Nonalcoholic Fatty Liver Disease and Measures of Early Brain Health in Middle-Aged Adults: The CARDIA Study

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**Objective:** To assess associations between nonalcoholic fatty liver disease (NAFLD) and measures of brain health in a population-based sample of adults.

**Methods:** Participants from the CARDIA study (Y25 exam; age 43-55 years) with concurrent computed tomography quantification of liver fat, visceral adipose tissue (VAT), and brain magnetic resonance (MR) images were included (n = 505). NAFLD was identified after exclusion of other causes of liver fat. Total tissue volume (TTV) and gray matter cerebral blood flow (GM-CBF) were estimated using 3T brain MR images.

**Results:** NAFLD prevalence was 18%. NAFLD was associated with lower TTV and GM-CBF after adjusting for intracranial volume, demographics, and health behaviors (P < 0.04 for all). In models with additional adjustment for cardiovascular risk factors, the association of NAFLD with GM-CBF remained significant (P = 0.04) but was attenuated after adjustment for VAT (P = 0.06) and eliminated with BMI (P = 0.20). NAFLD was not associated with TTV after adjustment for cardiovascular risk factors (P = 0.10) or additional adjustment for VAT (P = 0.14) or BMI (P = 0.05).

**Conclusions:** NAFLD is negatively associated with early brain health as assessed by MR measures of structure (TTV) and perfusion (GM-CBF). BMI and VAT attenuated this relationship, providing insight into the potential metabolic role of liver fat in brain health and disease.

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**Author Contributions:** Drs. VanWagner and Terry developed the proposal and analytic plan. Dr. Launer designed the brain MR protocol. Dr. Carr designed the abdominal CT protocol. Drs. Terry and Kang analyzed data. Dr. VanWagner drafted the manuscript. All authors were involved in interpretation of results and manuscript revision and had final approval of the submitted and published versions.

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Cognitive decline with age is common and may be caused by a variety of conditions, including Alzheimer’s disease and vascular dementia. A growing body of literature now recognizes that deterioration in vascular function over time is a major contributor to the process of cognitive aging. Liver disease, particularly NAFLD, may be involved in the process of cognitive aging through several potential mechanisms (Figure 1). First, persons with NAFLD have a high prevalence of individual vascular risk factors (e.g., hypertension) that contribute to the progression of cognitive aging. Additional risk factors that may accelerate vascular aging include microvascular endothelial dysfunction in concert with high levels of liver-derived gamma glutamyltransferase and insulin-like growth factor-1 (IGF-1) (14). Second, the concurrent presence of chronic systemic inflammation and metabolic syndrome, which are highly prevalent in NAFLD, has been shown to contribute to cognitive impairment in older persons (15). Finally, obesity, in particular visceral adiposity, is related to neurodegenerative, vascular, and metabolic processes that affect brain structures underlying cognitive function (16,17). Despite these associations, there is little evidence for whether the presence of NAFLD per se is associated with markers of early brain health independent of visceral adiposity and metabolic risk factors.

Our central hypothesis is that prevalent NAFLD is a marker for underlying pathologic processes that lead to vascular cognitive aging independent of general measures of adiposity and cardiovascular risk factors. Thus, the objective of the current study was to quantify associations between the presence of NAFLD and global imaging measures of brain structure and physiology. We further aimed to investigate whether obesity and other components of the metabolic syndrome attenuate this association.

Methods

Study population

Participants were enrolled in the Coronary Artery Risk Development in Young Adults (CARDIA) study, a longitudinal study to investigate the determinants and development of CVD in young adults. Details of the recruitment of the study sample have been previously described (18). Of the 5,115 adults enrolled in CARDIA, 3,498 (72% of survivors) were evaluated at the 25-year (Y25) follow-up exam (2010-2011), when a subsample participated in the CARDIA Brain Magnetic Resonance Imaging (MRI) substudy. The sample was enrolled at the time Y25 appointments were made, with the aim of achieving a balance within four strata of ethnicity/race (black, white) and sex from three of the CARDIA field centers: Birmingham, AL, Minneapolis, MN, and Oakland, CA. The CARDIA Brain MRI substudy was designed to investigate the morphology, pathology, physiology, and function of the brain with MRI technology. Exclusion criteria were a contraindication to MRI or a body size that was too large for the MRI scanner. Of those who were eligible for the substudy, our target was scans in 700 individuals; we obtained brain MRI scans of 719 individuals. The present study includes participants from the CARDIA Brain MRI substudy who also underwent concurrent computed tomography (CT) scanning of the abdomen as part of the Y25 core examination. Participants were excluded from the CT exam if they were pregnant or were unable to fit within the CT gantry.

Of 679 participants with available brain MRI and abdominal CT images, we further excluded participants with a medically verified history of stroke (n = 4), a self-reported history of hepatitis C or cirrhosis (n = 21), a risk factor for chronic liver disease, or with a potential cause of secondary hepatic steatosis: alcohol consumption ≥ 20 g/d in women and ≥ 30 g/d in men (1) (n = 108), self-reported human immunodeficiency virus (n = 6), prior intravenous drug use (n = 28), and medications known to cause hepatic steatosis (e.g., valproic acid, methotrexate, tamoxifen, or amiodarone; n = 7). The remaining 505 participants formed the sample population (Figure 2).

All participants provided written informed consent at each exam, including separate participant consent for the CARDIA Brain MRI substudy. Institutional review boards from each field center, the coordinating center, and the Intramural Research Program at the National Institute on Aging annually approved the CARDIA study.

Measurements

Standardized protocols for data collection and quality control were used across study centers and have been described in detail previously (18). Obesity was defined as body mass index (BMI) ≥ 30 kg/m². Metabolic syndrome was defined according to Adult Treatment Panel III criteria (19). To quantify physical activity (reported as exercise units), the CARDIA physical activity history questionnaire was used, which was an interviewer-based self-report of duration and intensity of participation in 13 categories of exercise over the previous 12 months (20). As a reference, 300 exercise units approximates 150 minutes of moderate-intensity activity per week (20).

Brain MRI protocol

Brain MRI were acquired on 3T MR scanners (Oakland: Siemens 3T Tim® MAGNETOM Trio™/VB 15 platform; Minneapolis: Siemens 3T Tim® MAGNETOM TrioTM/VB 15 platform; and Birmingham: Philips 3T Achieva/2.6.3.6 platform). Details of the training of MRI technologists at the different sites, implementation of study protocols, and quality assurance of scanner stability and performance have been previously published (21). Image processing was performed at a centralized reading center (University of Pennsylvania, Philadelphia, PA). A quality control protocol was employed prior to scan processing through an automated pipeline (21).

Structural MR brain images were processed using previously described methods (22-24). An automated multispectral computer algorithm classified total tissue volume into gray matter (GM), white matter (WM), and cerebral spinal fluid. The GM of the brain contains the cell bodies, dendrites, and axon terminals of all the neurons in the brain, whereas the WM contains all the connecting tracts between the various GM regions of the brain. Total, GM, and WM tissue volumes were further characterized as normal (NTV) and abnormal (ATV) tissue volumes and then into specific regions of interest (98 in the normal tissue and 94 in the abnormal tissue). Abnormal WM includes tissue damage due to ischemia, demyelination, and inflammation. Abnormal GM includes infarcted cortical tissue. NTV was adjusted for intracranial volume (ICV; total brain volume [TBV] plus cerebrospinal fluid).

The imaging protocol also included an axial pseudo-continuous arterial spin labeling (pCASL), which measures cerebral blood flow...
(CBF) (25). CBF is the volume of blood traversing a brain region per unit of time (mL/100 g/min). The mean perfusion volume from the pCASL was quantified into CBF units using the model and software described in Wang et al. (26), resulting in a CBF map. Due to technical difficulties, the CBF measures from the Birmingham site were not included in this analysis (n = 131). Here we present the estimate for the GM-CBF, as it is more reliably obtained than measures in WM. The technical error of measurement, an accuracy index that reflects measurement quality of both acquisition and processing of scans, was estimated from scans of three persons measured three times in the three centers; results were 1.2% for TBV, 27.8% for abnormal WM, and 7.3% for GM-CBF.

CT imaging protocol
The CT protocol included the heart and abdomen, using a non-contrast CT scan performed using GE (GE 750HD 64 and GE LightSpeed VCT 64, Birmingham and Oakland, respectively; GE Healthcare, Waukesha, WI) or Siemens (Sensation 64, Minneapolis; Siemens Medical Solutions, Erlangen, Germany) multi-detector CT scanners, and has been described previously (7). Quality control and image analysis was performed at a core reading center (Wake Forest University Health Sciences, Winston-Salem, NC).

NAFLD was defined as liver attenuation (LA) < 51 Hounsfield units (HU, equivalent to a liver/spleen ratio < 1.0) after exclusion of other causes of liver fat (Figure 2) (7,8). Measurement of LA was performed in the right lobe of the liver using CT slices through the upper abdomen and was reported as the average of nine measurements on three slices using circular regions of interest of 2.6 cm². The interclass correlation coefficient between different readers on a randomly selected sample of 156 participants was 0.975 for LA, indicating high reproducibility of CT-measured LA in this study. The methods for assessment of adiposity within CARDIA have also been described previously (7).

Statistical analysis
Participant characteristics were described using mean and proportion as appropriate. Tests for differences by NAFLD status included t test and Wilcoxon rank sum test for continuous variables and χ² test for categorical variables. Linear regression models were used to quantify cross-sectional associations between the exposure (continuous CT LA or NAFLD) and the outcome variables (brain MRI measures). Covariates in the multivariable model were chosen a priori for clinical importance. Potential confounders included age, race, sex, study center, socioeconomic level, cardiovascular risk factors (e.g., smoking status, physical activity score, diabetes status, systolic
blood pressure, high-sensitivity C-reactive protein [hsCRP], cholesterol, antihypertensive medication use), and additional NAFLD risk factors (e.g., alcohol intake and high-density lipoprotein [HDL] cholesterol). Spearman correlation coefficients were computed between obesity measures and LA and between obesity and brain MRI measures. We verified the model assumptions of linearity, normality of residuals, homoscedasticity, and absence of collinearity. In addition, the variance inflation factors were < 2 for models including LA and

### TABLE 1
Demographic, behavioral, and clinical participant characteristics by NAFLD status in the CARDIA Brain MRI substudy, year 25 exam (2010-2011)

| Overall sample (n = 505) | No NAFLD (n = 413) | NAFLD (n = 92) | P value* |
|--------------------------|-------------------|--------------|---------|
| Age, y                   | 50.1 (3.6)        | 50.0 (3.6)   | 50.7 (3.6) | 0.13    |
| Female, %                | 55.8%             | 57.9%        | 46.7%    | 0.05    |
| White, %                 | 55.2%             | 55.4%        | 54.3%    | 0.85    |
| Education, y             | 15.5(2.4)         | 15.5(2.4)    | 15.5(2.4)| 0.84    |
| Physical activity, exercise units/wk | 285 (142.0, 510.0) | 297.0 (144.0, 537.5) | 237.5 (97.5, 410.5) | 0.02    |
| Smoking, %               |                   |              |          | 0.79    |
| Never                    | 64.5%             | 64.7%        | 63.3%    |         |
| Past                     | 21.9%             | 21.4%        | 24.4%    |         |
| Current                  | 13.6%             | 13.9%        | 12.2%    |         |
| Alcohol drinkers, %      | 74.0%             | 74.0%        | 73.9%    | 0.98    |
| Alcohol use, mL/d        | 0 (0, 9.2)        | 2.4 (0, 9.7) | 0.0 (0, 7.4) | 0.03    |
| Anthropometric measures  |                   |              |          |         |
| BMI, kg/m²               | 29.1(5.9)         | 28.1 (5.4)   | 33.6 (5.5) | <0.0001 |
| Waist circumference, mm  | 91.9(13.6)        | 88.9 (12.1)  | 105.5 (11.6) | <0.0001 |
| CT fat measures          |                   |              |          |         |
| Total abdominal fat, cm³ | 458.5 (196.2)     | 422.1 (182.6) | 623.3 (170.3) | <0.0001 |
| Subcutaneous fat, cm³    | 319.3 (154.4)     | 298.8 (150.2) | 412.1 (139.0) | <0.0001 |
| Visceral fat, cm³        | 122.4 (67.3)      | 108.2 (58.7) | 186.6 (67.0) | <0.0001 |
| Liver attenuation, HU    | 56.9 (10.1)       | 60.4 (6.1)   | 40.8 (8.9) | <0.0001 |
| Comorbidities, %         |                   |              |          |         |
| Obesity                  | 37.6%             | 29.1%        | 76.1%    | <0.0001 |
| Diabetes                 | 9.7%              | 6.3%         | 25.0%    | <0.0001 |
| Hypertension             | 28.5%             | 25.7%        | 41.3%    | 0.003   |
| Metabolic syndromea     | 15.2%             | 10.4%        | 37.0%    | <0.0001 |
| Diastolic BP, mm Hg      | 73.6 (11.0)       | 72.7 (10.9)  | 77.6 (10.8) | 0.0001  |
| Systolic BP, mm Hg       | 118.0 (14.7)      | 117.1 (14.2) | 121.8 (16.1) | 0.01    |
| HTN treatment, %         | 23.0%             | 21.1%        | 31.5%    | 0.03    |
| Metabolic variables      |                   |              |          |         |
| Total cholesterol, mg/dL | 193.2 (36.5)      | 194.5 (36.1) | 187.4 (37.5) | 0.10    |
| Triglycerides, mg/dL     | 89.0 (65.0, 129.0)| 85.0 (62.0, 123.0)| 117.5 (84.5, 166.0) | <0.0001 |
| LDL cholesterol, mg/dL   | 114.0 (32.8)      | 114.9 (33.2) | 110.2 (31.0) | 0.21    |
| HDL cholesterol, mg/dL   | 57.5 (16.9)       | 59.4 (17.2)  | 48.7 (12.4) | <0.0001 |
| High TG/low HDL, %       | 32.1%             | 27.7%        | 52.2%    | <0.0001 |
| Cholesterol treatment, % | 13.1%             | 11.9%        | 18.5%    | 0.09    |
| Fasting glucoseb, mg/dL  | 91.3 (8.9)        | 90.7 (8.7)   | 94.8 (9.0) | 0.005   |
| Fasting insulinb, mg/dL  | 9.3 (6.8)         | 8.1 (5.6)    | 15.7 (9.1) | <0.0001 |
| Hba1c, %                 | 5.4 (0.4)         | 5.4 (0.4)    | 5.5 (0.3) | 0.016   |
| HOMA-IR scoreb, mean     | 2.2 (1.0, 2.8)    | 1.9 (1.0, 2.4)| 3.8 (2.2, 4.3) | <0.0001 |
| hsCRP, mg/dL             | 1.2 (0.6, 2.7)    | 1.0 (0.5, 2.2)| 2.6 (1.3, 4.7) | <0.0001 |

NAFLD = liver attenuation < 51 Hounsfield units (HU) after exclusion for secondary causes of liver fat (alcohol/medications).

*Results are expressed as mean (standard deviation), median (25th, 75th percentile), or %; t test or Wilcoxon rank sum for continuous variables, chi-square test for categorical variables for the difference between NAFLD and no NAFLD.

aDefined using the National Cholesterol Education Program’s Adult Treatment Panel III report (ATP III) criteria.

bParticipants with a diagnosis of diabetes or those on diabetes medications were excluded from analyses for glucose, insulin, HbA1c, and HOMA-IR score.

NAFLD, nonalcoholic fatty liver disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; BMI, body mass index; CT, computed tomography; BP, blood pressure; HTN, hypertension; HOMA-IR, homeostatic model assessment of insulin resistance.
Results
Clinical, fat distribution, and metabolic characteristics
Table 1 compares the clinical characteristics of NAFLD participants to those without NAFLD. NAFLD prevalence was 18.2%. Mean age across the population was 30.1 ± 3.6 years, 55.8% female, and 55.2% white. NAFLD participants had higher BMI (33.6 vs. 28.1 kg/m²), waist circumference (105.5 vs. 88.9 mm), and obesity prevalence (76.1% vs. 29.1%) than non-NAFLD (P < 0.0001 for all). NAFLD participants were also more likely to have dyslipidemia (e.g., high triglycerides, low HDL), hypertension, and diabetes mellitus, and 37.0% met criteria for metabolic syndrome (P < 0.001 for all). When compared to participants without NAFLD, participants with NAFLD exhibited higher levels of hsCRP, fasting insulin, glucose, and insulin resistance as defined by the homeostatic model assessment of insulin resistance (HOMA-IR). Notably, there was no significant difference in low-density lipoprotein, total cholesterol, or prevalence of cholesterol treatment use between NAFLD groups. Total physical activity level and median alcohol use were lower among participants with NAFLD. Finally, NAFLD participants had higher volumes of CT-defined abdominal, subcutaneous, and visceral fat and higher levels of liver fat indicated by lower levels of mean LA (P < 0.0001).

Association of NAFLD with brain MRI measures of structure and physiology: univariate analyses
No significant differences in NTV or ATV were observed between participants with and without NAFLD (Table 2). Utilizing quartiles of total-CBF, we assessed differences in the severity of subclinical changes in brain perfusion (Figure 3). NAFLD participants were more likely to have significantly lower levels of total-CBF than those without NAFLD (P = 0.004).

Multivariable analyses
In multivariable linear regression analyses, lower LA (e.g., higher liver fat) was associated with lower total tissue volume and total-NTV and decreased total-CBF and GM-CBF after adjustment for ICV or total tissue volume, demographics, and health behaviors (Table 3). Lower LA remained associated with decreased total-CBF and GM-CBF after adjustment for CVD risk factors. After additional adjustment for VAT, the association between LA and GM-CBF remained significant (P < 0.04). Additional adjustment for SAT further attenuated associations between LA and measures of brain perfusion.

Table 2: Brain MRI measures of structure and physiology by NAFLD status in the CARDIA Brain MRI substudy, year 25 exam (2010-2011)

| Measures of brain structure | No NAFLD (n = 413) | NAFLD (n = 92) | P valuea |
|-----------------------------|------------------|----------------|--------|
| Total tissue volume, cm³    | 979.1 (1.4)      | 973.4 (2.9)    | 0.08   |
| Normal tissue volume (NTV), cm³ | 978.4 (1.4)     | 972.9 (2.9)    | 0.09   |
| Total-NTV                   | 514.8 (1.1)      | 513.9 (2.3)    | 0.73   |
| WM-NTV                      | 463.6 (1.2)      | 458.9 (2.5)    | 0.09   |
| Abnormal tissue volume (ATV), cm³ | 0.69 (0.07)     | 0.61 (0.15)    | 0.63   |
| Total-ATV                   | 0.17 (0.02)      | 0.13 (0.04)    | 0.30   |
| WM-ATV                      | 0.51 (0.06)      | 0.47 (0.13)    | 0.77   |

NAFLD = liver attenuation < 51 Hounsfield units (HU) after exclusion for secondary causes of liver fat (alcohol/medications).
aResults are expressed as mean (standard error), linear regression analysis adjusted for intracranial volume (structure models) or total tissue volume (function models), center, age, race, and sex. Bold font indicates significant at P < 0.05.

- Cerebral blood flow (CBF), mL/100 g/min
- Total-CBF: 51.0 (0.6) vs. 53.6 (1.2), P = 0.004
- GM-CBF: 57.8 (0.6) vs. 53.6 (1.2), P = 0.003

Visceral adipose tissue (VAT) or BMI, suggesting that multicollinearity did not interfere with model fit. All models for tissue volumes were adjusted for ICV, as a measure of head size. Models for CBF were adjusted for total tissue volume, as a measure of total brain tissue perfused. Five models were fitted: Model 1 (base model); ICV (tissue volume models) or total tissue volume (CBF models), study center, age, race, sex, education level, alcohol intake (mL/d), smoking status (current vs. never/former), and physical activity score; Model 2: Baseline covariates (BMI); Model 3: Baseline covariates + VAT; Model 4: Model 2 + subcutaneous adipose tissue (SAT); Model 5: Model 2 + BMI. We tested for interactions between NAFLD and age, race, sex, visceral blood pressure or diabetes status, and levels of VAT volume or BMI in terms of total tissue volume, total-NTV, total-CBF, and GM-CBF. Because no interactions approached significance (P > 0.2), we did not include these in the final models. A P value < 0.05 was considered statistically significant. Analyses were performed using SAS® 9.4 (SAS Institute, Cary, NC).
TABLE 3 Multivariable association of continuous liver attenuation\textsuperscript{a} with brain MRI measures of structure and physiology

| Model                  | Measures of brain structure | Measures of brain physiology |
|------------------------|-----------------------------|-----------------------------|
|                        | TTV, cm\textsuperscript{3} | Total-NTV, cm\textsuperscript{3} | GM-NTV, cm\textsuperscript{3} | WM-NTV, cm\textsuperscript{3} | Total-CBF, mL/100 g/min | GM-CBF, mL/100 g/min |
| Base model             | \beta (SE) 0.339 (0.139)   | \beta (SE) 0.337 (0.139)   | \beta (SE) 0.156 (0.105)   | \beta (SE) 0.181 (0.117)   | 0.135 (0.056) 0.02     | 0.165 (0.062) 0.008    |
| Model adjusted R\textsuperscript{2} |                      |                             |                             |                             |                           |
| + CVD factors          | \beta (SE) 0.275 (0.154)   | \beta (SE) 0.277 (0.154)   | \beta (SE) 0.154 (0.116)   | \beta (SE) 0.123 (0.131)   | 0.136 (0.062) 0.03     | 0.168 (0.069) 0.02     |
| Model adjusted R\textsuperscript{2} |                      |                             |                             |                             |                           |
| + CVD + VAT            | \beta (SE) 0.279 (0.164)   | \beta (SE) 0.284 (0.164)   | \beta (SE) 0.140 (0.124)   | \beta (SE) 0.144 (0.139)   | 0.130 (0.067) 0.05     | 0.157 (0.074) 0.03     |
| Model adjusted R\textsuperscript{2} |                      |                             |                             |                             |                           |
| + CVD + SAT            | \beta (SE) 0.333 (0.159)   | \beta (SE) 0.334 (0.159)   | \beta (SE) 0.180 (0.121)   | \beta (SE) 0.153 (0.135)   | 0.113 (0.065) 0.08     | 0.137 (0.072) 0.06     |
| Model adjusted R\textsuperscript{2} |                      |                             |                             |                             |                           |
| + CVD + BMI            | \beta (SE) 0.342 (0.161)   | \beta (SE) 0.344 (0.161)   | \beta (SE) 0.183 (0.122)   | \beta (SE) 0.160 (0.137)   | 0.097 (0.067) 0.15     | 0.121 (0.074) 0.11     |
| Model adjusted R\textsuperscript{2} |                      |                             |                             |                             |                           |

\textsuperscript{a}When interpreting $\beta$ coefficients, note that LOW liver attenuation is associated with HIGH levels of liver fat. All models are per single Hounsfield unit (HU) decrease in liver attenuation. Base model: Intracranial volume (structure models) or TTV (physiology models), center, age, race, sex, education, smoking, physical activity, and alcohol (mL/d); CVD factors: systolic blood pressure, hypertension treatment, high-density lipoprotein cholesterol, cholesterol treatment, diabetes, and high-sensitivity C-reactive protein. Bold font indicates significant at $P<0.05$. BMI, body mass index; CBF, cerebral blood flow; CVD, cardiovascular disease; GM, gray matter; MRI, magnetic resonance imaging; SAT, subcutaneous adipose tissue; TTV, total tissue volume; VAT, visceral adipose tissue; WM, white matter; NTV, normal tissue volume; SE, standard error.
physiology (e.g., CBF), and when adjusted for BMI, LA was no longer statistically associated with CBF. In contrast, the relationship between LA and several measures of brain structure (e.g., total tissue volume, total-NTV) was attenuated when adjusted for CVD risk factors but remained significant when adjusted for BMI or SAT (Table 3, Figure 4). In fully adjusted models for markers of adiposity that included both BMI and SAT, the associations between LA and total-CBF, GM-CBF, and NTV were further weakened ($P < 0.13$, $P < 0.09$, $P < 0.05$, respectively).

Table 4 displays the associations between the presence of CT-defined NAFLD and MRI measures of brain structure and physiology. Similar to the findings with continuous LA, CT-defined NAFLD was associated with lower total tissue volume and total-NTV and decreased total-CBF and GM-CBF when adjusted for ICV, demographics, and health behaviors (base model, $P < 0.04$ for all). Associations of NAFLD with measures of brain physiology were attenuated when adjusted for CVD risk factors. Additional adjustment for VAT had little effect on the associations of NAFLD with CBF, but adjustment for BMI or SAT left the associations nonsignificant. Associations of NAFLD with measures of brain structure (e.g., total tissue volume, total-NTV) were nonsignificant when adjusted for CVD risk factors or VAT; however, there was a trend toward statistical significance when adjusted for SAT ($P = 0.06$) or BMI ($P = 0.05$). Finally, we examined associations between liver fat from any cause (e.g., including participants with

Figure 4 Multivariable association between continuous liver attenuation and either (A-D) GM-CBF or (E-H) total-NTV adjusted for: (A,E) CVD risk factors alone; (B,F) CVD risk factors + BMI; (C,G) CVD risk factors + VAT; and (D,H) CVD risk factors + SAT. All models adjusted for either TTV (GM-CBF models) or intracranial volume (total-NTV models), center, age, race, sex, education, smoking, physical activity, and alcohol (mL/d). CVD risk factors included systolic blood pressure, hypertension treatment, high-density lipoprotein cholesterol, cholesterol treatment, diabetes, and high-sensitivity C-reactive protein. BMI, body mass index; CVD, cardiovascular disease; GM-CBF, gray matter cerebral blood flow; NTV, normal tissue volume; TTV, total tissue volume; VAT, visceral adipose tissue; subcutaneous adipose tissue.

Table 4 displays the associations between the presence of CT-defined NAFLD and MRI measures of brain structure and physiology. Similar to the findings with continuous LA, CT-defined NAFLD was associated with lower total tissue volume and total-NTV and decreased total-CBF and GM-CBF when adjusted for ICV, demographics, and health behaviors (base model, $P < 0.04$ for all). Associations of NAFLD with measures of brain physiology were attenuated when adjusted for CVD risk factors. Additional adjustment for VAT had little effect on the associations of NAFLD with CBF, but adjustment for BMI or SAT left the associations nonsignificant. Associations of NAFLD with measures of brain structure (e.g., total tissue volume, total-NTV) were nonsignificant when adjusted for CVD risk factors or VAT; however, there was a trend toward statistical significance when adjusted for SAT ($P = 0.06$) or BMI ($P = 0.05$). Finally, we examined associations between liver fat from any cause (e.g., including participants with
| Model                          | Measures of brain structure | Measures of brain physiology |
|-------------------------------|-----------------------------|------------------------------|
|                              | TTV, cm³                    | Total-NTV, cm³               | GM-NTV, cm³                   | WM-NTV, cm³                   | Total-CBF, mL/100 g/min | GM-CBF, mL/100 g/min |
|                              | β (SE)                      | P                            | β (SE)                        | P                            | β (SE)                    | P                            |
| Base model                   | −3.69 (1.69)                | 0.03                         | −3.65 (1.69)                  | 0.03                         | −1.40 (1.28)              | 0.27                         | −2.25 (1.41)              | 0.11                        | −1.37 (0.63)              | 0.03                         | −1.60 (0.70)              | 0.02                        |
| Model adjusted R²            | 0.933                       |                               | 0.933                        |                               | 0.848                      |                              | 0.846                      |                              | 1.46                        |                              | 0.197                       |                              | 0.197                       |                              |
| + CVD factors                | −2.98 (1.78)                | 0.10                         | −2.98 (1.79)                  | 0.10                         | −1.29 (1.35)               | 0.34                         | −1.69 (1.51)              | 0.27                        | −1.31 (0.68)              | 0.05                         | −1.52 (0.75)              | 0.04                        |
| Model adjusted R²            | 0.934                       |                               | 0.934                        |                               | 0.850                      |                              | 0.845                      |                              | 1.69                        |                              | 0.199                       |                              | 0.197                       |                              |
| + VAT                        | −2.83 (1.89)                | 0.14                         | −2.86 (1.89)                  | 0.13                         | −1.10 (1.43)               | 0.44                         | −1.76 (1.60)              | 0.27                        | −1.39 (0.73)              | 0.06                         | −1.54 (0.81)              | 0.06                        |
| Model adjusted R²            | 0.934                       |                               | 0.934                        |                               | 0.850                      |                              | 0.845                      |                              | 1.95                        |                              | 0.195                       |                              | 0.192                       |                              |
| + SAT                        | −3.50 (1.85)                | 0.06                         | −3.50 (1.85)                  | 0.06                         | −1.59 (1.41)               | 0.26                         | −1.90 (1.57)              | 0.23                        | −1.22 (0.70)              | 0.08                         | −1.34 (0.78)              | 0.09                        |
| Model adjusted R²            | 0.934                       |                               | 0.934                        |                               | 0.850                      |                              | 0.845                      |                              | 2.09                        |                              | 0.21                        |                              | 0.204                       |                              |
| + BMI                        | −3.63 (1.86)                | 0.05                         | −3.64 (1.86)                  | 0.05                         | −1.55 (1.41)               | 0.27                         | −2.09 (1.58)              | 0.19                        | −0.91 (0.71)              | 0.21                         | −1.01 (0.79)              | 0.20                        |
| Model adjusted R²            | 0.934                       |                               | 0.934                        |                               | 0.850                      |                              | 0.845                      |                              | 0.24                        |                              | 0.193                       |                              |                          |                              |

NAFLD = liver attenuation < 51 Hounsfield units (HU) after exclusion for secondary causes of liver fat (alcohol/medications).
Base model: Intracranial volume (structure models) or TTV (physiology models), center, age, race, sex, education, smoking, physical activity, and alcohol (mL/d); CVD factors: systolic blood pressure, hypertension treatment, high-density lipoprotein cholesterol, cholesterol treatment, diabetes, and high-sensitivity C-reactive protein. Bold font indicates significant at P < 0.05.
BMI, body mass index; CBF, cerebral blood flow; CVD, cardiovascular disease; GM, gray matter; MRI, magnetic resonance imaging; TTV, total tissue volume; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WM, white matter; normal tissue volume; SE, standard error.
heavy alcohol use) and brain MRI measures of structure and physiology. The pattern and magnitude of all observed associations were unchanged (Supporting Information Table S1).

Discussion

In a large, population-based, cross-sectional study of black and white middle-aged adults without prevalent cerebrovascular disease, these data indicate that the presence of NAFLD is associated with less favorable subclinical MRI measures of brain health even when controlled for traditional CVD risk factors and central adiposity. To the best of our knowledge, this is the first study to consider the effect of VAT, a potential effect modifier of the association between NAFLD and vascular cognitive aging, on these associations.

A growing body of literature demonstrates that asymptomatic patients who have risk factors for CVD, such as hypertension and diabetes, show significant structural and physiologic brain imaging changes (27). Furthermore, the presence of an increasing number of individual vascular risk factors (e.g., the metabolic syndrome) appears to increase the magnitude of these brain imaging changes (28). We now add the observation that the degree of liver fat and presence of NAFLD are associated with unfavorable brain imaging changes even when controlled for recognized vascular risk factors for cognitive aging.

Higher levels of adiposity including BMI, waist circumference, SAT, and VAT have all been associated with lower TBV (16,29). BMI, in particular, has been associated with regional brain GM volume decreases, although the location and magnitude of these decreases have been inconsistent (30-33). Some investigations have reported lower GM volume only in those with obesity (BMI ≥ 30 kg/m²) (29), whereas others have reported significant GM volume reductions in those who are merely overweight (BMI ≥ 25 kg/m²) (30,32). There is no consensus on the association of adiposity (particularly BMI) with WM volumetric changes (34). In the current study, there was no significant association between continuous LA or CT-defined NAFLD and GM or WM tissue volumes; nor was there an interaction between NAFLD status and BMI and tissue volumes. In contrast, CT-defined NAFLD was associated with global loss of brain tissue volume. After controlling for BMI, continuous LA remained associated with decreased total tissue volumes, and there was a trend toward significance after adjustment for BMI in the models using CT-defined NAFLD. These findings suggest that the relationship between liver fat and decreased total brain tissue volume is not explained by global adiposity alone.

An important aspect of the present study was the inclusion of VAT as a potential effect-modifying variable. Different fat compartments carry differential metabolic risks (35), and there is evidence that abdominal obesity and visceral fat are more correlated with vascular risk than global body mass (36). In the Framingham Offspring Study, the inverse association between VAT and TBV was the strongest and most robust compared to associations between TBV and BMI, waist circumference, or SAT (37). However, the mechanisms underlying the inverse association between VAT with TBV are speculative and have not included the potential role of liver fat. In the current study, the association between NAFLD and MR measures of brain structure was no longer statistically significant after adjustment for VAT. Thus, the association between NAFLD and decreased brain tissue volume is not wholly independent of VAT, and whether NAFLD is a mediator of the relationship between VAT and brain MR volume requires further study. On the other hand, the association of continuous LA with physiologic changes in brain imaging (e.g., total and GM-CBF) remained significant after adjusting for VAT and vascular risk factors, and there was a trend toward significance between NAFLD and GM-CBF when controlled for VAT. The direction of these associations was congruent between continuous LA and GM-CBF, with NAFLD and GM-CBF further strengthening the hypothesis that VAT may only partially explain the relationship between liver fat and measures of brain physiology. Notably, there was also no interaction between NAFLD and VAT in our analysis. In contrast, when vascular risk factor models were adjusted for BMI, NAFLD (and continuous LA) was no longer associated with decreased CBF. These observations provide important insight into the potential pathophysiologic mechanisms of increased adiposity in the development of vascular cognitive aging.

To date, few studies have been published evaluating the effects of adiposity on brain blood flow (38,39). In one study of 36 adults, authors investigated the effects of high BMI on regional CBF using single photon emission CT imaging in healthy subjects (38). They observed that higher BMI was associated with lower CBF in the prefrontal cortex, which is the area of the brain responsible for executive function (38). This finding is supported by other studies that have reported diminished metabolic activity in the prefrontal cortex with high BMI (40). In another study of 25 healthy lean participants and 23 participants with overweight/obesity, investigators revealed significantly lower CBF in both the prefrontal cortex and the hypothalamus (area of the brain that produces hormones that govern body weight hemostasis) after intranasal insulin administration compared with placebo, pointing to selective central insulin resistance (39). In this study, the magnitude of hypothalamic response correlated with the amount of VAT independent of other tissue volumes but did not account for liver fat volume (39). We have observed an inverse association between NAFLD, a condition highly associated with systemic insulin resistance, and total GM-CBF in a large population of asymptomatic adults. Our finding raises important questions about the potential role of central insulin resistance in the development of vascular cognitive aging (Figure 1).

This study has several strengths. It provides the first data on a large, community-based, biracial, middle-age (50 years) cohort on the relationship between NAFLD and a range of MRI characteristics in relation to key risk factors for cerebrovascular disease. In addition, we provide the modifying effects of total (BMI) and visceral adiposity (VAT) on these observed associations. Such studies on this age cohort are important as they will provide clues of early changes in the brain that may eventually predict who is at risk for vascular cognitive decline. The main limitation in this study is its cross-sectional study design, by which we cannot infer causal direction. An additional follow-up is planned. We also cannot extrapolate the differential effects of VAT versus liver fat on brain structure and physiology, and the distinctions between these relationships warrant further study. NAFLD prevalence in CARDIA is on the lower end of the reported spectrum of disease, which is likely attributable to differences in population-level reporting of NAFLD (e.g., ultrasound vs. CT imaging). In addition, those with morbid obesity in whom NAFLD is highly prevalent were excluded from analysis if they were unable to fit in the CT or MR scanner. Finally, brain physiology was only assessed by CBF. The relationship between NAFLD and other markers...
of early brain dysfunction, such as alterations in default mode networks or functional connectivity, requires further study.

Conclusion

In conclusion, the presence of NAFLD is negatively associated with measures correlating with brain health in a population-based, middle-age sample of black and white adults. BMI attenuates the association between NAFLD and physiologic measures of brain health, whereas VAT attenuates the association between NAFLD and brain structure. An important clinical manifestation of vascular risk-factor-related structural and physiologic brain imaging changes in otherwise healthy persons is cognitive impairment. The evolving body of literature in this area suggests that in asymptomatic at-risk persons, physiologic brain imaging changes compared with structural changes, specifically reductions in CBF and glucose metabolism during rest, affect cerebral hemodynamics via impairment in the microcirculation before the occurrence of detectable structural changes, which can then lead to cognitive impairment. Future studies are needed to assess the longitudinal effect of NAFLD on progression of structural and physiologic brain changes and on cognitive function.

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