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

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Objective: Endothelial dysfunction in childhood obesity may precede cerebrovascular damage and cognitive impairment in adulthood. A noninvasive proxy of microvascular health is required to identify the risk for microvascular damage in obese children.

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

Results: Compared to controls, obese adolescents had smaller retinal arterioles (8.3% decrease, \( P < 0.05 \)) and wider venules (5.4% increase, \( P < 0.01 \)). Larger retinal arteriole diameters were associated with less global cerebral atrophy (\( B = -0.24 \) [95% confidence interval, CI: \(-0.48, -0.002\)]) and larger hippocampal volumes (\( B = 0.01 \) [95% CI: 0, 0.02]). Venule diameters (\( B = 84.2 \) [95% CI: 30.3, 138.1]) were predicted by inflammation (fibrinogen). Arteriolar diameters were predicted by insulin resistance, indicated by logHOMA (homeostatic model assessment, HOMA) values (\( B = -17.03 \) [95% CI: \(-28.25, -5.81\)]) and body mass index (BMI) (\( B = -0.67 \) [95% CI: \(-1.09, -0.24\)]). All analyses were adjusted for mean arterial pressure, sleep apnea, and vessel diameter.

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

Introduction

Within 30 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 gray matter integrity relative to obese controls (7). We previously reported that nondiabetic 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, noninvasive proxies of microcirculation. Obese adolescents with IR demonstrate both micro- and macrovascular 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, IR, inflammation, and hypertension on retinal vessel diameters among healthy weight and obese nondiabetic 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**

**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 of 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 as 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) of ≥30. The group characteristics of participants fulfilling study inclusion criteria are summarized in Table 1.

**Blood tests and insulin resistance assessment**

After a 10-h 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 sample (Instrumentation Laboratory, Beckman Coulter Inc., Fullerton, USA) (11). Fasting glucose and insulin values were used to compute homeostatic model assessment (HOMA)-IR, a validated measure of insulin sensitivity (12).

**BMI, anthropometric measurements, and SA assessment**

To calculate BMI (kg/m²), 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 min 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 nonmydriatic 45 fundus camera (Canon CR4-45NM, Canon EOS Rebel 6.1MPix). Subjects were seated in a dimly lit room for 5-10 min to allow appropriate pupillary dilation. The digital photographs were centered on the optic disk for each eye using standardized settings and were processed as described previously (15). We measured each of the six 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

| TABLE 1 Description of the lean and obese groups |
|------------------------------------------------|
| Lean (n = 40) | Obese (n = 62) |
|----------------|----------------|
| Age (years) | 17.25 ± 1.56 | 17.71 ± 1.58 |
| No. of females/males | 20/20 | 41/21 |
| BMI (kg/m²)* | 22.14 ± 3.1 | 37.84 ± 6.34 |
| SA score | 0.17 ± 0.13 | 0.23 ± 0.15 |
| HOMA-IR* | 1.44 ± 0.71 | 3.78 ± 2.25 |
| Glucose (mg/dl) | 75.0 ± 6.67 | 77.53 ± 8.47 |
| Insulin (μU/mL)* | 7.81 ± 3.68 | 19.66 ± 11.33 |
| HbA1c (%)* | 5.22 ± 0.3 | 5.45 ± 0.46 |
| Systolic BP (mm Hg)* | 103.15 ± 9.72 | 113.82 ± 12.65 |
| DBP (mm Hg)* | 63.10 ± 7.36 | 69.13 ± 9.79 |
| Mean BP (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)* | 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) | 1,214.92 ± 114.94 | 1,193.51 ± 128.79 |
| Global brain atrophy (cc)** | 30.10 ± 10.83 | 43.98 ± 22.86 |
| Hippocampal volume (cc)** | 5.84 ± 0.72 | 5.49 ± 0.72 |

Unless noted, values are expressed as mean ± SD. *Significant group differences (P < 0.05). 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.

**Significance based on log-transformed values, mean reported on nontransformed data.**

**Significance based on the values residualized to intracranial vault size, mean reported on nonresidualized data.**

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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 was then used in our analyses. Our method is highly reproducible, with an inter-rater 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. To estimate the absolute size of the vessels and to account for refractive errors, we measured the disk diameter and adjusted vessel measurements assuming a standardized disk 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 the measurement of global atrophy and to exclude gross pathology. MPRAGE sequence parameters were as follows: TR, 1,300 ms; TE, 4.38 ms; TI, 800 ms; flip angle, 15°; acquisition matrix, 256 x 256; field of view (FOV), 256 mm; NEX, 1; 192 slices, and slice thickness, 1.0 mm. FLAIR sequence parameters were as follows: TR, 9,000 ms; TE, 97 ms; TI, 2,500 ms; acquisition matrix, 256 x 256; FOV, 210 mm; 50 slices; slice thickness, 3 mm; no gaps; NEX, 1; flip angle, 180°.

The MPRAGE study procedure accounted for individual variability in brain size by measuring intracranial 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 as 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 >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 0.05. 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) was supported by the literature and/or (2) survived exploratory stepwise regression analyses in our data set. 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 (for the results of exploratory analyses, see Appendix A). Although SA was not significant in our exploratory analyses, we included it in the final model based on the 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 first step, vessel diameter (estimated by CRAE) in the second step, logFibrinogen in the third step, and BMI in the fourth and last step. To predict CRAE, we adjusted for MAP and SA in the first step, vessel diameter (estimated by CRVE) in the second step, logHOMA in the third step, and BMI in the last step. To determine the contribution of BMI to vessel caliber (CRAE or CRVE) independent of inflammation (logFibrinogen) and IR (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 first step, vessel diameter (CRAE when the predictor of interest was CRVE, and vice versa) in the second step, and retinal vessel caliber (CRVE or CRAE) in the third step.

Data were analyzed using SPSS for Windows, version 17.0 (SPSS Inc., Chicago, Illinois, USA).
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) |
|----------------------|----------------------|---------------|------------------------|-------------|
|                      | \(\Delta R^2\) | \(B(95\%)\) CI | \(\Delta R^2\) | \(B(95\%)\) CI | \(\Delta R^2\) | \(B(95\%)\) CI | \(R^2\) | SE  |
| CRVE                 | 0.022               | 0.15 (−0.52, 0.82) | 0.31<sup>a</sup> | 0.75 (0.47, 1.03) | 0.099<sup>b</sup> | 84.2 (30.3, 138.1) | 0.06<sup>b</sup> | 0.96 (0.21, 1.71) | 0.479 | 18.53 |
|                      |                     |               |                        |             |                     |                  |        |     |

Steps of the regression are shown separated by the columns. \(\Delta R^2\) 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.

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.
<sup>a</sup>P value for the \(\Delta R^2\), significant at \(P < 0.01\).
<sup>b</sup>P value for the \(\Delta R^2\), significant at \(P < 0.05\).

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 the previous studies, we included it in Table 1 as a point of reference. Groups differed significantly by BMI (kg/m<sup>2</sup>), BP (systolic, diastolic and MAP, mm Hg), low-density lipoprotein (LDL), glucose (mg/dl), CRP (mg/l), and triglycerides (mg/dl) \((P < 0.05)\). Obese adolescents had greater global cerebral atrophy (cc) and smaller hippocampal volumes (cc) \((P < 0.05)\). Table 1 summarizes the differences between the normal BMI and the obese group. Obese adolescents also had significantly lower CRAE (185.22±17.04 μm), and higher CRVE (291.71±25.91 μm) than normal BMI controls \((P < 0.05)\). 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 % \((P < 0.05)\).

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 (CRVE) | Step 3 (logHOMA) | Step 4 (BMI) |
|----------------------|----------------------|---------------|-----------------|-------------|
|                      | \(\Delta R^2\) | \(B(95\%)\) CI | \(\Delta R^2\) | \(B(95\%)\) CI | \(\Delta R^2\) | \(B(95\%)\) CI | \(\Delta R^2\) | \(B(95\%)\) CI | \(R^2\) | SE  |
| CRAE                 | 0.09<sup>b</sup> | −0.60 (−1.05, −0.16) | 0.33<sup>a</sup> | 0.41 (0.29, 0.53) | 0.15<sup>a</sup> | −26.3 (−36.27, −16.31) | 0.05<sup>a</sup> | −0.67 (−1.09, −0.24) | 0.607 | 12.36 |

Steps of the regression are shown separated by the columns. \(\Delta R^2\) 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.

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.
<sup>a</sup>P value for the \(\Delta R^2\), significant at \(P < 0.01\).
<sup>b</sup>P value for the \(\Delta R^2\), significant at \(P < 0.05\).
Factors in the final regression models were based on the 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 (first step), and CRAE (second step), logFibrinogen (third step) explained 9% of the variance \( B = 84.2 \) [95% CI: 30.3, 138.1], \( P < 0.01 \). Furthermore, BMI (fourth step) explained an additional 6% of variance in CRVE. When we reversed the last two steps of this regression analysis, BMI (third step) explained 12% of the variance in CRVE \( B = 1.26 \) [95% CI: 0.57, 1.95], \( P < 0.01 \), but logFibrinogen (fourth step) did not explain any additional variance.

In the prediction of CRAE, after controlling for MAP and SA (first step), CRVE (second step), logHOMA (third step) uniquely explained 15.0% of the variance \( B = -26.3 \) [95% CI: –36.27, –16.31], \( P < 0.01 \). In addition, BMI (fourth step) explained an additional 5% of the variance in CRAE \( B = -0.67 \) [95% CI: –1.09, –0.24], \( P < 0.01 \). Reversing the last two steps of the hierarchal regression analysis, BMI (third step) explained 15% \( B = -1.01 \) [95% CI: –1.39, –0.63], \( P < 0.01 \), and logHOMA (fourth step) explained an additional 4% of the variance in CRAE \( B = -17.03 \) [95% CI: –28.25, –5.81], \( P < 0.01 \).

In parallel analyses, predicting ICV-adjusted global cerebral atrophy, we controlled for MAP and SA (first step), CRVE (second step), and CRAE (third step): CRAE significantly accounted for 5% of variance in global cerebral atrophy \( B = -0.24 \) [95% CI: –0.48, –0.002], \( P < 0.05 \) (Table 4). With ICV-adjusted hippocampal volume as the dependent variable, and after adjusting for the same potential confounders, CRAE showed a statistical trend \( \Delta R^2 = 0.04, B = 0.01 \) [95% CI: 0, 0.02], \( P = 0.05 \). Scatter plots of CRAE versus 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 more than 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

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**TABLE 4 Association of CRAE with global cerebral atrophy, hippocampal volume, controlling for MAP, SA, and vessel diameter (CRVE)**

|                      | Step 1 (MAP/SA) |   |   |   |   |   |   |   |   |   |   |
|----------------------|-----------------|-----|---|---|---|-----|---|---|---|---|---|
|                      | \( \Delta R^2 \) | \( B(95\% \text{ CI}) \) | \( \Delta R^2 \) | \( B(95\% \text{ CI}) \) | \( \Delta R^2 \) | \( B(95\% \text{ CI}) \) | \( R^2 \) | SE |
|----------------------|-----------------|-----|---|---|---|-----|---|---|---|---|---|
| Cerebral Atrophy     | 0.09*           | 0.55(0.15, 0.95) | 1.97(–23.6, 27.6) | 0.00 | –0.01(–0.15, 0.12) | 0.05* | –0.24(–0.48, –0.002) | 0.133 | 16.09 |
|                      |                 | 1.01(0.06, 2.48) | 0.15(–0.87, 1.2)  | 0.01 | 0.002(–0.003, 0.01) | 0.04  | 0.01(0, 0.02)   | 0.092 | 0.625 |

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

MAP, mean arterial blood pressure; SA, sleep apnea; CRAE, central retinal vessel equivalent for arterioles; CRVE, central retinal vessel equivalent for venules.

*\(^*P < 0.01.

\(^{\text{b}}P < 0.05.

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**FIGURE 3** Scatter plot showing global atrophy versus CRAE. Global cerebral atrophy residualized for ICV size (cc). CRAE: central retinal vessel equivalent for arterioles.
Brain infarcts and white matter lesions more than 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 IR 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 IR, 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 ($P < 0.05$) and hippocampal atrophy ($P = 0.054$), 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 prediabetes.

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 among 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 noninvasive proxy for brain health in adolescents.

In healthy children of 6-9 years, higher BMIs are associated with larger retinal venular caliber, possibly as compensation for increased blood volume in obesity (28,29). Obese preadolescents 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 IR (logHOMA) contribute independently to retinal arteriolar caliber (CRAE). Similarly, we previously published the findings in which IR and cerebral atrophy were associated with poor retinal vessel health, independent of hypertension and age among nondiabetic adults (15).

Adults and adolescents with IR and T2DM show impairments in endothelial-dependent vasodilation in peripheral tissues (30). In childhood obesity, inflammation, oxidative stress, and IR lead to endothelial dysfunction (9,31). Similarly, we found inflammation (fibrinogen levels) and IR (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 article in which IR 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. Although 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, although 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 this 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. Finally, the omission of an overweight group from this study and the use of BMI may present a disadvantage in the detection of IR 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 also clearly a significant predictor of IR, 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). As 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, although the skull sutures are still open, greatly contributes to the skull size or ICV volume. 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). As 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, SA, and MAP, 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, IR, and diabetes in childhood.

Conclusions

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, whereas retinal arteriolar caliber reductions are independently associated with decreased IR and higher BMI. Most importantly, lower CRAE among obese adolescents is associated with MRI-based cerebral atrophy and reduced hippocampal volumes.

APPENDIX A

Exploratory analyses for variables of interest with CRAE and CRVE

| CRAE | CRVE |
|------|------|
|      |      |
| Age  | −0.05| 0.60| −0.01| 0.95 |
| Ethnicity | −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| 0.00 |
| CRVE* | 0.49| 0.00| − | − |

Pearson’s bivariate correlations for variables of interest age, ethnicity, sex, mean arterial blood pressure (MAP), sleep apnea, log-Fibrinogen, logHOMA, and BMI with 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.

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