
115 brain imaging to test the hypothesis that cardiovascular risk profiles are already
116 correlated with variation in vessel morphology and white matter hyperintensity
117 lesions in young adulthood.

118

119

120 **Methods**

121 **Study design and participants**

122 This was a cross-sectional observational study completed between August 2014 and
123 May 2016. The South Central Research Ethics Committee for the National Health
124 Service Health Research Authority (NHS HRA) approved the study (14/SC/0275). All
125 participants gave written informed consent. Measurements were completed at the
126 Oxford Cardiovascular Clinical Research Facility and Oxford Centre of Clinical
127 Magnetic Resonance Research, John Radcliffe Hospital, University of Oxford, United
128 Kingdom. Image analysis performed using pipelines developed at the Hotchkiss
129 Brain Institute, University of Calgary and Wellcome Centre for Integrative
130 Neuroimaging, University of Oxford¹⁴⁻¹⁸. Final data collection was completed on the
131 31st of May 2016.

132

133 Participants aged 18 to 40 years were recruited through active and passive
134 recruitment¹⁹ including local advertising, invitation from local birth cohort studies and
135 invitation from the Oxford University Hospital Hypertension Service. Strategies were
136 designed to recruit adults with a heterogeneity in risk factors known to be present in
137 young adult populations including traditional risk factors such as hypertension and
138 more novel factors such as gestational age. Participants were excluded if they had
139 previous cardiovascular or cerebrovascular events, renal dysfunction or metabolic
140 disease including diagnosis of hyperlipidaemia. Participants with secondary causes
141 of hypertension such as renal vascular disease, vascular anomalies or adrenal
142 dysfunction were excluded following assessment in Oxford University Hospital
143 Hypertension Service. Recruitment was continued to 125 participants to ensure over
144 90% power at $P=0.05$ to identify a 0.70-SD difference in vessel density, vessel
145 caliber and white matter lesion count between lowest and highest cardiovascular risk
146 tertile groups. The subgroup of 52 participants with ASL measures provides 80%
147 power to detect 10% difference in perfusion²⁰.

148 **Procedures**

149 **Cardiovascular Risk Assessment**

150 Participants attended a research clinic in the morning after a 12-hour fast to complete
151 a detailed cardiovascular risk assessment (Supplementary Data eMethods 1).

152 Measurements included: body size, fasting blood samples, clinic and 24-hour blood
153 pressure, as well as peak oxygen uptake and exercise blood pressure (from
154 cardiopulmonary exercise testing). In addition, participants completed a detailed
155 lifestyle questionnaire and had seven complete days of objectively measured
156 physical activity.

157

158 **Brain Imaging and Analysis**

159 Individuals underwent multimodality brain MRI scanning (3.0T Trio Tim, Siemens,
160 Munich, Germany). The MRI protocol included T1-weighted structural, T2-weighted
161 Fluid-Attenuated Inversion Recovery (FLAIR), Diffusion Tensor Imaging (DTI) and
162 Time-of-Flight (TOF) MR Arteriogram (MRA) (Supplementary Data eMethods 2). MR
163 imaging was completed fasted and prior to exercise testing. Complete acquisition
164 and analysis methods are presented in the on-line supplement.

165

166 T1-weighted images were processed using FMRIB Software Library (FSL) tools²¹.

167 Brain vessel segmentation was completed on TOF MRA using previously described
168 automated segmentation tools (Figure 1)^{14, 18}. Binary segmentations were used to
169 determine vessel density, caliber and tortuosity.

170

171 White matter hyperintensity (WMH) lesions were segmented using the Brain Intensity
172 AbNormality Classification Algorithm (BIANCA) a fully-automated, supervised
173 method for WMH detection^{13, 22}. BIANCA classifies image voxels based on their
174 intensity and spatial features, where the intensity features were extracted from T2-
175 weighted FLAIR, T1-weighted and DTI fractional anisotropy (FA) images, FA images

176 were generated using DTI tools, FSL topup, FSL eddy and DTIFit^{21, 23-25}. WMH
177 masks were manually segmented from 10 images to use as the training set for
178 BIANCA, these were independently verified by a neurologist (TS) and radiologist
179 (DM) blinded to participant risk profile. Lesion count was selected as the most
180 sensitive outcome of white matter change in young adults in whom a single lesion,
181 independent of volume, could be considered abnormal²⁶. Minimum lesion size used
182 in analysis was 1 mm³.

183

184 A subgroup of 52 participants also had multi-delay vessel-encoded
185 pseudocontinuous Arterial Spin Labelling (ASL), identical to a previously published
186 protocol¹⁵. Cerebral blood flow and blood arrival time were estimated from ASL
187 images using a previously described analysis pipeline^{15, 17}. Gray matter masks were
188 used to calculate the average cerebral blood flow after linear registration of the ASL
189 MRI to the T1-weighted MRI dataset.

190

191 **Statistical Analysis**

192 Existing literature on risk predictors of brain health was used to define an a priori set
193 of potential correlates of MRI brain health in young adults^{5, 6, 12, 27-29}. These were
194 grouped as: 1) non-modifiable, including age, sex, gestational age, and 2) modifiable,
195 including systolic blood pressure, body mass index (BMI), peak exercise capacity
196 (oxygen uptake ml/min/kg), peak exercise diastolic blood pressure, weekly vigorous
197 activity, alcohol consumption, smoking history, lipid profile, glucose and insulin
198 resistance, and current hypertension medication.

199

200 Univariable analysis was completed to investigate correlation between the defined
201 cardiovascular risk markers and brain outcomes. Multivariable analysis was
202 completed using a forced entry linear regression model. To reduce multiple testing
203 and potential interaction between the variables, the prediction model was restricted

204 to a subset of variables (resting systolic blood pressure, body mass index, vigorous
205 physical activity, alcohol consumption and smoking). This model was adjusted for
206 non-modifiable factors including age, sex and gestational age.

207

208 To investigate correlation between risk markers and brain outcomes, participants
209 were scored for positive traits in modifiable risk profiles: BMI <25 kg/m²; highest
210 tertile cardiovascular fitness and/or physical activity; alcohol <8 drinks/week; non-
211 smoker for > 6 months; blood pressure on awake ambulatory monitoring <130/80
212 mmHg; a non-hypertensive diastolic response to exercise (peak diastolic blood
213 pressure <90 mmHg), total cholesterol <200mg/dL; and fasting glucose <100mg/dL⁵.
214 ^{6, 12, 27-29}. Two models were created to represent: 1) simple modifiable health score
215 determined from lifestyle measures recorded in clinic (physical activity, BMI,
216 smoking, alcohol), and 2) detailed modifiable health score that additionally included
217 clinical investigations (exercise testing, blood samples and ambulatory blood
218 pressure). Relationships between scores and brain outcomes were studied using
219 linear regression adjusted for age and sex. Secondary sensitivity analysis assessed
220 minimum number and combinations of factors required to maintain model
221 significance.

222

223 In addition, univariable analysis was completed to investigate correlation between
224 vessel morphology and white matter hyperintensity lesion count and in a subgroup
225 (n=52), blood arrival time and cerebral blood flow. These relationships were further
226 investigated with fixed entry linear regression models adjusted for modifiable and
227 non-modifiable factors used in the models above (Supplementary data eTable 2-4).

228

229 Statistical analysis was undertaken using Statistical Product and Service Solutions
230 (SPSS) Version 22 (Armonk, New York, U.S). Normality of variables was assessed
231 by visual assessment of curves. If normally distributed, results are presented as

232 mean \pm standard deviation for continuous variables, otherwise median and
233 interquartile range. For categorical variables, number and percentage are presented.
234 Comparison between groups for continuous variables was performed with a 2-sided,
235 independent-sample Student's *t* test. Multivariable analysis was completed using
236 forced entry linear regression. All multivariable analyses were adjusted for age and
237 sex. P-values <0.05 were considered statistically significant and all results were
238 considered exploratory. Results are presented as point estimate and 95% confidence
239 intervals stated in units appropriate to the risk factor and brain outcome being
240 reported. Graphpad Prism 7 software was used for statistical figures and mean with
241 95% confidence intervals presented.

242

243 **Results**

244 125 participants completed the brain MRI protocol and cardiovascular risk
245 assessment study measures. The mean age of participants was 24.7 ± 5.0 years, 61
246 participants were female (49%), the mean gestational age was 36.6 ± 4.3 weeks,
247 educational attainment was high with 86 completing University level education
248 (68.8%), 29 participants had prior history of hypertension of which 21 were on anti-
249 hypertension medications (16.8%) (Table 1).

250

251 **Modifiable risk factors and association with brain vessel structure and white** 252 **matter hyperintensity lesions**

253 Univariable correlations between risk factors (SBP, BMI, smoking pack years, Ex
254 DBP, Cholesterol/HDL ratio, Hypertension treatment) and brain vessel density and
255 caliber are presented in Table 2. Vessel tortuosity only varied with gestational age in
256 both univariable and multivariable models (0.005 unit tortuosity change/gestational
257 week, 95%CI 0.001 to 0.009, $p=0.007$) (Supplementary Data, eTable 1). In the
258 multivariable models, systolic blood pressure (-0.2 vessels/cm³ per 10mmHg, 95%CI
259 -0.004 to -0.4 , $p=0.04$), smoking (2 vessels/cm³ per 10 pack years, 95%CI 0.6 to 3.0,

260 $p=0.04$) and Body Mass Index (-0.1 vessels/cm³ per 1kg/m², 95%CI -0.01 to -0.15 ,
261 $p=0.02$) remained independent correlates of vessel density, while vessel caliber was
262 independently correlated with systolic blood pressure ($-6\mu\text{m}$ per 10mmHg, 95%CI $-$
263 0.5 to -10.0 , $p=0.03$) and smoking ($40\mu\text{m}$ per 10 pack years, 95%CI 2.0 to 80.0 ,
264 $p=0.04$). In univariable models, white matter hyperintensities also correlated with
265 smoking, exercise diastolic blood pressure and, in addition, alcohol intake
266 (Supplementary Data, eTable 2).

267

268 Modifiable behavioural risk scores provide an overall assessment of risk profile
269 based on: high physical activity; not smoking in the last 6 months; body mass index
270 <25 kg/m²; and alcohol consumption <8 drinks/week demonstrated that vessel
271 density increased by 0.5 vessels/cm³ for each additional score point (95%CI 0.2 to
272 0.8 , $p=0.002$) and vessel caliber by $10\mu\text{m}$ (95%CI 2.0 to 17.0 , $p=0.01$) (Table 3). The
273 more complex cardiovascular risk model based on a cumulative score across 8
274 parameters also correlated with vessel morphology. Each increase in score
275 associated with a 0.3 vessels/cm³ higher vessel density (95%CI 0.1 to 0.5 , $p=0.003$)
276 and $8\mu\text{m}$ greater vessel caliber (95%CI 3.0 to 13.0 , $p=0.01$). Similarly, white matter
277 hyperintensity lesion count correlated with scores in Model 1 and 2, reducing by 2.2
278 lesions per additional positive score on the simple grading (95%CI -0.5 to 4.0 ,
279 $p=0.01$), and 1.6 fewer white matter hyperintensity lesions per unit of the complex
280 score (95%CI -0.5 to 3.0 , $p=0.006$). Differences in vessel morphology and white
281 matter hyperintensity lesions between tertiles of the study group, divided based on
282 the complex score, are presented in Figure 2.

283

284 In exploratory secondary analysis, a sensitivity analysis was performed removing
285 individual components from the modifiable health scores. The minimum combination
286 of components required to maintain significant correlations were 3 factors, with
287 alcohol consumption and body mass index being essential in each score (data not

288 presented). Models 1 and 2 also correlated with the total volume of white matter
289 hyperintensity adjusted for brain size with a 61 mm³ reduction in white matter
290 hyperintensity lesion volume for each additional score on model 1 (95%CI -5 to -117
291 mm³, p=0.03) and a 51 mm³ lower white matter hyperintensity lesion volume per
292 additional score on model 2 (95%CI -15 to -87 mm³ p=0.006).

293

294 **Vessel Morphology and brain MRI biomarkers of cerebral blood flow, arrival** 295 **time and white matter lesion count**

296 To explore whether cerebral blood flow also varied with cardiovascular risk factors, a
297 subgroup (n=52) analysis was performed in those with cerebral blood flow measures
298 (mean cerebral blood flow 60 ml/100g/min (SD 11.5) and mean blood arrival time
299 1.01 seconds (SD 0.08)). In univariable analysis, slower blood arrival time and
300 reduced cerebral blood flow were correlated with increased BMI (Supplementary
301 Data, eTable 2). Cerebral blood flow was also lower in correlation with anti-
302 hypertensive medication 11 ml/100g/min (95%CI -3 to -18, p=0.007). When cerebral
303 blood flow and blood arrival time was modelled using the simple modifiable risk
304 score, blood arrival time was 0.03 second faster for each additional point (95%CI -
305 0.007 to -0.05, p=0.009) and cerebral blood flow 4 ml/100g/min higher (95%CI 0.5 to
306 7.6, p=0.03) (Table 3).

307

308 In multivariable analysis, controlling for modifiable risk factors (SBP, BMI, VPA,
309 smoking, alcohol intake) blood arrival time and cerebral blood flow varied with
310 cerebral vessel density, with each additional vessel per cm³ correlating with a 0.015
311 seconds faster blood arrival time (95%CI -0.002 to -0.03, p=0.02) and 3 ml/100g/min
312 increase in cerebral blood flow (95%CI 0.7 to 5.4, p=0.01). Vessel density was
313 inversely correlated with white matter hyperintensity count with a reduction of 1.5
314 lesions per unit increase in vessel density per cm³ (95%CI -0.4 to -2.7, p=0.01).
315 (Supplementary Data eTables 3-4).

316 **Discussion**

317 This study demonstrates adverse modifiable cardiovascular risk profiles in young
318 people are associated with differences in brain vessel structure and function as well
319 as an increased number of white matter hyperintensity lesions. This suggests
320 cerebrovascular pathology may be accumulating earlier than previously anticipated.

321

322 Modifiable risk factors such as blood pressure, BMI, smoking and lipid profile are
323 known to drive systemic vascular disease in young people in part through biological
324 vascular disorders including endothelial dysfunction and oxidative stress³⁰⁻³². The
325 current study suggests the cerebrovasculature may be similarly affected. Novel early
326 life factors, such as preterm birth, have also been linked with early vascular disease³³
327 as the third trimester and early neonatal period are hypothesized to be times of
328 significant vascular remodelling. Gestational age did predict vessel tortuosity,
329 consistent with previous reports in infants³⁴, but not other cerebrovascular measures.
330 Further work is needed to understand whether this was because participants were
331 largely born late preterm or because cardiovascular risk profile overwhelms this early
332 exposure.

333

334 To capture the complete risk profile of each participant, ideal modifiable
335 cardiovascular risk scores were developed. Such scores are established prediction
336 tools for future cardiovascular and cerebrovascular disease in older populations^{5, 27,}
337 ³⁵. In this study, the simple risk score correlated with variation in all of the
338 cerebrovascular measures including vascular structure, brain blood flow and white
339 matter hyperintensities. The difference in white matter lesion burden between lowest
340 and highest modifiable risk scores was around 20%. No longitudinal outcome studies
341 have tracked white matter hyperintensities from similar age groups but the typical
342 rate of progression of white matter hyperintensity lesions per year in older
343 populations is 10 to 20%^{36, 37}. Adverse modifiable cardiovascular risk factors are

344 major determinants of this progression³⁸ with small lesions increasing in size or
345 clustering into confluent lesions^{39, 40}. Accumulation of lesions from an early age might
346 explain why, by mid-life, white matter hyperintensity lesion volume is an established
347 predictor of future stroke risk⁷. If a 20% difference between groups were maintained
348 into older adult life, this would be associated with a 2 to 3-fold increased risk of
349 stroke, dementia and all-cause mortality⁷.

350

351 However, it has been proposed that early small lesions, as observed in this study,
352 may be reversible^{41, 42}. Reducing multiple risk factors can change risk trajectories and
353 reduce vascular disease burden⁴³. Individuals with higher cardiovascular fitness have
354 a greater number of small vessels⁴⁴ and exercise interventions are associated with
355 beneficial effects on cerebral perfusion⁴⁵⁻⁴⁸ as well as short-term benefits for brain
356 volume^{49, 50}. In addition, sustained lifestyle intervention and active blood pressure
357 lowering in patients with diabetes, or following a stroke, significantly reduces the
358 burden of white matter hypertensities and prevents accumulation of new lesions⁵¹⁻⁵⁴.
359 These interventions typically achieve 25% improvements in cardiovascular fitness
360 and 10 mmHg reductions in blood pressure, comparable to differences between high
361 and low risk groups in this study.

362

363 However, lifestyle-based primary cardiovascular prevention in young people requires
364 complex intervention design. Recent systematic review of interventions in young
365 hypertensives demonstrated that the optimal way to intervene is poorly understood
366 with lack of sustained effect⁵⁵. The alternative to lifestyle interventions would be
367 pharmacological treatment. Anti-hypertensive use in this study group was associated
368 with a trend towards increased brain vessel density^{17, 18}. However, there was not a
369 proportional increases in cerebral blood flow; a phenomenon previously described in
370 hypertensives and proposed to be a 'brain protective' response, as cerebral vessel
371 rarefaction drives an increase in blood pressure to maintain cerebral blood flow⁵⁶.

372 Further work to identify optimal interventions in young adults to maintain
373 autoregulation of cerebral blood flow, while reducing risk, may be required.

374

375 **Limitations**

376 This study has several limitations. First, a small sample recruited at a single site
377 increases risk of bias and the study may be underpowered to identify subtle
378 correlations with some risk factors. Second, mixed passive and active recruitment
379 strategies mean the sample is not population-based and could be considered similar
380 to a convenience sample. Therefore, it is not possible to generalise expected
381 prevalence of cerebrovascular changes to the wider population. Third, the study is
382 cross-sectional and causality of the observed relationships cannot be inferred.
383 Fourth, cerebral blood flow was only available in a subgroup so ability to understand
384 interactive effects of modifiable risk factors, vascular remodelling and perfusion on
385 white matter integrity is limited. Fifth, longitudinal follow up will be required to
386 comment on the clinical significance of the observed findings. As such, this study
387 should be considered preliminary and exploratory but does support a need for future
388 work. The complexity of the imaging protocol and associated financial costs may limit
389 its widespread use but large multi-centre studies with more focused protocols, and
390 extended follow up, will allow tracking of vascular remodelling and assessment of
391 impact on white matter and later disease. Randomised control trials will also allow
392 effects of both lifestyle and pharmacological intervention to be properly evaluated.

393 **Conclusion**

394 In this preliminary study involving young adults without clinical evidence of
395 cerebrovascular disease, modifiable cardiovascular risk factors were associated with
396 MRI indices of cerebral vessel structure and function, and white matter
397 hyperintensities. Further research is needed to determine the clinical importance of
398 these findings for the primordial prevention of cerebrovascular disease.

399

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406

407 **Authorship**

408 All authors meet criteria for authorship: WW, AL, HB, CF, HD, PL contributed to the
409 design of the study, secured funding and refined the overall study protocol and lead
410 the project delivery, NF, LG, TO, MJ, CM contributed to the development of the Brain
411 MRI protocol and related pipelines, AL, WW, OH, JF, SN contributed to image
412 acquisition and quality control, WW, NF, LG, TO, MJ, CM, JB, HB, TS, DM, RP
413 contributed to brain MRI image processing and analysis, AD advised on
414 accelerometer protocol for objective physical activity measurement and compressed
415 analysis of raw data, WW, AL, HB, OH, completed cardiovascular risk assessment
416 and analysis of measures, WW, CF, PL and EF contributed to the statistical analysis,
417 WW wrote the manuscript with support from LG, OH, AL, CF, NF, HD, PL. All
418 authors contributed to revision of the manuscript. PL completed the final edit of the
419 manuscript.

420

421 **Disclosures**

422 Dr. Okell reports grants from The Royal Academy of Engineering, during the conduct
423 of the study; In addition, Dr. Okell has a patent (US Patent 9,757,047) with royalties
424 paid from Siemens Healthcare. All other authors declare no competing interests.

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428 **Role of the funding source**

429 The funders of the study had no role design and conduct of the study; collection,
430 management, analysis, and interpretation of the data; preparation, review, or
431 approval of the manuscript; and decision to submit the manuscript for publication.

432 The corresponding author had full access to all the data in the study and takes
433 responsibility for the integrity of the data and the accuracy of the data analysis

434

435 **Access to data**

436 Dr. Williamson and Professor Leeson had full access to all of the data in the study
437 and take full responsibility for the integrity of the data and the accuracy of the data
438 analysis

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652

653

654 Table 1. Age, demographics and cardiovascular risk profile of study group.

	Study Group (n=125)
Demographics	
Age, mean (SD), years	24.7 (5.0)
Female, n, (%)	61 (49%)
Gestational Age, mean (SD), weeks	36.6 (4.3)
Smoking, n, (%)	19 (15.2)
Smokers' median pack years (IQR)	2.7 (6.7)
Alcohol, n, (%)	97 (77.6)
Alcohol consumers' median drinks per week (IQR)	4.0 (4.0)
Hypertension Diagnosis, n, (%)	29 (23.0)
FHx Stroke or CHD, n, (%)	10 (8)
Education Level	
Completed University, n, (%)	86 (68.8)
Anthropometrics	
Height, mean (SD), m	1.73 (0.1)
Weight, mean (SD), kg	70.9 (13.8)
BMI, mean (SD), kg/m ²	23.6 (3.7)
Blood pressure, mean (SD), mmHg	
Resting Systolic	122.0 (11.6)
Resting Diastolic	71.3 (9.55)
Ambulatory Awake Systolic	129.6 (11.8)
Ambulatory Awake Diastolic	76.9 (8.0)
Peak Exercise Systolic	174.8 (25.4)
Peak Exercise Diastolic	87.1 (12.4)
Fitness	
Peak VO ₂ , mean (SD), ml/kg/min	37.9 (9.6)
Peak Respiratory Exchange Ratio, mean (SD)	1.2 (0.06)
VPA, median (IQR), hours per week	0.74 (1.25)
MVPA, median (IQR), hours per week	14.73 (6.09)
Biochemistry	
Total Cholesterol, mean (SD), mg/dL	170.15 (29.0)
LDL, mean (SD), mg/dL	97.45 (25.9)
HDL, mean (SD), mg/dL	55.68 (11.2)
TChol:HDL ratio, mean (SD)	3.18 (0.85)
Triglyceride, median (IQR), mg/dL	74.4 (54.0)
Blood Glucose, mean (SD), mg/dL	88.2 (7)
HOMA-IR, mean (SD)	0.77 (0.46)
HsCRP, median (IQR), mg/L	0.57 (1.16)
Brain MRI Outcomes	
Brain vessel density, mean (SD), vessels/cm ³	8.3 (1.41)
Brain vessel calibre, mean (SD), µm	531 (36)
Brain vessel tortuosity, mean (SD)	1.49 (0.088)
Brain white matter hyperintensity lesion count, mean (SD)	20.9 (7.9)

655 Abbreviations: FHx, Family History, BMI, body mass index; SBP, systolic blood
656 pressure; DBP, diastolic blood pressure; Alcohol (1 drink per week = 2 units of
657 alcohol), Peak VO₂, Peak Oxygen Uptake; VPA, Vigorous Physical Activity; MVPA,
658 Moderate to Vigorous Physical Activity; LDL, low density lipoprotein; HDL, high
659 density lipoprotein; T Chol: total cholesterol; HsCRP, highly sensitive C reactive
660 protein; HOMA-IR, homeostatic model assessment of insulin resistance.
661

662

663 **Table 2. Univariable correlations and regression models for modifiable risk**
 664 **factors and brain vessel density and vessel caliber**

	Brain Vessel Density (vessels/cm ³)			Brain Vessel Caliber (µm)				
	Univariable		Adjusted	Univariable		Adjusted		
	Point Estimate (95 %CI)	P value	Point Estimate (95 %CI)	P value	Point Estimate (95 %CI)	P value	Point Estimate (95 %CI)	P Value
Gestational Age, weeks	-0.001 (-0.06 to 0.06)	.98	-0.02 (-0.08 to 0.03)	.42	-0.1 (-2.0 to 1.0)	.88	-1.0 (-3.0 to 0.5)	.16
Resting SBP, mmHg	-0.03 (-0.004 to -0.05)	.02	-0.02 (-0.0004 to -0.04)	.046	-0.4 (-1.0 to 2.0)	.15	-0.6 (-0.05 to -1.0)	.03
BMI, kg/m ²	-0.10 (-0.02 to -0.16)	.01	-0.08 (-0.01 to -0.15)	.02	-1.0 (-3.0 to 1.0)	.33	-1.0 (-3.0 to 1.0)	.42
VPA, hours per week	0.10 (-0.17 to 0.39)	.42	-0.04 (-0.28 to 0.20)	.75	1.0 (-6.0 to 8.0)	.73	-2.0 (-9.0 to 4.0)	.49
Alcoholic drinks per week	-0.10 (-0.008 to -0.025)	.31	-0.01 (-0.04 to 0.02)	.41	-0.1 (-1.0 to 1.0)	.70	-1.0 (-2.0 to 0.1)	.09
Smoking pack years	0.20 (0.06 to 0.30)	.004	0.17 (0.06 to 0.28)	.004	3.0 (-0.2 to 6.0)	.06	4.0 (0.2 to 8.0)	.04
Peak VO ₂ , ml/kg/min	0.01 (-0.02 to 0.04)	.5			0.4 (-0.2 to 1.0)	.19		
Peak Ex DBP, mmHg	-0.02 (-0.003 to -0.04)	.047			-1.0 (-0.4 to -1.0)	<.001		
Cholesterol/HDL Ratio	-0.40 (-0.06 to -0.69)	.02			-3.0 (-10.0 to 5.0)	.52		
HOMA IR	-0.56 (0.04 to -1.17)	.07			-14.0 (-30 to 1.0)	.08		
Hypertension Rx	0.75 (-0.01 to 1.5)	.05			10 (-9.0 to 31.0)	.27		
Model Statistics			R²=0.20	p=.009			R²=0.24	p=.001

665

666 The adjusted multivariable models are restricted to simple modifiable factors that can
 667 be assessed during a clinical consultation (resting systolic blood pressure, body
 668 mass index, participation in vigorous physical activity, alcohol consumption and
 669 smoking). The models were controlled for age, sex and gestational age.

670 Abbreviations and units: SBP, systolic blood pressure (mmHg); BMI, body mass
 671 index (kg/m²); VPA, Vigorous Physical Activity (hours per week); Alcohol (1 drink per
 672 week = 2 units of alcohol); Smoking (pack years); Peak VO₂, Peak Oxygen Uptake
 673 (ml/kg/min); Ex DBP, Peak exercise diastolic blood pressure (mmHg),
 674 Cholesterol/HDL ratio, ratio total cholesterol/high density lipoprotein; HOMA-IR,
 675 homeostatic model assessment of insulin resistance, Hypertension Rx participant
 676 taking prescription medications for hypertension (yes/no).

677

678 **Table 3. Modifiable health scores and correlation with brain vessel density,**
679 **vessel caliber, brain blood flow and white matter hyperintensity lesion count**

	Model 1 Simple Modifiable Health Score		Model 2 Detailed Modifiable Health Score	
	Change in point estimate per unit increase in score (95%CI)	P Value	Change in point estimate per unit increase in score (95%CI)	P Value
Brain Vessel Density, vessels/cm³	0.50 (0.19 to 0.81)	.002	0.31 (0.112 to 0.514)	.003
Brain Vessel Caliber, μm	10 (2.0 to 17.0)	.014	8.0 (3.0 to 13.0)	.002
Brain Vessel Tortuosity	0.004 (-0.02 to 0.02)	.97	0.005 (-0.008 to 0.18)	.44
Brain Blood Flow, ml/min/100g	4.0 (0.5 to 7.6)	.027	2.47 (-0.05 to 4.98)	.05
Brain Blood Arrival Time, seconds	-0.03 (-0.007 to -0.05)	.009	-0.014 (-0.03 to 0.001)	.07
Brain white matter hyperintensity lesion count, number	-2.16 (-0.46 to -3.86)	.013	-1.58 (-0.47 to -2.79)	.006

680

681

682 Model 1 uses a cumulative score for modifiable risk factors that can be assessed in a
683 single consultation based on 4 factors, given equal weight, with a positive score
684 assigned for: alcohol consumption <8 drinks/week; participating in >=75 minutes
685 vigorous physical activity or high moderate to vigorous activity; not smoking in last 6
686 months; and body mass index <25 kg/m². Model 2 uses a cumulative score across a
687 comprehensive assessment of modifiable risk factors including a score for: high
688 cardiovascular fitness and/or physical activity (measured as being in the top tertile of
689 peak oxygen uptake (110% predicted peak oxygen uptake or higher) or participating
690 in >=75 minutes vigorous physical activity); not smoking in last 6 months; ambulatory
691 awake blood pressure <130/80 mmHg; body mass index <25kg/m²; fasting total
692 cholesterol <200 mg/dL; fasting blood glucose <100 mg/dL; and diastolic blood
693 pressure at peak exercise <=90 mmHg. Models are adjusted for age and sex.

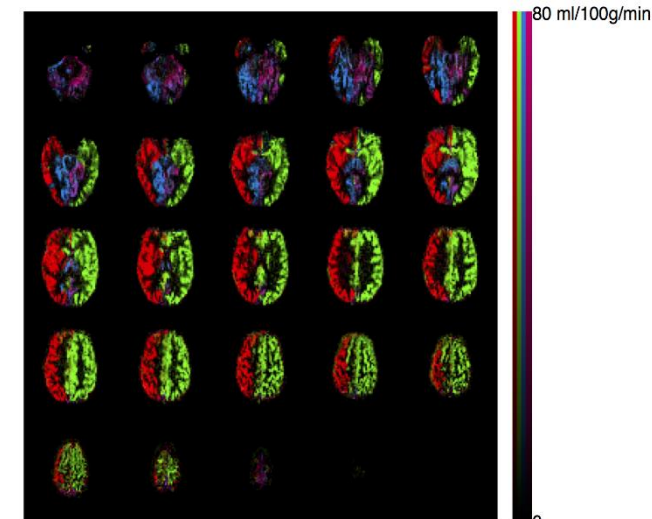
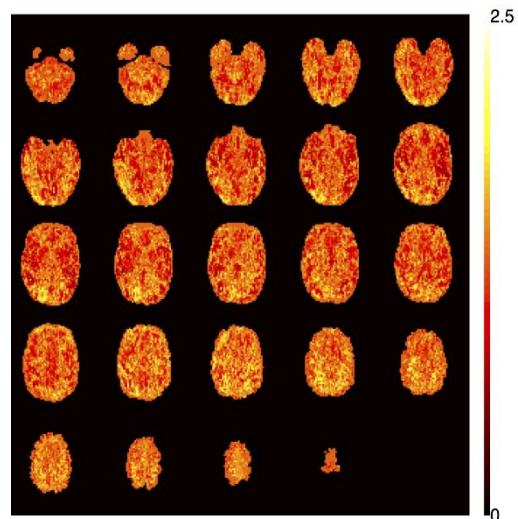
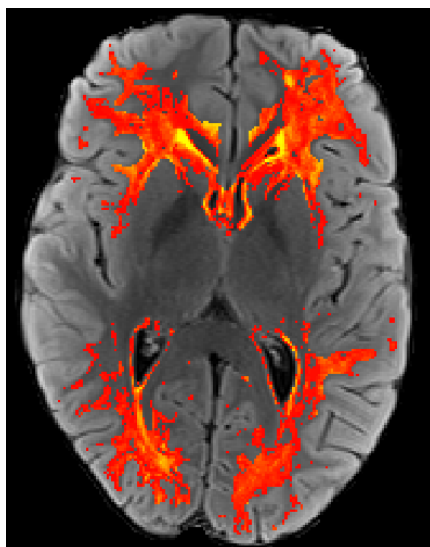
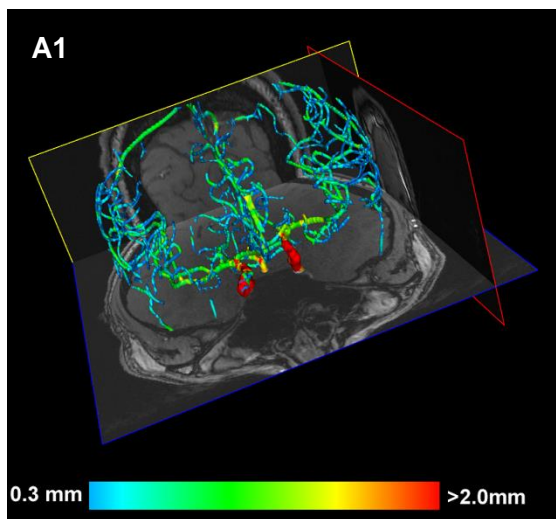
694 **Figure 1. Panels A1 and A2 provide a case comparison of the MRI imaging modalities and analysis tools used to assess brain vessel morphology,**
695 **white matter lesion count, cerebral perfusion and blood arrival time**

3D Reconstruction of Brain Vessels segmented from Time of Flight MRI arteriogram

Probability map of white matter hyperintensity lesions overlaid on Axial FLAIR image

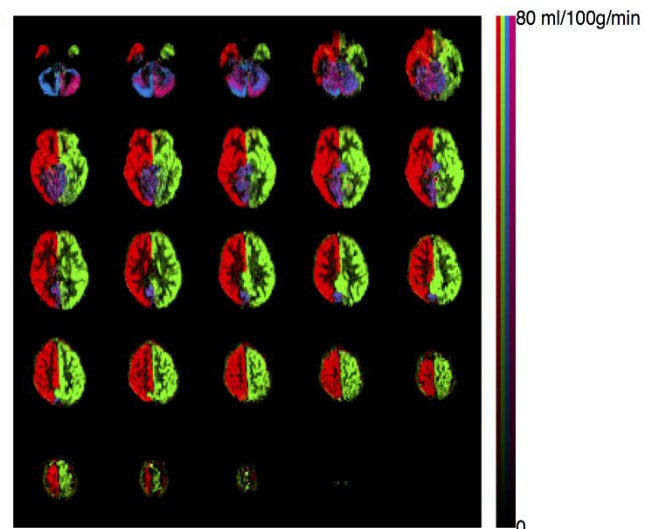
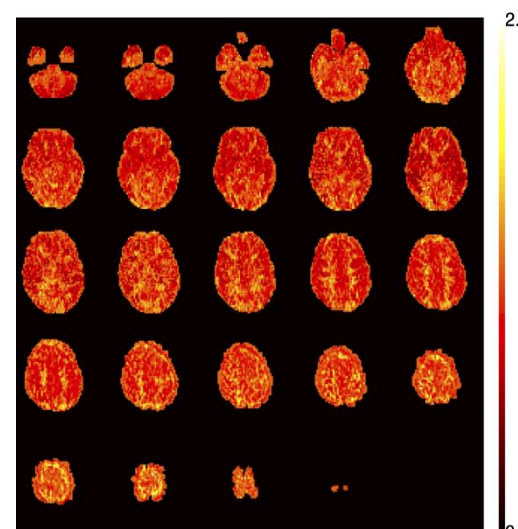
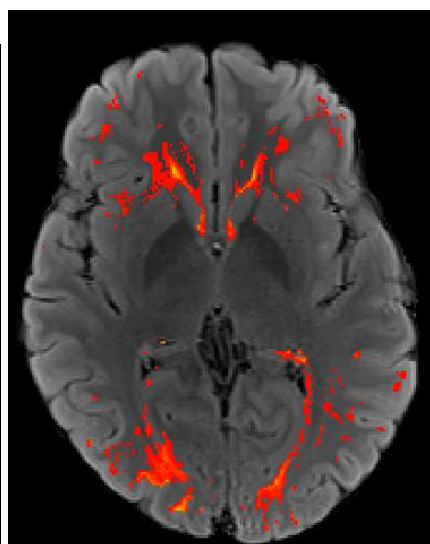
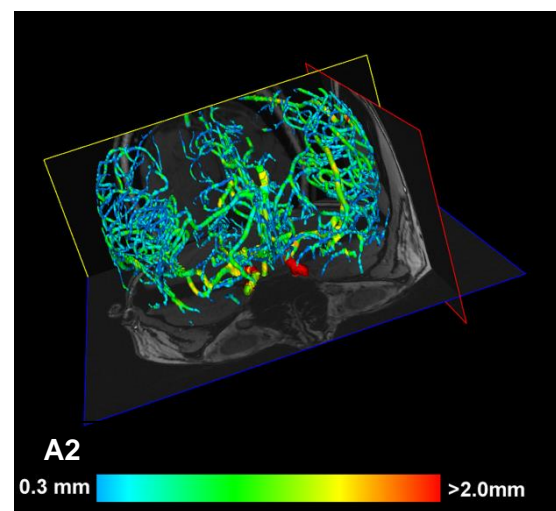
Axial arterial spin labeled images demonstrating brain blood flow

Axial arterial spin labeled images demonstrating brain blood arrival time



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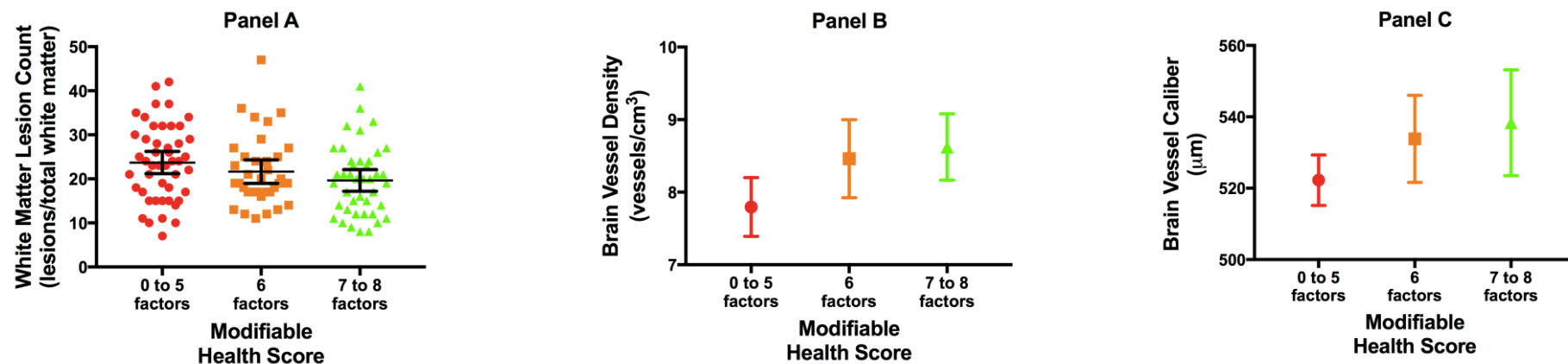
699 Time of Flight (TOF) magnetic resonance arteriogram was used to acquire images of the brain vessels, this was analyzed using automated tools generating
700 binary segmentations to determine overall vessel density, caliber and tortuosity. 3D reconstructions of segmented brain vessels are provided in column one of
701 Panels A1 and A2. Three image modalities T2 weighted Fluid Attenuated Inversion Recovery (FLAIR), Diffusion Tensor Imaging (DTI) and T1 weighted
702 structural images were used to optimise white matter segmentation and white matter hyperintensity lesion quantification using analysis tools from the Brain
703 Intensity AbNormality Classification Algorithm (BIANCA). BIANCA is a fully automated, supervised method for white matter hyperintensity detection, based on
704 the *k*-nearest neighbour (*k*-NN) algorithm. The BIANCA output is a probability map of the likelihood that the voxel being classified is a lesion. The probability
705 map is displayed in column 2 of panels A1 and A2, on a spectrum of orange to yellow, and overlaid on an axial FLAIR image for comparison. Voxels likely to
706 be white matter hyperintensity lesions are demonstrated as bright yellow. A threshold of 0.9 was applied to define the voxel as lesion or not which was then
707 fed into cluster analysis to identify individual lesions and quantify white matter hyperintensity volumes. White matter hyperintensity lesions are demonstrated
708 as bright yellow. In a subgroup of the study population (n=52) pseudocontinuous vessel selective arterial spin labelling (ASL) was acquired to allow the
709 assessment of blood flow to the brain. This provides two outputs, a measure of blood arrival time (seconds), demonstrated in column 3 and a measure of
710 volume of blood flow (ml/100g/min) demonstrated in column 4, of Panels A1 and A2.

711

712 Panel A1 and A2 provide a comparison between two cases with visible differences in vessel morphology and white matter intensity lesion count that may be
713 associated with observed differences in optimal risk profiles. Case A1 is a 21 year old male with BMI 26 kg/m³, resting blood pressure 144/81 mmHg, awake
714 ambulatory blood pressure 135/74 mmHg, 40 minutes of vigorous activity and 14 hours of moderate to vigorous activity per week measured on triaxial
715 accelerometer, non-smoker with alcohol intake greater than 8 drinks per week, blood pressure at peak exercise measured 200/70 mmHg, total cholesterol 178
716 mg/dl and fasting blood glucose 77 mg/dl. Case A1 vessel density measures 6.4 vessels/cm³, he has 30 white matter hyperintensity lesions measuring 1mm
717 or more, cerebral blood flow measuring 62ml/100g/min (lower intensity on colour scale in column 4) and blood arrival time of 1.26 second (more yellow on the
718 colour scale in column 3). Case A2 is a 24 year old female with BMI 23 kg/m³, resting blood pressure 134/81 mmHg, awake ambulatory blood pressure
719 122/77 mmHg, recording 20 minutes of vigorous activity and 21 hours of moderate to vigorous activity per week measured on triaxial accelerometer, non-
720 smoker with alcohol intake less than 2 drinks per week, blood pressure at peak exercise measured 180/90 mmHg, total cholesterol 127 mg/dl and fasting
721 blood glucose 84 mg/dl. Case A1 vessel density measures 12.6 vessels/cm³, she has 8 white matter hyperintensity lesions, cerebral blood flow measuring
722 83ml/100g/min (brighter intensity on colour scale) and blood arrival time of 1.07 second (more orange on the colour scale in column 3).

723

Figure 2. Comparison of white matter lesion count and vessel morphology between groups of participants based on their modifiable health score.



Model 2 modifiable health score provided a comprehensive assessment of modifiable risk factors based on a cumulative score for each of the following factors: high cardiovascular fitness (defined as physical activity measured in the top tertile of peak oxygen uptake ($\geq 110\%$ predicted peak oxygen uptake) or participating in ≥ 75 minutes vigorous physical activity per week); not smoking in last 6 months; ambulatory awake blood pressure $< 130/80$ mmHg; body mass index < 25 kg/m²; fasting total cholesterol < 200 mg/dL; fasting blood glucose < 100 mg/dL; and diastolic blood pressure at peak exercise ≤ 90 mmHg. The panels in figure 2 present comparisons between groups of participants who in Model 2 score 0 to 5 positive factors (n=47), 6 factors (n=36) and > 7 positive factors (n=42). Participants with > 7 factors have a mean vessel density 11% higher than participants with 0 to 5 positive traits (Panel B, 8.6 vessels/cm³ (SD 1.39) vs 7.8 vessels/cm³ (SD 1.21) p=0.007), a mean vessel caliber 3% higher (Panel C, 538 μm (SD 21) vs 522 μm (SD 45) p=0.02) and on average 20% lower white matter hyperintensity lesion counts (Panel A, 19.6 lesions (SD 7.8) vs 23.5 lesions (SD 8.6) p=0.03). Panels present group means and 95%CI and reported group differences are adjusted for age and sex.