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Retinal Vessel Abnormalities as a Possible Biomarker of Brain Volume Loss in Obese Adolescents

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Abstract

Objective—Endothelial dysfunction in childhood obesity may precede cerebrovascular damage and cognitive impairment in adulthood. Identifying risk for microvascular damage in obese children requires a non-invasive proxy of microvascular health.

Design and Methods—We assessed the associations of hippocampal volumes and global cerebral atrophy with retinal vessel caliber in 40 normal BMI controls and 62 obese age-matched non-diabetic adolescents and evaluated the contribution of inflammation, obesity and insulin resistance to retinal vessel caliber.

Results—Compared to controls, obese adolescents had smaller retinal arterioles (8.3% decrease, p<.05) and wider venules (5.4% increase, p<.01). Larger retinal arteriole diameters were associated with less global cerebral atrophy (B=−.24(95% CI:−.48,−.002) and larger hippocampal volumes (B=.01(95% CI:0,.02). Inflammation (fibrinogen) predicted venule diameters (B=84.2(95% CI:30.3, 138.1). Insulin resistance, indicated by logHOMA values (B=−17.03(95% CI:−28.25,−5.81) and body mass index (BMI) (B=−.67(95% CI:−1.09,−.24), predicted arteriolar diameters. All analyses were adjusted for mean arterial pressure, sleep apnea, and vessel diameter.

Conclusion—Measures of brain health, BMI, and insulin resistance are associated with retinal vessel caliber. If confirmed in larger studies, retinal arteriolar caliber may serve as a possible non-

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Conflicts of Interests

The authors of this paper report no conflicts of interest.

invasive proxy for brain atrophy in obese adolescents, and the identification of elevated risk for cerebral microvascular disease in adulthood.

Keywords

obesity; brain atrophy; adolescent; retina; hippocampus

Introduction

Within thirty years, childhood obesity rates have nearly tripled,[1] bringing with it a parallel rise in insulin resistance (IR), a precursor to type 2 diabetes mellitus (T2DM).[2] In adulthood, negative health consequences of T2DM include macro- and microvascular disease,[3] cognitive impairment,[4] cerebral atrophy,[5] and hippocampal volume reductions.[6] Many of these complications are also present in adolescents with T2DM. Furthermore, obese adolescents with T2DM demonstrate other brain complications, including reduced white and grey matter integrity relative to obese controls.[7] We previously reported that non-diabetic adolescents with metabolic syndrome (MetS) have neurocognitive abnormalities, including reduced hippocampal volumes, increased cerebral brain atrophy, lower cognitive scores, and compromised white matter microstructural integrity.[8]

Scalable interventions to prevent future macrovascular damage require robust, non-invasive proxies of microcirculation. Obese adolescents with IR demonstrate both micro- and macro-vascular endothelial dysfunction, which may progress to macrovascular disease in adulthood.[9] Despite increasingly high rates of IR among obese children, no studies have yet observed the impact of obesity and IR on retinal vasculature. More importantly, no studies have simultaneously evaluated retinal vasculature and brain health during adolescence, a critical time for brain maturation.[10]

In this study, we assess the impact of BMI, insulin resistance, inflammation, and hypertension on retinal vessel diameters among healthy weight and obese non-diabetic adolescents. We also assess the association between retinal vessel calibers and MRI-based hippocampal volume and global cerebral atrophy (adjusting for head size) and after accounting for mean arterial blood pressure (MAP) and sleep apnea (SA).

Methods and Procedures

Participants

This study was conducted at the NYU School of Medicine's Brain, Obesity, and Diabetes Laboratory (BODyLab). We evaluated 102 consecutively recruited adolescents without T2DM between the ages of 14 and 20. Subjects were recruited on the internet or from a related study aiming to prevent diabetes among obese adolescents. Parental written informed consent was obtained from all participants under 18 years of age, and written informed consent from those 18 years or older. All study subjects were compensated for their time and inconvenience. Participants underwent medical, retinal, medical (endocrine), and brain MRI evaluations. Individuals with significant neurological, medical (other than hypertension or dyslipidemia), psychiatric conditions (including depression or substance abuse), or

diagnosed with T2DM were excluded from participation. No subjects were on antihypertensive medications or other drugs known to affect retinal blood vessels such antiglaucoma medications or diuretics. None of the participants admitted to regular cigarette smoking. The study protocol followed the tenets of the Declaration of Helsinki and was approved by the Institutional Board of Research Associates of the New York University School of Medicine.

Categorization into the obese group required a body mass index (BMI) ≥30. The group characteristics of participants fulfilling study inclusion criteria are shown in Table 1.

Blood Tests and Insulin Resistance Assessment

After a 10-hour overnight fast all study participants had a blood sample taken for glucose and insulin, HbA1c, blood count, comprehensive metabolic and lipid profiles, and high sensitivity C-reactive protein (CRP) and fibrinogen levels. CRP was measured in plasma using an enzymatic immunoassay (Vitros CRP slide, Ortho Clinical Diagnostics, Amersham, England). Plasma fibrinogen was measured by the prothrombin-time derived method with reference to the Clauss fibrinogen assay using ACL TOP 500 CTS coagulation analyzer with closed tube sampling (Instrumentation Laboratory, Beckman Coulter Inc., Fullerton, United States).[11] Fasting glucose and insulin values were used to compute HOMA-IR, a validated measure of insulin sensitivity.[12]

BMI, anthropometric measurements, and sleep apnea (SA) assessment

To calculate BMI (kg/m^2), height and weight were assessed using a Seca 700 beam scale of 500 lbs capacity (with height-rod), calibrated prior to each individual measurement. Subjects completed a questionnaire to evaluate symptoms of SA and the severity of sleep related symptoms.[13]

Blood pressure assessment and definition of hypertension

We measured sitting blood pressure (BP) twice with a random-zero sphygmomanometer utilizing an appropriately sized adult arm cuff. The first reading was performed 30 minutes after the subjects arrived, at the beginning of the physical exam. A second reading was obtained at the end of the physical examination. These two readings were then averaged. Mean arterial BP was defined as DBP + (SBP - DBP)/3.[14]

Retinal vessel caliber measurements

We obtained digital retinal photographs using a non-mydriatic 45 fundus camera (Canon CR4-45NM, Canon EOS Rebel 6.1MPix). Subjects were seated in a dimly lit room for 5–10 minutes to allow appropriate pupillary dilation. The digital photographs were centered on the optic disc for each eye using standardized settings and were processed as described previously.[15] We measured each of the 6 largest arterioles and venules for each eye (Figure 1).

We summarized individual measurements of arterioles and venules into indices based on the Revised Parr-Hubbard formula for summarizing retinal vessel diameters. We then derived a single number called the central retinal vessel equivalent for arterioles (CRAE) and venules

(CRVE), which captures average vascular cross-sections, adjusted for branching, of the arteriolar and venular systems.[16,17] For all participants, retinal vessel measurements were performed on both eyes and averaged. Mean CRAE and CRVE for both eyes were then used in our analyses. Our method is highly reproducible, with an interrater intraclass correlation coefficient of 0.83 for CRAE and 0.88 for CRVE.[15] Given the general absence of cataracts in this age group, all retinal photographs were gradable. In order to estimate the absolute size of the vessels and to account for refractive errors, we measured the disc diameter and adjusted vessel measurements assuming a standardized disc diameter of 1.850 mm.[15,16]

Brain MRI analyses

All adolescents were scanned on a 1.5 T Siemens Avanto System. We utilized a Magnetization Prepared Rapid Gradient Echo (MPRAGE) and a Fast Fluid Attenuated Inversion Recovery (FLAIR) sequence for measurement of global atrophy and to exclude gross pathology. MPRAGE sequence parameters were: TR 1300 ms; TE 4.38 ms; TI 800 ms; flip angle 15°; acquisition matrix 256×256; FOV 256 mm; NEX 1; 192 slices and slice thickness of 1.0 mm. FLAIR sequence parameters were: TR 9000 ms; TE 97 ms; TI 2500 ms; acquisition matrix 256 ° 256; FOV 210 mm; 50 slices; slice thickness 3 mm; no gaps; NEX 1; flip angle 180 degrees.

The MPRAGE study procedure accounted for individual variability in brain size by measuring intra-cranial vault (ICV) size within the supratentorial compartment, following margins of the dura and tentorium. We used a thresholding procedure to estimate the CSF portion of the ICV volume, and this value was used as a measure of global cerebral atrophy, per our published reliable methods.[18] Using our locally developed Multimodal Image Data Analysis System software, the hippocampus and superior temporal gyrus (control region) were outlined on coronal images using our published parcellation methods.[18,19] To account for individual variability in brain size, measures of hippocampal volume and global cerebral atrophy were residualized to ICV (Figure 2).

MRI analyses were conducted blind to subject identity and/or retinal measurements results. No participants had any white matter hyperintensities on their FLAIR images.

Statistical Analyses

We regarded individuals as outliers when they had greater than 3 standard deviations from the group mean for HOMA, BMI, CRAE, and/or CRVE, and excluded them from analyses. We tested for normality with the Kolmogorov-Smirnov test, employing an alpha of .05. Non-normal variables (CRP, Fibrinogen and HOMA) were logarithmically (base 10) transformed. Differences between groups were analyzed using independent t-tests and chi-square tests of independence. We used hierarchal multiple regression analyses to predict CRVE, CRAE and ICV-adjusted brain volumes. Independent variables that were either 1) significant in exploratory stepwise regression analyses or 2) conceptually important based on our review of the literature were included in the final regression analyses.

We utilized a conservative approach for determining which variables would be included in the final regression models. Predictor variables were included in the final regression model if their relationship to retinal vessel caliber 1) were supported by the literature and/or 2)

survived exploratory stepwise regression analyses in our dataset. Our exploratory analyses displayed significant results for MAP, vessel diameter (CRAE when the variable of interest was CRVE and vice versa), logFibrinogen, logHOMA, and BMI, and thus we included them in the final model (please see Appendix A for results of exploratory analyses). Although SA was not significant in our exploratory analyses, we included it in the final model based on relationships between SA and obesity and brain found in the literature.[10,20,21]

In the following analyses, we accounted for shared variance by adjusting for vessel diameter (controlling for CRVE in CRAE analyses and vice versa).[22] To predict CRVE, we adjusted for MAP and SA scores in the 1st step, vessel diameter (estimated by CRAE) in the 2nd step, logFibrinogen in the 3rd step, and BMI in the 4th and last step. To predict CRAE, we adjusted for MAP and SA in the 1st step, vessel diameter (estimated by CRVE) in the 2nd step, logHOMA in the 3rd step, and BMI in the last step. To determine the contribution of BMI to vessel caliber (CRAE or CRVE) independent of inflammation (logFIbrinogen) and insulin resistance (logHOMA), we reversed steps 3 and 4. Predicting how well the retinal vessel measurements predicted hippocampal volume and global brain atrophy we also used hierarchal multiple regression analyses, controlling for MAP and SA in the 1st step, vessel diameter (CRAE when the predictor of interest was CRVE, and vice versa) in the 2nd step, and retinal vessel caliber (CRVE or CRAE) in the 3rd step.

Data were analyzed using SPSS for Windows, version 17.0 (SPSS Inc., Chicago, Ill., USA).

Results

Obese and normal BMI control adolescent groups were well-matched on age and sex. Although none of the regression analyses involved arteriolar-to-venular diameter ratio (AVR), because it has been used in previous studies, we included it in Table 1 as a point of reference. Groups differed significantly by BMI (kg/m^2), blood pressure (systolic, diastolic and MAP, mmHg), LDL (mg/dL), HDL (mg/dL), Fibrinogen (mg/dL), CRP (mg/L), and triglycerides (mg/dL) ($p<.05$). Obese adolescents had greater global cerebral atrophy (cc) and smaller hippocampal volumes (cc) ($p<.05$). Table 1 describes differences between the normal BMI and obese group. Obese adolescents also had significantly lower CRAE (185.22 μm ± 17.04), and higher CRVE (291.71 μm ± 25.91) than normal BMI controls ($p<.05$). Although all study participants had fasting glucose and hemoglobin A1C (HbA1c%) levels below 100 mg/dL and 6.10%, respectively, groups also differed on fasting insulin (μIU/mL) and glucose (mg/dL), HOMA-IR, and HbA1C % ($p<.05$).

Factors in the final regression models were based on results from exploratory stepwise regression analyses predicting the retinal variables (CRAE or CRVE) and/or the brain variables (ICV-adjusted hippocampal or brain atrophy volumes). In these exploratory analyses MAP, vessel diameter (CRAE when the factor was CRVE and vice versa), BMI, logFibrinogen (for CRVE dependent variable), and logHOMA (for CRAE as dependent variable) were retained in the models (Appendix A). We also included SA in the explanatory models below, based on its strong associations with obesity and brain in the literature [10,20,21].

In the prediction of CRVE, after controlling for MAP, SA, and age (1st step), and CRAE (2nd step), logFibrinogen (3rd step) explained 9% of the variance (B=84.2(95% CI: 30.3,138.1), p<.01). Furthermore, BMI (4th step) explained an additional 6% of variance in CRVE. When we reversed the last two steps of this regression analysis, BMI (3rd step) explained 12% of the variance in CRVE (B=1.26(95% CI:.57,1.95), p<.01), but logFibrinogen (4th step) did not explain any additional variance.

In the prediction of CRAE, after controlling for MAP and SA (1st step), CRVE (2nd step), logHOMA (3rd step) uniquely explained 15.0% of the variance (B=−26.3(95% CI: −36.27,−16.31), p<.01). In addition, BMI (4th step) explained an additional 5% of the variance in CRAE (B=−.67(−1.09,−.24), p<.01). Reversing the last two steps of the hierarchal regression analysis, BMI (3rd step) explained 15% (B=−1.01(95% CI:−1.39,−.63), p<.01), and logHOMA (4th step) explained an additional 4% of the variance in CRAE (B= −17.03(95% CI:−28.25,−5.81), p<.01).

In parallel analyses, predicting ICV-adjusted global cerebral atrophy, we controlled for MAP and SA (1st step), CRVE (2nd step), and CRAE (3rd step): CRAE significantly accounted for 5% of variance in global cerebral atrophy (B=−.24(95% CI:−.48,−.002), p<. 05) (Table 4). With ICV-adjusted hippocampal volume as the dependent variable, and after adjusting for the same potential confounders, CRAE showed a statistical trend (ΔR^2=.04, B=.01(95% CI:0,.02), p=.05). Scatterplots of CRAE vs. ICV-adjusted cerebral global atrophy and hippocampal volume showed no indication of a cohort effect, as the distributions for healthy weight and obese groups overlapped (Figures 3 and 4).

Discussion

In adults, retinopathy may be a potential biomarker for neurological outcomes or brain structural damage that may impact cognitive performance.[23] In the Women's Health Initiative study, the presence of baseline retinopathy significantly increased the risk for overall cognitive decline and parietal and total brain atrophy over a 10-year span.[23] In the prospective Atherosclerosis Risk in Communities Brain Magnetic Resonance Imaging Study, baseline retinal microvascular abnormalities increased the risk of subclinical MRI brain infarcts and white matter lesions over a 10.5-year span.[24] These adult lesions are not likely reversible, highlighting the need for prevention among youth.[25] Evident retinopathy may be a marker of neurological pathology in adults. However, should retinal evaluations be implemented as a useful marker of obesity-associated metabolic disease among youth, we need to target more subtle forms of retinal pathology, such as general arteriolar narrowing without pronounced signs of microvascular damage (retinal hemorrhages, exudation and arteriovenous nicking). Moreover, merely measuring BMI is not a sufficient measure of obesity. The interplay between obesity and insulin resistance predicts measures of brain health. Thus, it is important to concentrate on more subtle forms of both metabolic disease and retinal vessel pathology as markers for prevention in adolescents, particularly among obese adolescents with T2DM, or insulin resistance, who are most at risk for developing early cerebrovascular disease.

In this study, obese adolescents showed greater brain atrophy and reductions in hippocampal volumes relative to normal BMI controls. Among the obese group, smaller retinal arteriolar calibers were associated with greater cerebral global atrophy ($p<.05$) and hippocampal atrophy ($p=.054$), independent of MAP, SA, and retinal vessel diameter. The presence of cerebral atrophy among obese adolescents in the absence of overt diabetes implies systemic damage may occur during pre-diabetes.

We acknowledge that obese populations, as well as our obese group, may include "metabolically unhealthy obese" and "metabolically healthy obese" individuals. To describe the sample, we contrasted the lean and obese groups. As metabolic dysregulation is a continuum, and cut scores are somewhat arbitrary, all analyses between metabolic parameters, retinal measurements and brain were performed continuously. Moreover, the definitions of metabolically "healthy" or "unhealthy" among adolescents are not firmly established.

Our group recently published a study observing lower cognitive performance and reductions in brain structural integrity among adolescents with MetS. Interestingly, results indicated the presence of a dose effect, in which adolescents with more abnormal metabolic parameters demonstrated more brain dysfunction. Those findings, in addition to providing some of the rationale for our statistical approach also suggest that even relatively short-term impairments in metabolism may be occurring among adolescents in the absence of overt, clinically manifest vascular disease, and may give rise to brain complications.[7]

Autopsy studies have demonstrated close associations between retinal and cerebral pathology.[26,27] Similarly, our findings suggest brain atrophy and retinal arteriolar narrowing may share pathological mechanisms. These results, if confirmed in larger studies, support the notion that CRAE may serve as a non-invasive proxy for brain health in adolescents.

In healthy children ages 6 to 9, higher BMIs are associated with larger retinal venular caliber, possibly as compensation for increased blood volume in obesity.[28,29] Obese pre-adolescents also display narrowed retinal arteriolar diameters.[22] In this study, we found BMI significantly mediates the relationship between inflammation (logFibrinogen) and retinal venule caliber (CRVE), whereas both BMI and insulin resistance (logHOMA) contribute independently to retinal arteriolar caliber (CRAE). Similarly, we previously published findings in which insulin resistance and cerebral atrophy were associated with poor retinal vessel health, independent of hypertension and age among non-diabetic adults.[15]

Adults and adolescents with IR and T2DM show impairments in endothelial-dependent vasodilatation in peripheral tissues.[30] In childhood obesity, inflammation, oxidative stress, and insulin resistance lead to endothelial dysfunction.[9,31] Similarly, we found inflammation (fibrinogen levels) and insulin resistance (HOMA values) to be associated with CRVE and CRAE, respectively.

We used fibrinogen as our marker of inflammation as it is associated with neuroinflammation.[32] Moreover, our group previously published a study in 2010, "Obesity-mediated inflammation may damage the brain circuit that regulates food intake",[33] in which we describe the potential damage by adiposity-related fibrinogen on the integrity of some of

the brain structures involved in reward and feeding behaviors. We also published a recent paper in which insulin resistance in adolescence was associated with acute phase reactants CRP and fibrinogen without elevations of inflammatory cytokines,[34] suggesting obesity-related inflammation in adolescents may vary from that observed in adults. While the prediction of CVD by fibrinogen has been actively researched, a CVD risk cut-score value for fibrinogen in adolescents has yet to be determined. In the 13-year longitudinal Coronary Artery Risk Development in Young Adults (CARDIA) study, authors observed associations between fibrinogen and traditional risk factors for CVD.[35] The findings supported the use of fibrinogen as a disease marker of CVD risk, rather than a causative factor for CVD. Moreover, the CARDIA study observed strong inverse relationships between modifiable CVD risk factors (i.e. weight loss and smoking cessation) and change in 13-year fibrinogen levels in middle age. In summary, while fibrinogen levels in adolescence may predict CVD in adulthood, adaptation of a healthy lifestyle may attenuate the risk for CVD regardless of high fibrinogen levels in adolescence. In this study, the obese group had significantly higher mean fibrinogen levels compared to the normal BMI group, as supported by the literature. This difference may be indicative of higher risk for future CVD, as well higher disposition for other obesity-related cerebral microvascular damage in the obese group.

This study has some limitations. Retinal photographs were not synchronized to the cardiac cycle, though it is unlikely the data were systematically biased as retinal photographs were likely randomly distributed within the cardiac cycle. In addition, we did not assess intraocular pressure, but no associations between intraocular pressure and retinal vessel diameters have been shown and elevations in intraocular pressure are very rare in adolescence.[36] Although low birth weight and markers of poor early life growth have been associated with narrower retinal arteriolar calibers,[37] we did not have access to these historical data. Furthermore, this is a cross-sectional study and causality between obesity and IR and reductions in CRAE and increased brain atrophy and decreased hippocampal volume cannot be conferred. Lastly, the omission of an overweight group from this study and the use of BMI may present a disadvantage in the detection of insulin resistance among participants with less adiposity. Our group recently published a study in which we observed the best predictor of HOMA-IR was a combination of waist circumference and body fat percentage. Although BMI was clearly also a significant predictor of insulin resistance, it did so less robustly. However, the added discriminatory capacity of waist circumference and percent body fat over BMI was mostly among leaner participants.[38] Because our main goal in this study was to ascertain the associations between retinal and brain measurements and metabolic dysregulation, we chose to use BMI in the model to establish the nature of those associations after accounting for excess weight.

Brain development, while the skull sutures are still open, greatly contributes to the skull size or intracranial vault volume (ICV). It is unlikely that different developmental trajectories could have contributed to our CSF (brain atrophy) findings. For instance, compared to children of normal BMI, obese children are taller, display significantly larger mandibular and maxillary dimensions,[39] and also undergo earlier sexual maturation (among girls).[40] Since the increase in CSF reflects brain loss from where the brain was for maximal skull size, it is unlikely that our increase in CSF, even after adjusting for individual ICV, reflects different developmental trajectories between lean and obese children.

Our study presents a number of strengths. Adolescent participants were carefully matched on age and sex. Our method of measuring CRAE and CRVE is highly reliable. Furthermore, we controlled for vessel diameter, sleep apnea, and mean arterial blood pressure, all of which have been associated with retinal vascular integrity.

Our novel results support the need for aggressive interventions for weight and IR management among obese adolescents. In this novel study, we show retinal vascular abnormalities and increased global and hippocampal atrophy in obese adolescents without diabetes. After confirmation from future larger studies, basic monitoring of retinal vasculature may serve as a proxy for identifying early signs of cerebrovascular disease linked to obesity, insulin resistance, and diabetes in childhood.

In conclusion, this study demonstrates retinal CRAE narrowing and CRVE widening in obese adolescents. In this population, retinal venular caliber increases are associated with higher fibrinogen levels while retinal arteriolar caliber reductions are independently associated with decreased insulin resistance and higher BMI. Most importantly, lower CRAE among obese adolescents is associated with MRI-based cerebral atrophy and reduced hippocampal volumes.

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AT, AC and CL collected data and analyzed retinal and brain images. WT created the image analyses tools and the tools for retinal evaluations. MD analyzed the data and drafted the manuscript. AC and CL conceived the study and AC obtained funds for and directed the study. All authors were involved in writing the paper and had final approval of the submitted and published versions.

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Appendix

Appendix A. Exploratory Analyses for Variables of Interest with CRAE and CRVE.

	CRAE		CRVE	
	r	P	r	P
Age	−0.05	0.60	−0.01	0.95
Etdnicity	−0.02	0.84	−0.03	0.80
Sex	−0.03	0.79	−0.10	0.31
MAP	−0.37	0.00	0.74	0.46
SA	−0.11	0.30	0.05	0.70
LogFibrinogen	−0.15	0.23	0.35	0.00
LogHOMA	−0.43	0.00	0.12	0.22
BMI	−0.37	0.00	0.26	0.01
CRAE*	-	-	0.49	.000
CRVE*	0.49	0.00	-	-

Pearson's bivariate correlations for variables of interest age, etdnicity, sex, mean arterial blood pressure (MAP), sleep apnea, logFibrinogen, logHOMA, and BMI witd CRAE and CRVE.

* CRAE correlated to CRVE as a measure of vessel caliber. Pearson's correlation coefficient (r) and significance (P).

MAP: mean arterial blood pressure; SA: sleep apnea; logFibrinogen: log-transformed Fibrinogen; logHOMA: log-transformed homeostatic model assessment for insulin resistance; BMI: body mass index; CRAE: central retinal vessel equivalent for arterioles; CRVE: central retinal vessel equivalent for venules.

What is already known about this subject:

- Obesity is associated with retinal arteriolar narrowing
- Endothelial dysfunction in childhood obesity may precede cerebrovascular damage and cognitive impairment in adulthood.
- Non-diabetic adolescents with metabolic syndrome demonstrate neurocognitive abnormalities, including reduced hippocampal volumes, increased cerebral brain atrophy, lower cognitive scores, and compromised white matter microstructural integrity compared to adolescents without metabolic syndrome.
- Scalable interventions to prevent future macrovascular damage will require robust, non-invasive proxies of microcirculation that identify individuals at risk.

What this study adds:

- -Retinal arteriolar caliber reductions among non-diabetic obese adolescents are independently associated with increased insulin resistance and higher BMI.
- -Retinal arteriolar narrowing is associated with insulin resistance and obesity in adolescence and it explains reductions in hippocampal volumes and increases in brain atrophy
- -Inflammation as reflected in increased fibrinogen levels is associated with an increase in retinal venules, but not arterioles.

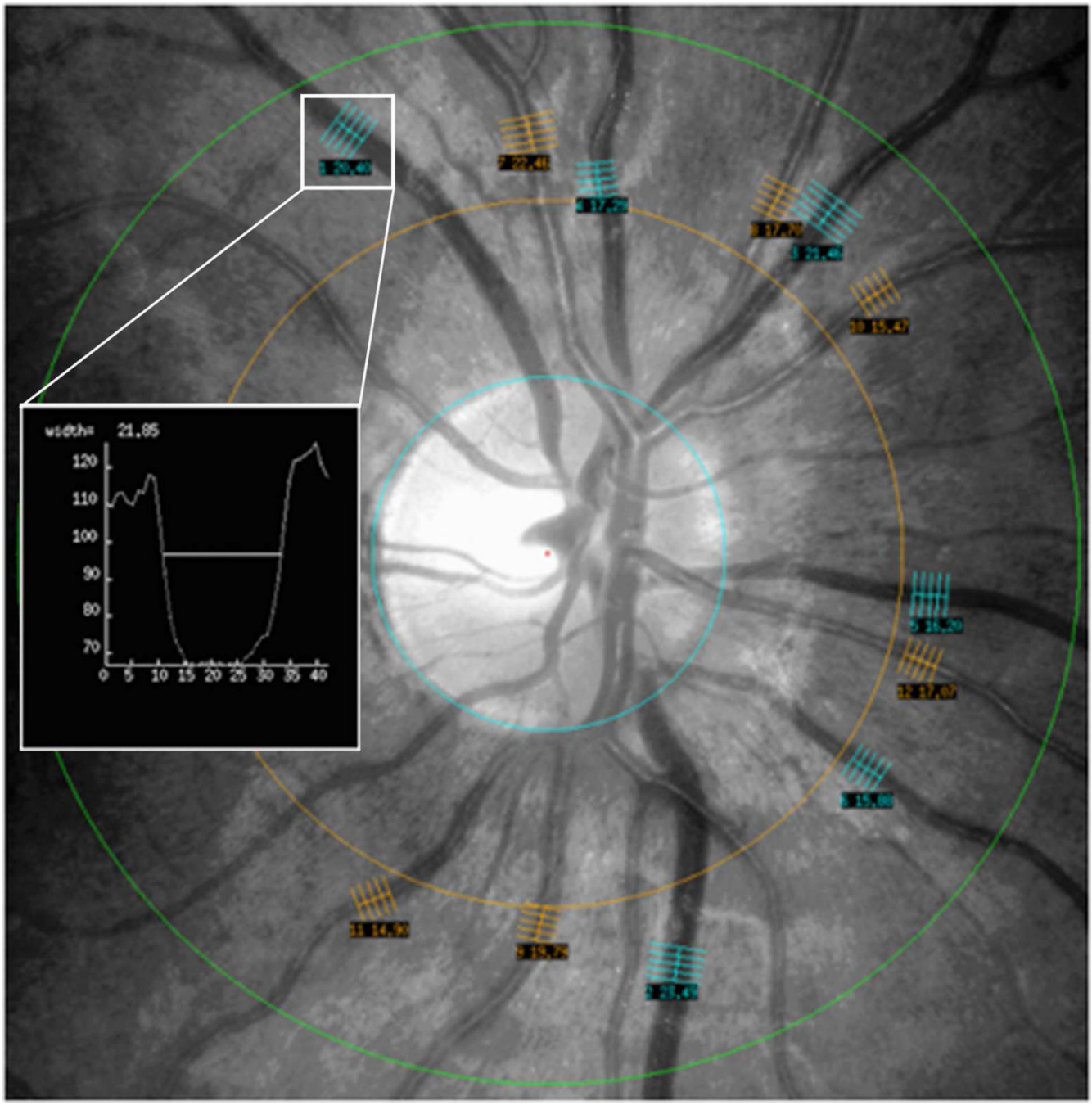

Figure 1.

Digital retinal image with grid defining Zone B : 0.5–1.0 disc diameters from the disc margin. Retinal vessel diameter measurements (6 arterioles and 6 venules) in Zone B and pixel histogram are shown. The average widths of 5 equidistant measures are displayed.

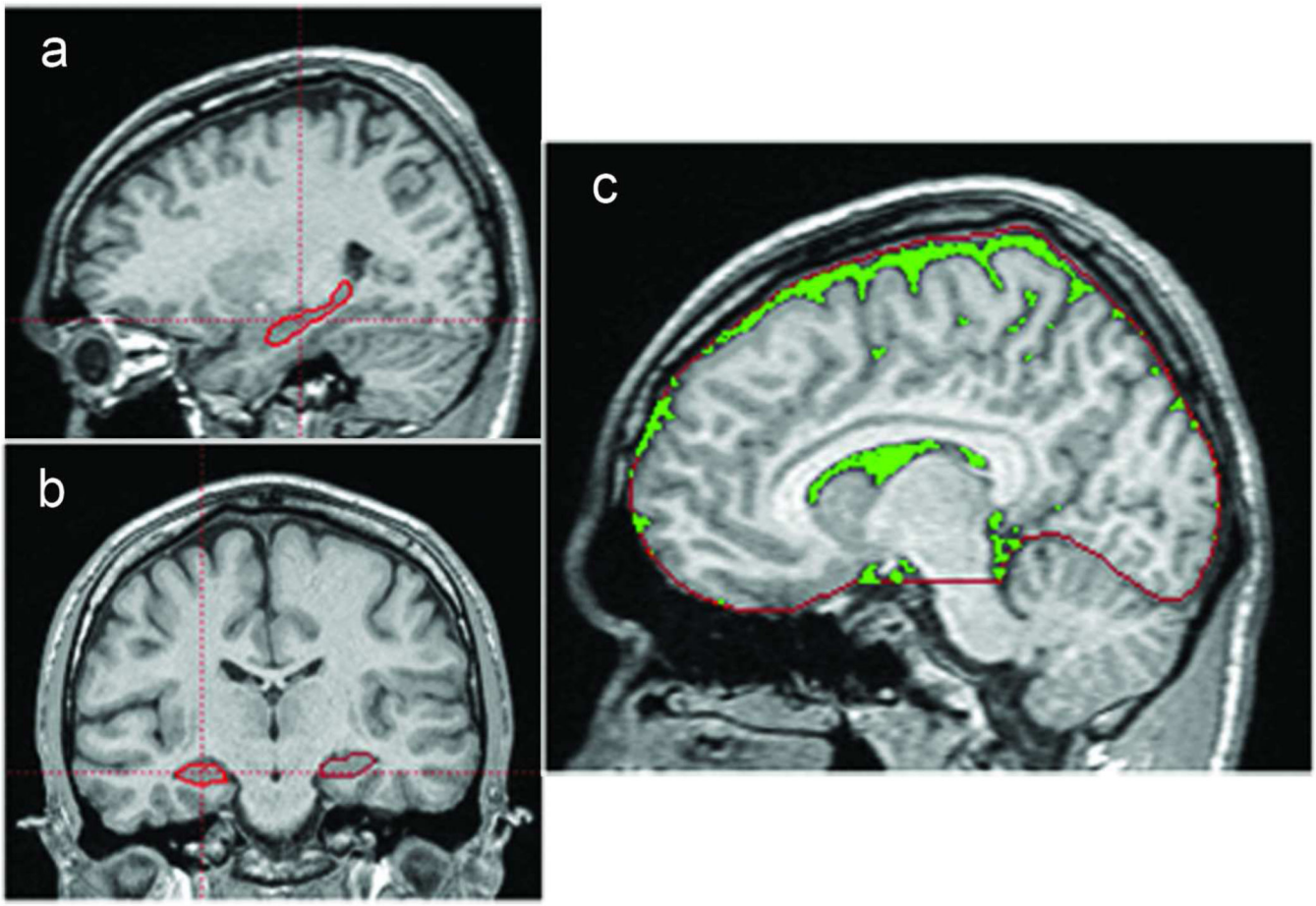

Figure 2.
Hippocampal volumetric measurements from the sagittal (a) and coronal (b) planes. Intracranial vault and overall Global Brain Atrophy (c).

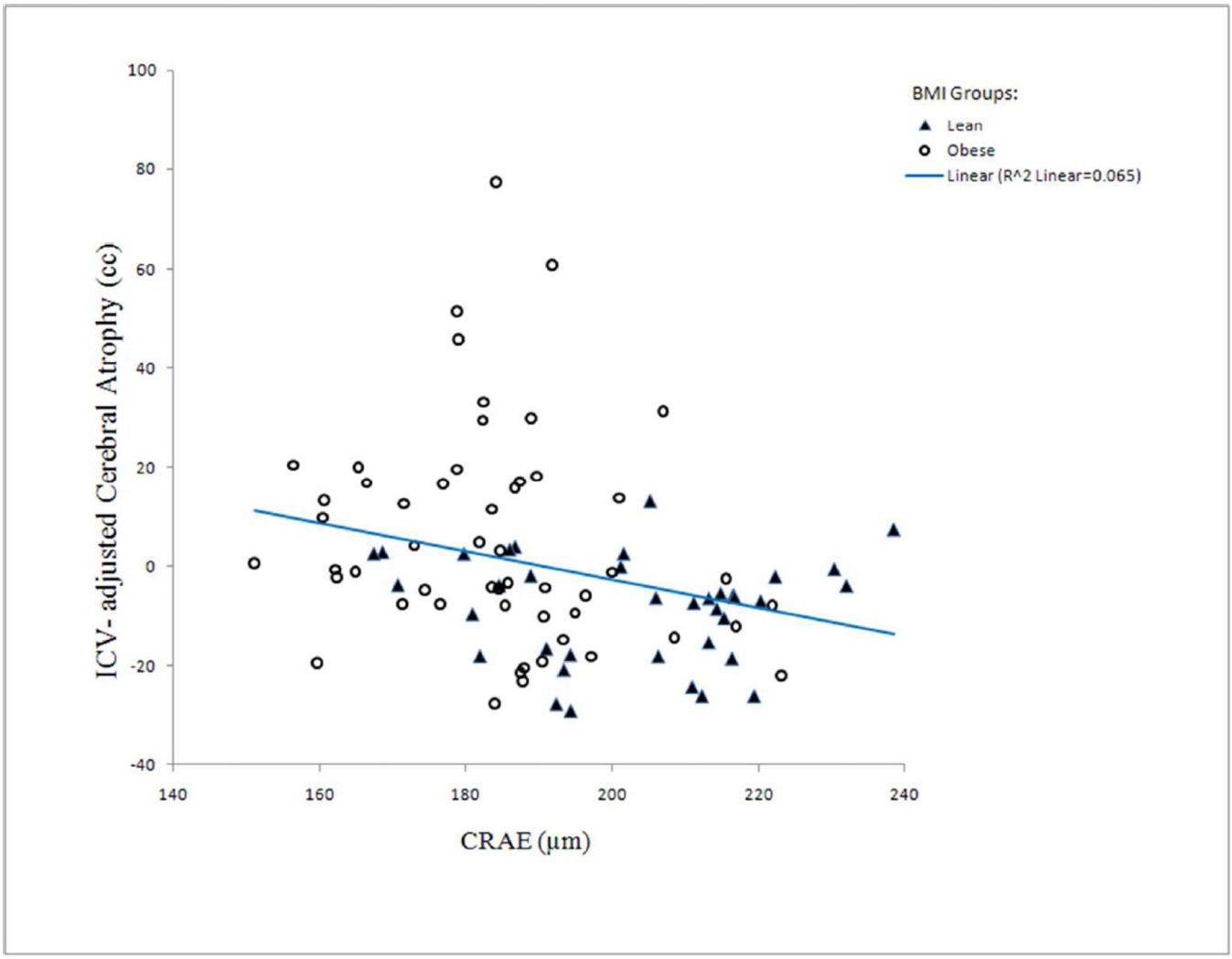

Figure 3.
Scatterplot displaying global atrophy vs. CRAE. Global cerebral atrophy residualized for intracranial vault size (cc). (CRAE: central retinal vessel equivalent for arterioles)

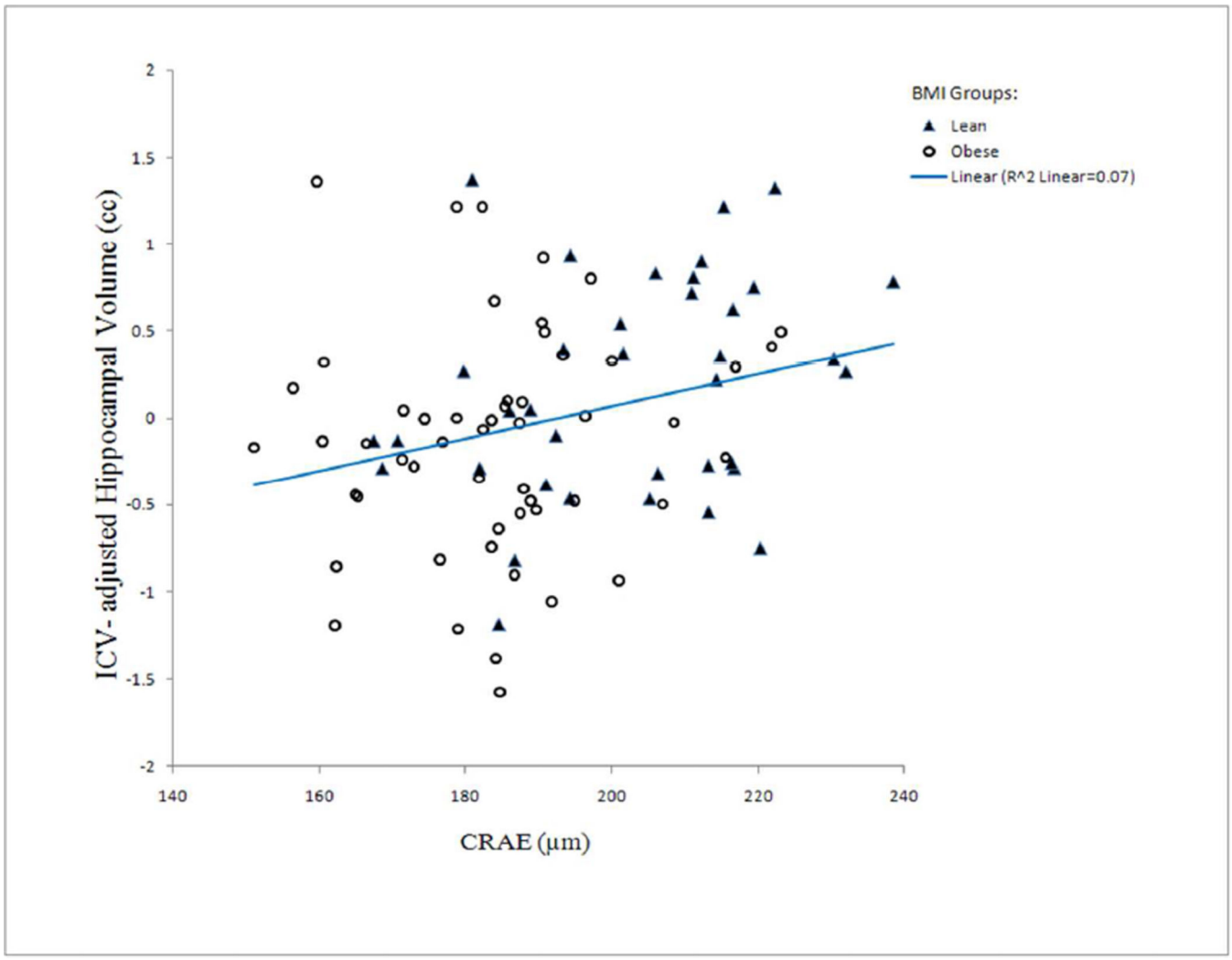

Figure 4.

Scatterplot displaying hippocampal volume vs. CRAE. Hippocampal volume residualized for intracranial vault size (cc). (CRAE: central retinal vessel equivalent for arterioles)

Table 1

Description of the lean and obese groups.

	Lean (n=40)	Obese (n=62)
Age (yr)	17.25 ± 1.56	17.71 ± 1.58
No. of females/males	20/20	41/21
BMI (kg/m^2)*	22.14± 3.1	37.84 ± 6.34
Sleep apnea score	0.17±0.13	0.23±0.15
HOMA-IR*[a]	1.44± 0.71	3.78± 2.25
Glucose (mg/dl)	75.0 ± 6.67	77.53± 8.47
Insulin (μIU/ml)*	7.81 ± 3.68	19.66 ± 11.33
HbA1c (%)*	5.22± 0.3	5.45 ± 0.46
Systolic Blood Pressure (mm Hg)*	103.15 ± 9.72	113.82 ± 12.65
Diastolic Blood Pressure (mm Hg)*	63.10 ± 7.36	69.13 ± 9.79
Mean Blood Pressure (mm Hg)*	76.45 ± 7.04	84.03 ± 9.75
Cholesterol (mg/dL)	154.46 ± 28.42	161.87 ± 22.81
LDL (mg/dL)*	87.85 ± 24.01	100.65± 20.97
HDL (mg/dL)*	52.59± 11.75	43.17 ± 8.52
Fibrinogen (mg/dL)*	282.44 ±39.31	361.24± 89.91
CRP (mg/L)*[b]	0.85 ± 1.53	3.6± 2.5
Triglyceride (mg/dL)*	70.51 ± 28.55	90.2 ± 40.1
AVR*	0.74 ± 0.06	0.63 ± 0.04
CRAE (μm)*	201.96 ±17.97	185.22 ±17.04
CRVE (μm)*	276.67 ±30.47	291.71 ±25.91
Intracranial Vault Size (cc)	1214.92 ±114.94	1193.51 ±128.79
Global Brain Atrophy (cc)*[c]	30.10 ± 10.83	43.98± 22.86
Hippocampal Volume (cc)*[c]	5.84 ± .72	5.49 ± .72

Unless noted, values are expressed as mean ± SD.

*Significant group differences (P<0.05).

[a]Significance based on log-transformed values, mean reported on non-transformed data.

[b]CRP>10 mg/L excluded from analysis.

[c]Significance based on values residualized to intracranial vault size, mean reported on non-residualized data.

(BMI: body mass index; HOMA-IR: homeostatic model assessment for insulin resistance; HbA1C: hemoglobin A1C; LDL: low density lipoprotein; HDL: high density lipoprotein; CRP: c-reactive protein; AVR: arteriole to venule diameter ratio; CRAE: central retinal vessel equivalent for arterioles; CRVE: central retinal vessel equivalent for venules)

Table 2

Association of BMI and Fibrinogen with CRVE, controlling for MAP, SA, age, and vessel diameter (CRAE).

	Step 1(MAP/SA/Age)		Step 2 (CRAE)		Step 3 (logFibrinogen)		Step 4 (BMI)			
	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	R^2	SE
CRVE	.022	.15(−.52,.82) 21.9(−21.4,65.3) .5(−3.36,4.35)	.31[a]	.75(.47, 1.03)	.09[a]	84.2(30.3, 138.1)	.06[b]	.96(.21, 1.71)	.479	18.53
	Step 1(MAP/SA/Age)		**Step 2 (CRAE)**		**Step 3 (BMI)**		**Step 4 (logFibrinogen)**			
	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	R^2	SE
CRVE	.022	.15(−.52,.82) 21.9(−21.4, 65.3) .5(−3.36, 4.35)	.31[a]	.75(.47, 1.03)	.12[a]	1.26(.57, 1.95)	.03	53.1(−3.93, 110.14)	.479	18.53

Steps of the regression are shown separated by the columns. ΔR^2 is the change in R^2, B(95%CI) is the B coefficient and 95% confidence interval ranges, R^2 for total R^2 of the model, and SE is the standard error of the estimate of the final model.

P value for the ΔR^2, significant at [a]$P < 0.01$ [b]$P < 0.05$.

(MAP: mean arterial blood pressure; SA: sleep apnea; BMI: body mass index; logFibrinogen: log-transformed fibrinogen; CRAE: central retinal vessel equivalent for arterioles; CRVE: central retinal vessel equivalent for venules)

Table 3

Association of BMI and HOMA with CRAE, controlling for MAP, SA, age, and vessel diameter (CRVE).

	Step 1(MAP/SA/Age)		Step 2 (CRAE)		Step 3 (logFibrinogen)		Step 4 (BMI)			
	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	R^2	SE
CRVE	.09[b]	−.60(−1.05,−.16) −.9.55(−37.24,18.14) −.52(−3.1,2.06)	.33[a]	.41(.29,.53)	.15[a]	−.26.3(−36.27,−16.31)	.05[a]	−.67(−1.09,−.24)	.607	12.36
	Step 1(MAP/SA/Age)		**Step 2 (CRAE)**		**Step 3 (BMI)**		**Step 4 (logFibrinogen)**			
	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	R^2	SE
CRVE	.09[b]	−.60(−1.05,−.16) 92212;9.55(−37.24,18.14) −.52(−3.1,2.06)	.33[a]	.41(.29,.53)	.15[a]	−1.01(−1.39,−.63)	.04[a]	−17.03(−28.25,−5.81)	.607	12.36

Steps of the regression are shown separated by the columns. ΔR^2 is the change in R^2, B(95%CI) is the B coefficient and 95% confidence interval ranges, R^2 for total R^2 of the model, and SE is the standard error of the estimate of the final model.

P value for the ΔR^2, significant at [a]$P < 0.01$ [b]$P < 0.05$.

(MAP: mean arterial blood pressure; SA: sleep apnea; BMI: body mass index; logHOMA: log-transformed homeostatic model assessment for insulin resistance; CRAE: central retinal vessel equivalent for arterioles; CRVE: central retinal vessel equivalent for venules)

Table 4

Association of CRAE with global cerebral atrophy, hippocampal volume, controlling for MAP, SA, and vessel diameter (CRVE).

	Step 1(MAP/SA/Age)		Step 2 (CRAE)		Step 3 (CRAE)			
	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	ΔR^2	B(95% CI)	R^2	SE
Cerebral Atrophy	.09[a]	.55(.15,.95) 1.97(−23.6,27.6)	.00	−.01(−.15,.12)	.05[b]	−.24(−.48,−.002)	.133	16.09
Hippocampal Volume	.04	−.02 (−.03,.001) .15 (−.87,1.2)	.01	.002(-.003,.01)	.04	.01(0,.02)	.092	.625

Global cerebral atrophy and hippocampal volume are adjusted to intracranial vault size. Steps of the regression are shown separated by the columns. ΔR^2 is the change in R^2, B(95%CI) is the B coefficient and 95% confidence interval ranges, *P* value for the ΔR^2, R^2 for total R2 of the model, and SE is the standard error of the estimate of the final model.

[a]*P* <0.01 [b]*P* <0.05.

(MAP: mean arterial blood pressure; SA: sleep apnea; CRAE: central retinal vessel equivalent for arterioles; CRVE: central retinal vessel equivalent for venules)