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RESEARCH ARTICLE

Leptin bioavailability and markers of brain atrophy and vascular injury in the middle age

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Abstract

INTRODUCTION: We investigated the associations of leptin markers with cognitive function and magnetic resonance imaging (MRI) measures of brain atrophy and vascular injury in healthy middle-aged adults.

METHODS: We included 2262 cognitively healthy participants from the Framingham Heart Study with neuropsychological evaluation; of these, 2028 also had available brain MRI. Concentrations of leptin, soluble leptin receptor (sOB-R), and their ratio (free leptin index [FLI]), indicating leptin bioavailability, were measured using enzyme-linked immunosorbent assays. Cognitive and MRI measures were derived using standardized protocols.

RESULTS: Higher sOB-R was associated with lower fractional anisotropy (FA, $\beta = -0.114 \pm 0.02$, $p < 0.001$), and higher free water (FW, $\beta = 0.091 \pm 0.022$, $p < 0.001$) and peak-width skeletonized mean diffusivity (PSMD, $\beta = 0.078 \pm 0.021$, $p < 0.001$). Correspondingly, higher FLI was associated with higher FA ($\beta = 0.115 \pm 0.027$, $p < 0.001$) and lower FW ($\beta = -0.096 \pm 0.029$, $p = 0.001$) and PSMD ($\beta = -0.085 \pm 0.028$, $p = 0.002$).

DISCUSSION: Higher leptin bioavailability was associated with better white matter (WM) integrity in healthy middle-aged adults, supporting the putative neuroprotective role of leptin in late-life dementia risk.

KEYWORDS
cognition, DTI, fractional anisotropy, free leptin index, free water, leptin, leptin bioavailability, leptin perturbations, leptin receptor, MarkVCID, mean diffusivity, MRI, neuropsychological evaluation, peak width of skeletonized mean diffusivity, the Framingham Heart Study, white matter microstructural integrity

Highlights

- Higher leptin bioavailability was related to better preservation of white matter microstructure.
- Higher leptin bioavailability during midlife might confer protection against dementia.
- Potential benefits might be even stronger for individuals with visceral obesity.
- DTI measures might be sensitive surrogate markers of subclinical neuropathology.

1 | BACKGROUND

Alzheimer's disease (AD) is the leading cause of dementia, impacting the lives of millions of people worldwide.[1] Accruing epidemiological evidence suggests that midlife obesity is an important contributor to the risk of developing AD.[2] This has created a growing interest in disentangling the mechanisms linking obesity to AD, which potentially extend through vascular,[3] genetic,[4] and metabolic pathways.[5]

The study of adipose tissue has led to significant insights. Once viewed as a passive reservoir for energy storage, adipose tissue is now considered a part of the endocrine system, secreting a group of bioactive peptides, known as adipokines, that exert pleiotropic autocrine, paracrine, and endocrine effects in the periphery as well as the central nervous system.[6]

Leptin, a cardinal adipokine responsible for central control of food intake and energy homeostasis, has been implicated in a variety of neurophysiological functions, including brain development, neurogenesis, and neuroprotection.[7] Due to these effects, it has been considered a plausible mechanistic intermediary in the pathway leading from obesity to AD. This hypothesis has been substantiated by findings relating higher leptin levels to lower risk for incident AD and mild cognitive impairment (MCI),[8,9] as well as better structural brain indices in older adults.[8] However, studies conducted in younger individuals have not detected associations between leptin and late-life dementia risk or AD endophenotypes, such as neuropsychological test scores or brain volume measures.[10,11] Given the long latency period between the onset of pathological changes in the brain and the manifestation of clinical symptoms that characterize AD,[12] these incongruent findings could indicate that decreased leptin levels might actually represent a consequence rather than a cause of AD-related pathobiological processes. This highlights the importance of studying the associations of leptin markers with cognitive and neuroimaging outcomes in younger individuals, who are less likely to have accumulated AD pathology.

Important to consider is also the fact that in certain individuals, particularly those who are obese, high levels of circulating leptin might be linked to the development of central leptin resistance, ultimately reflecting a state of relative leptin deficiency from a brain-signaling standpoint.[13] Measuring the free leptin index (FLI) has been proposed as a way to account for leptin resistance and reflect leptin bioavailability[14–16]; therefore, FLI might be more sensitive than circulating leptin levels to capture potential relationships between leptin signaling states and health-related outcomes.

In the present study, we aimed to gain further insights into the potential relationships of leptin with neurodegenerative and cerebrovascular burden. To this end, we investigated the associations of leptin, its soluble receptor, and leptin bioavailability with cognitive function and magnetic resonance imaging (MRI) markers of brain atrophy and vascular injury in cognitively healthy, community-dwelling middle-aged adults from the Framingham Heart Study (FHS). We further sought replication in a diverse cohort of the MarkVCID study.

2 | METHODS

2.1 | Participants

The present cross-sectional investigation included cognitively healthy participants from the Third Generation Cohort of the FHS,[17] who provided blood samples at the first examination cycle (2002–2005) and underwent brain MRI and neuropsychological testing at the second examination cycle (2008–2011). Those with missing data on demographic characteristics or cardiovascular risk factors were excluded.

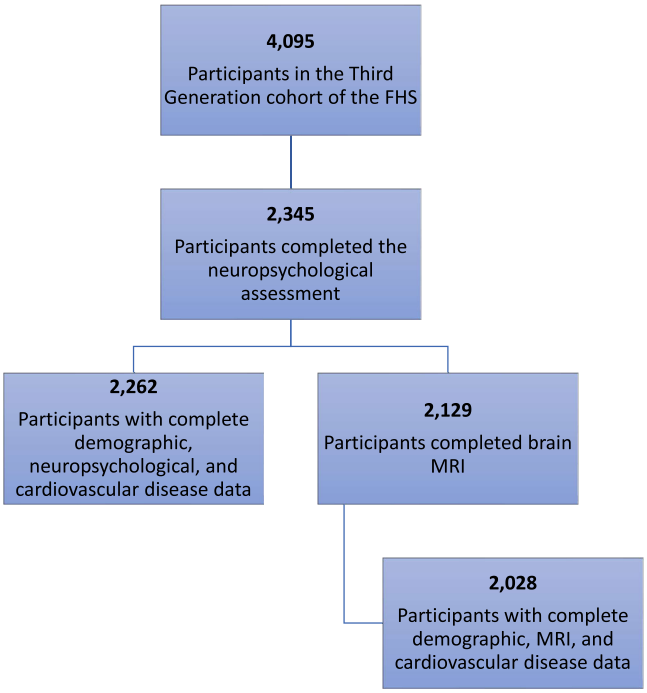

FIGURE 1 Flowchart of the Framingham Heart Study (FHS) sample.

RESEARCH IN CONTEXT

1. **Systematic review**: The literature was reviewed using traditional (e.g., PubMed) sources. Leptin perturbations have been associated with dementia and cognitive decline in older adults. However, due to the long preclinical stages that characterize these disorders, previous findings might be confounded by reverse causality.
2. **Interpretation**: Our findings suggest that higher leptin bioavailability during midlife is related to better preservation of white matter microstructure, and therefore, it might confer protection against the development of cognitive decline and dementia later in life by maintaining cognitive network efficiency.
3. **Future directions**: This study provides insights into the putative neuroprotective role of leptin in late-life dementia risk. Interventions that correct leptin perturbations during midlife might be important strategies for dementia risk reduction and should be further studied, ideally, through clinical trials.

Furthermore, participants with a contraindication for MRI, stroke by the time of MRI acquisition, or other neurological findings that could substantially influence the measurement of MRI outcomes were excluded from MRI analyses. The final analytic sample consisted of 2262 participants for neurocognitive analyses, and 2028 participants for MRI analyses (Figure 1). All participants have provided written informed consent. Study protocols and consent forms have been approved by the institutional review board of the Boston University Medical Center.

2.2 | Measurement of leptin markers

Blood samples were collected after an overnight fast and were stored at -80°C until processing. The concentrations of leptin and soluble leptin receptor (sOB-R) were measured from serum using a standard enzyme-linked immunosorbent assay (ELISA). The intra-assay coefficients of variation were 4.97% for the leptin assay and 4.01% for the sOB-R assay.[18] The FLI was calculated as the ratio of leptin to sOB-R. Higher FLI values indicate greater leptin bioavailability.[14–16]

2.3 | Neuropsychological evaluation

Cognitive function was assessed with a neuropsychological test battery consisting of validated tests, designed to evaluate different cognitive domains. The following cognitive domains were assessed (using the respective tests): abstract reasoning (Wechsler Adult Intelligence Scale [WAIS] similarities test), processing speed (Trail Making Test-TMT part A [TMT-A]), executive functioning (TMT part B [TMT-B]), episodic memory (logical memory delayed recall), and visuospatial skills (Hooper Visual Organization Test [HVOT]). TMT-A and TMT-B scores were multiplied by -1 for better interpretability (i.e., higher scores reflect better cognitive performance).

2.4 | Neuroimaging indices

Study participants underwent brain MRI on a variety of machines with 1.5 Tesla field strength. The detailed brain MRI protocol has been described elsewhere.[19] Briefly, three-dimensional T1-weighted, fluid-attenuated inversion recovery (FLAIR), and diffusion tensor imaging (DTI) sequences were obtained. Total brain, cortical gray matter, hippocampal, and white matter hyperintensity (WMH) volumes were derived using standard protocols.[19] Total cranial volume (TCV) was determined using a convolutional neural network method.[20] Volumetric measures were divided by TCV to correct for head size.

White matter (WM) microstructural integrity measures of free water (FW), free water-corrected fractional anisotropy (FA), and peak width of skeletonized mean diffusivity (PSMD) were derived from DTI sequences using previously described algorithms.[21–23] Briefly, FW and FA were derived from the WM mask, which was defined by thresholding the FSL FA template at a value of 0.3 to reduce cerebrospinal fluid (CSF) partial volume contamination.[23,24] PSMD was calculated using a publicly available script (PSMD Marker,[25] version 0.95), as the difference between the 95th and 5th percentiles of the voxel-based mean diffusivity (MD) values within the subject's MD skeleton, after application of FA threshold and custom-made masks to avoid

contamination of the skeleton by CSF partial volume effects.[22,23] The rationale for selecting the above-mentioned DTI measures was twofold. First, their inter-rater reliability, test-retest repeatability, and inter-scanner reproducibility have been previously explored and ascertained.[23] Second, the used algorithms for FA and PSMD calculation account for CSF contamination of the diffusion signal, to provide tissue-specific DTI indices and consequently allow for more accurate inferences about the underlying WM tissue structure. [21,22,26]

2.5 | Cardiovascular risk factors and other covariates

All participants undergo standardized assessments at each examination cycle, where anthropometric measurements are obtained, and information regarding medication records and clinical history are updated. Diabetes mellitus (DM) was defined by either a fasting blood glucose of ≥126 mg/dL, or the use of glucose-lowering medications. Body mass index (BMI) was calculated as weight divided by the height squared (kg/m^2). Systolic blood pressure (SBP) was derived from the average of 2 blood pressure readings obtained 10 min apart with the participant resting in a sitting position. Smoking was defined as current smoking within the year preceding the assessment. Waist-to-hip ratio (WHR) was calculated by dividing the participant's waist circumference by hip circumference (cm/cm), and was ranked into quartiles within the individual sex groups; subsequently, WHR was dichotomized by the upper quartile (WHR > 1.01 for men and WHR > 0.92 for women) to indicate excess WHR. Prevalent cardiovascular disease (CVD) was defined as a positive history of transient ischemic attack, coronary heart disease, congestive heart failure, or peripheral vascular disease.

2.6 | Statistical analysis

Continuous variable distributions were graphically explored using Q-Q plots. The variables expressing serum leptin concentration, serum sOB-R concentration, FLI, WMH volume, FW, PSMD, TMT-A, TMT-B, and HVOT scores were natural log-transformed to normalize their distributions. Subsequently, the natural log-transformed leptin marker variables were standardized within sex to account for differences in their distributions between men and women.[27]

2.6.1 | Primary analyses

Associations of leptin markers with cognitive and MRI indices were explored using linear regression models with the respective neuropsychological or brain imaging measure of interest as the outcome and leptin markers as the main predictors (in separate models for each marker). Models were adjusted for age, sex, BMI, DM, SBP, antihypertensive medication use, smoking, prevalent CVD, and the time interval between blood draw and neuropsychological evaluation or brain MRI acquisition. Cognitive models were further adjusted for educational attainment; MRI models including DTI-derived outcomes were further adjusted for TCV to account for potential influences of head size on DTI measures.[28]

The false discovery rate (FDR) was controlled at < 5% using the Benjamini-Hochberg procedure to account for multiple hypothesis testing,[29] with cognitive and MRI outcomes considered separately.

2.6.2 | Replication

To examine the replicability and external validity of primary analysis findings, we used data from the MarkVCID-1 study. MarkVCID-1 was established as a consortium of 7 sites across the United States, aiming to identify and validate fluid- and MRI-based biomarkers for vascular injury associated with vascular contributions to cognitive impairment and dementia (VCID).[30] This replication analysis includes data from the San Antonio site with Olink Explore panel and neurological outcome data (imaging and cognitive) measured at the baseline visit (version dated January 17, 2022). Specifically, significant associations of leptin makers with cognitive or MRI outcomes in FHS were also assessed in a sample of 89 dementia-free Mexican American participants. The standardized clinical, imaging, and biobanking protocols of the MarkVCID-1 study have been described elsewhere.[30,31] Briefly, MRI measures were derived using similar protocols to the FHS cohort.[23] Processing speed and executive functioning were assessed similarly to the FHS (using the TMT-A and TMT-B tests, respectively), and episodic memory was assessed with the Craft Story 21 Recall (Delayed).[32] The similarities and HVOT tests were not part of the MarkVCID-1 neuropsychological test battery. Leptin and leptin receptor measurements were derived from the Olink cardiometabolic panel (included as part of the Explore panel). Olink assays report protein levels in a proprietary relative quantification unit, called Normalized Protein eXpression (NPX), that is not directly comparable between different proteins/analytes. Algorithms that convert these values to conventional protein concentration units are not available; therefore, FLI calculation was not feasible. However, here we report results for leptin and leptin receptor, with the latter representing an indirect measure of leptin bioavailability. Because NPX is in a $\log_2$ scale, a 1 NPX difference can be interpreted as a doubling of protein concentration. Models were similarly structured to those described in Section 2.6.1, and were adjusted for age, sex, BMI, DM, SBP, antihypertensive medication use, smoking, and TCV.

2.6.3 | Exploratory analyses

Considering that leptin expression in humans exhibits sexual dimorphism and prior findings suggesting that obesity might influence the relationships between leptin and cognitive function,[11,13,27] we tested for interactions between leptin markers and sex or excess WHR on neurological outcomes in secondary analyses. A two-step approach was followed. First, as an omnibus test, the product term of the respective leptin marker and the moderator of interest (M)

was entered in a model including the same set of confounders as the primary analyses, as well as M by confounder interaction terms. If the product term was significant at $p \leq 0$ .1, then potential effect modification by M was further explored by conducting stratified analysis at the different levels of M; M was considered an effect modifier if stratified analysis for the association of interest indicated either the presence of a significant association in one stratum but not the other, or significant associations in both strata with different stratum-specific estimates.[33]

Statistical analyses were performed using SAS Software, version 9.4 (SAS Institute). A 2-sided p-value < 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Participant characteristics in FHS

Participant characteristics of the FHS sample are presented in Table 1. In the largest (cognitive) sample, the mean age was 40.4 (standard deviation [SD] = 8.6) years, and 1186 (52.4%) participants were women. Most participants (> 99%) were non-Hispanic Whites. Current smoking and use of antihypertensive medications were reported by 13.2% and 7.7% of study participants, respectively. The prevalence of DM was 2.6%, and of CVD 0.9%. The mean SBP was 116.9 (SD = 13.9), and the mean BMI was 26.9 (SD = 5.4). The MRI sample displayed similar characteristics, although with a slightly better cardiovascular profile.

3.2 | Primary analyses

3.2.1 | Associations with cognitive measures

Associations of leptin markers with neuropsychological test scores in the FHS are presented in Table 2. For the reported associations in which the response variable was log-transformed, the respective beta coefficients can be interpreted as indicating the relative change in the response variable for an increase in the predictor variable by 1 SD unit (i.e., in a model of the form $\ln y = \beta_1 \times \chi + \beta_0$, for a one-increment increase in χ, the relative change in y is equal to $e^{\beta_1} - 1$, which can be approximated to β_1 for values of β_1 close to zero, using the first two terms of a Maclaurin series expansion). Although there were nominal associations of higher FLI with worse performance in TMT-A and better performance in the logical memory delayed recall, these did not remain significant after correction for multiple testing (Table 2). Relationships between leptin markers and other neuropsychological test scores were not significant.

3.2.2 | Associations with MRI measures

Associations of leptin markers with MRI markers of brain atrophy and vascular injury in the FHS are presented in Table 3. No associations were observed between serum leptin concentrations and MRI indices. An increase in ln(sOB-R) concentrations by 1 SD was associated with a 0.11-unit decrease in FA (β = -0.114, standard error [SE] = 0.02, $p < 0.001$), a 9.1% increase in FW ($\beta = 0.091$, SE = 0.022, $p < 0.001$), and a 7.8% increase in PSMD ($\beta = 0.078$, SE = 0.021, $p < 0.001$), indicating overall worse WM microstructural integrity. Correspondingly, a 1 SD increase in ln(FLI), reflecting an increase in the concentration of bioavailable leptin, was associated with a 0.12-unit increase in FA ($\beta = 0.115$, SE = 0.027, $p < 0.001$), a 9.6% decrease in FW (β = -0.096, SE = 0.029, $p = 0.001$), and a 8.5% decrease in PSMD (β = -0.085, SE = 0.028, $p = 0.002$), indicating overall better WM microstructural integrity. These associations remained significant after correction for multiple testing. Nominal associations of lower sOB-R concentrations and higher FLI with lower WMH volume were also observed but did not survive correction for multiple testing.

3.3 | Replication

Participant characteristics of the MarkVCID-1 sample are presented in Table 4. Mean age was 70.5 (SD = 7.3) years, and 66 (74.2%) study participants were women. Overall, prevalence of cardiovascular risk factors, including use of antihypertensive medications (51.7%), diabetes (27%), and current smoking (24.7%), was higher compared to the FHS sample. Mean SBP (134.2, SD = 17.9) and BMI (30.3, SD = 6.4) were also higher.

A twofold increase in leptin receptor concentration was associated with a 10% increase in PSMD ($\beta = 0.099$, SE = 0.031, $p = 0.002$). No associations were observed with FA (β = -0.002, SE = 0.004, $p = 0.64$) or FW ($\beta = 0.034$, SE = 0.036, $p = 0.36$).

3.4 | Exploratory analyses

3.4.1 | Interaction and effect modification by sex

Interaction and effect modification analyses for sex are presented in Table S1 and Table 5, respectively. The product terms of all leptin markers with sex were significant in models with HVOT score as the outcome, suggesting the potential presence of interaction. Stratified analyses revealed that in men, a 1 SD increase in ln(Leptin) concentration was associated with an 11.2% increase in HVOT scores ($\beta = 0.112$, SE = 0.047, $p = 0.02$), and a 1 SD increase in ln(FLI) was associated with a 12.3% increase in HVOT scores ($\beta = 0.123$, SE = 0.044, $p = 0.006$); these associations were not present in women or the total sample. In addition, the product term of FLI with sex was significant in models with TMT-B score as the outcome. In stratified analyses, a 1 SD increase in ln(FLI) was associated with a 9.2% increase in TMT-B scores in men ($\beta = 0.092$, SE = 0.044, $p = 0.04$), whereas no such association was observed in women or the total sample. Finally, although the omnibus test was significant, suggesting a potential interaction between leptin concentration and sex on total brain volume, no significant associations were detected in stratified analyses.

TABLE 1 Participant characteristics in the Framingham Heart Study.

Demographic, anthropometric, and clinical characteristics	NP sample ($n = 2262$)	MRI sample ($n = 2028$)
Sex, *n* (%)		
Women	1186 (52.4)	1070 (52.8)
Men	1076 (47.6)	958 (47.2)
Age, years, mean (SD)	40.4 (8.6)	40.2 (8.7)
Race, *n* (%)		
Black	3 (0.1)	3 (0.1)
White	2251 (99.5)	2018 (99.5)
Other	8 (0.4)	7 (0.4)
Ethnicity		
Hispanic	2 (0.1)	2 (0.1)
Non-Hispanic	2260 (99.9)	2026 (99.9)
Education *n* (%)		
Bachelor's degree	843 (37.3)	767 (37.9)
Graduate degree	380 (16.8)	347 (17.2)
Highschool or less	310 (13.7)	268 (13.3)
Some college	729 (32.2)	640 (31.7)
Blood draw to NP or MRI interval, years, mean (SD)	7.8 (1.1)	7.8 (1.0)
BMI, kg/m^2, mean (SD)	26.9 (5.4)	26.7 (5.2)
WHR, mean (SD)	0.92 (0.08)	0.92 (0.08)
Excess WHR, *n* (%)		
No	1705 (75.5)	1543 (76.2)
Yes	553 (24.5)	483 (23.8)
Systolic blood pressure, mmHg, mean (SD)	116.9 (13.9)	116.7 (13.9)
Antihypertensive medication use, *n* (%)	174 (7.7)	156 (7.7)
Diabetes mellitus, *n* (%)	58 (2.6)	50 (2.5)
Current smoking, *n* (%)	299 (13.2)	257 (12.7)
Prevalent cardiovascular disease [a], *n* (%)	20 (0.9)	14 (0.7)
Leptin markers		
Leptin, ng/mL, median (IQR)	7280 (3645, 14,973)	7330 (3623, 14,861)
sOB-R, ng/mL, median (IQR)	17.7 (12.1, 23.7)	18.2 (12.3, 24.1)
FLI, median (IQR)	423.0 (193.5, 970.1)	418.3 (188.4, 961.7)
Neuropsychological test scores		
Similarities, mean (SD)	17.34 (3.06)	17.36 (3.09)
TMT part A, min, median (IQR)	0.40 (0.32, 0.48)	0.38 (0.32, 0.47)
TMT part B, min, median (IQR)	0.93 (0.75, 1.18)	0.93 (0.75, 1.17)
Logical memory delayed recall, mean (SD)	11.79 (3.58)	11.79 (3.59)
HVOT, median (IQR)	27.0 (25.5, 28.0)	27.0 (25.5, 28.0)
MRI measures ($n = 2129$)		
Total cranial volume, cm^3, mean (SD)	1264.1 (125.3)	1263.1 (125.8)
Total brain volume, cm^3, mean (SD)1	998.4 (102.6)	997.9 (102.8)
Cortical gray matter volume, cm^3, mean (SD)	480.2 (46.6)	480.0 (46.7)
Hippocampal volume, cm^3, mean (SD)	6.86 (0.72)	6.86 (0.72)
WMH volume, cm^3, median (IQR)	0.42 (0.21, 0.88)	0.42 (0.21, 0.86)
Fractional anisotropy, mean (SD)	0.62 (0.01)	0.63 (0.01)
Free water, median (IQR)	0.20 (0.19, 0.21)	0.20 (0.19, 0.21)
PSMD $\times 10^{-4}$, median (IQR)	2.16 (2.01, 2.36)	2.16 (2.01, 2.36)

Abbreviations: BMI, body mass index; FLI, free leptin index; HVOT, Hooper Visual Organization Test.; IQR, interquartile range; MRI, magnetic resonance imaging; NP, neuropsychological evaluation; PSMD, peak width of skeletonized mean diffusivity; SD, standard deviation; sOB-R, soluble leptin receptor; TMT, Trail Making Test; WHR, waist-to-hip ratio; WMH, white matter hyperintensity.

[a]Prevalent cardiovascular disease includes history of transient ischemic attack, coronary heart disease, congestive heart failure, or peripheral vascular disease.

TABLE 2 Associations of leptin markers with neuropsychological test scores ($n = 2262$).

	Leptin[a]			sOB-R[a]			FLI[a]		
Cognitive outcome	***β***	**SE**	***p*-value**	***β***	**SE**	***p*-value**	***β***	**SE**	***p*-value**
Similarities	0.004	0.031	0.90	−0.001	0.022	0.95	0.003	0.029	0.92
TMT part A[a,b]	−0.057	0.030	0.06	0.036	0.021	0.09	−0.068	0.028	0.02
TMT part B[a,b]	0.026	0.031	0.39	−0.018	0.022	0.40	0.032	0.029	0.26
Logical memory delayed recall	0.057	0.030	0.06	−0.029	0.021	0.18	0.062	0.028	0.03
HVOT[a]	0.032	0.031	0.30	−0.011	0.022	0.62	0.031	0.029	0.29

Note: Results from linear regression models with the respective neuropsychological test score as the outcome and the respective leptin marker as the main predictor. Models are adjusted for age, sex, education, diabetes mellitus, body mass index, systolic blood pressure, antihypertensive medication use, current smoking, prevalent cardiovascular disease, and time interval between blood draw and neuropsychological assessment. Values represent beta coefficients (β), and their corresponding standard errors (SE) and raw *p*-values (p). Statistically significant findings after false discovery rate control at 5% using the Benjamini-Hochberg procedure are indicated in bold.

Abbreviations: FLI, free leptin index; HVOT, Hooper Visual Organization Test.;sOB-R, soluble leptin receptor; TMT, Trail Making Test.

[a]These variables have been natural log-transformed to normalize their distributions.

[b]Scores were multiplied by −1 so that higher scores reflect better cognitive performance.

TABLE 3 Associations of leptin markers with MRI markers of brain atrophy and vascular injury ($n = 2028$).

	Leptin[a]			sOB-R[a]			FLI[a]		
MRI outcome	***β***	**SE**	***p*-value**	***β***	**SE**	***p*-value**	***β***	**SE**	***p*-value**
Total brain volume[b]	0.008	0.029	0.78	−0.013	0.020	0.52	0.016	0.027	0.56
Cortical gray matter volume[b]	0.023	0.028	0.41	−0.009	0.020	0.66	0.022	0.026	0.40
Hippocampal volume[b]	0.011	0.033	0.73	−0.012	0.024	0.62	0.017	0.031	0.58
WMH volume[a,b]	−0.034	0.029	0.24	0.040	0.021	0.05	−0.054	0.027	0.05
Fractional anisotropy[c]	0.041	0.029	0.16	**−0.114**	**0.020**	**<0.001**	**0.115**	**0.027**	**<0.001**
Free water[a,c]	−0.039	0.031	0.21	**0.091**	**0.022**	**<0.001**	**−0.096**	**0.029**	**0.001**
PSMD[a,c]	−0.038	0.029	0.20	**0.078**	**0.021**	**<0.001**	**−0.085**	**0.028**	**0.002**

Note: Results from linear regression models with the respective MRI marker as the outcome and the respective leptin marker as the main predictor. Models are adjusted for age, sex, diabetes mellitus, body mass index, systolic blood pressure, antihypertensive medication use, current smoking, prevalent cardiovascular disease, and time interval between blood draw and MRI acquisition. Values represent beta coefficients (β), and their corresponding standard errors (SE) and raw *p* values (p). Statistically significant findings after false discovery rate control at 5% using the Benjamini-Hochberg procedure are indicated in bold.

Abbreviations: FLI, free leptin index, MRI, magnetic resonance imaging; PSMD, peak width of skeletonized mean diffusivity.; sOB-R, soluble leptin receptor; WMH, white matter hyperintensity.

[a]These variables have been natural log-transformed to normalize their distributions.

[b]as percentage of total cranial volume.

[c]Models are further adjusted for total cranial volume.

3.4.2 | Interaction and effect modification by WHR

Interaction and effect modification analyses for WHR are presented in Table S2 and Table 6, respectively. The product terms of WHR with sOB-R and FLI were significant in models with PSMD as the outcome. Stratified analyses revealed that the effect size for the association of sOB-R with PSMD was larger in participants with excess WHR compared to those without excess WHR as well as the total sample. Moreover, the association of higher FLI with lower PSMD observed in the total sample was only present in participants with excess WHR.

4 | DISCUSSION

In this study, higher leptin bioavailability, indicated by decreased concentrations of its soluble receptor and higher FLI, was associated with better WM integrity in cognitively healthy, community-dwelling middle-aged adults. Considering the relatively young age, and good cognitive and cardiovascular health of the FHS participants, these observations may suggest that leptin perturbations are present in individuals with early subclinical neuropathology.

4.1 | Leptin markers and cognitive measures

In AD, neuropathological changes begin many years before the emergence of clinical symptoms.[12] Similarly, vascular brain damage can exist without evident cognitive impairment, and such asymptomatic individuals are at increased risk for future decline.[34] During these preclinical disease stages, although neuropathology may already be present, cognitive function remains within the population norms. This

TABLE 4 Participant characteristics in the MarkVCID-1 sample ($n = 89$).

Demographic, anthropometric, and clinical characteristics	*n* (%)
Sex	
Women	66 (74.2)
Men	23 (25.8)
Age, years, mean (SD)	70.5 (7.3)
Education, years, mean (SD)	14.3 (2.4)
BMI, kg/m^2, mean (SD)	30.3 (6.4)
Systolic blood pressure, mmHg, mean (SD)	134.2 (17.9)
Antihypertensive medication use, *n* (%)	46 (51.7)
Diabetes mellitus, *n* (%)	24 (27.0)
Current smoking, *n* (%)	22 (24.7)
Leptin markers	
Leptin, NPX, mean (SD)	−0.2 (1.4)
Leptin receptor, NPX, mean (SD)	0.1 (0.3)
Neuropsychological test scores	
TMT part A, min, median (IQR)	0.57 (0.43, 0.70)
TMT part B, min, median (IQR)	1.49 (1.21, 2.29)
Craft Story 21 Recall (Delayed), mean (SD)	17.2 (6.5)
MRI measures	
Total cranial volume, cm^3, mean (SD)	1349.8 (142.0)
Total brain volume, cm^3, mean (SD)	1049.2 (109.7)
Total gray matter volume, cm^3, mean (SD)	582.8 (54.2)
WMH volume, cm^3, median (IQR)	1.91 (0.87, 3.22)
Fractional anisotropy, mean (SD)	0.50 (0.01)
Free water, median (IQR)	0.18 (0.17, 0.21)
PSMD × 10^{-4}, median (IQR)	3.25 (2.94, 3.49)

Abbreviations: BMI, body mass index; IQR, interquartile range; MRI, magnetic resonance imaging; NPX, Normalized Protein eXpression; PSMD, peak width of skeletonized mean diffusivity; SD, standard deviation; TMT, Trail Making Test.; WMH, white matter hyperintensity.

likely corresponds to the settings of the present study, considering the inclusion of predominantly young and healthy participants, and might account for the lack of associations between leptin bioavailability and cognitive performance measures. Nevertheless, longitudinal studies are warranted to investigate whether leptin perturbations in cognitively normal individuals increase the risk of future cognitive decline.

It is also possible that relationships between leptin signaling and cognition are modulated by sex. In stratified analyses, higher leptin bioavailability was associated with better visuospatial skills and executive function in men. These associations were not observed in the total sample but became evident in subgroup analyses despite the lower power, indicating that sex might act as an effect modifier in the relationships between leptin and cognition. In line with this hypothesis, excessive leptin per unit fat was positively associated with cognitive performance in white men but not women in the Dallas Heart Study.[35]

4.2 | Leptin markers and volumetric brain indices

Higher plasma leptin concentrations were associated with larger total cerebral brain volume but not with temporal horn volume in a subsample of 198 cognitively healthy participants with a mean age of 79 years from the Original cohort of the FHS.[8] Furthermore, in 527 Dutch participants 70–82 years of age from the Pravastatin in elderly individuals at risk for vascular disease (PROSPER) study, higher serum leptin was associated with higher volume of the amygdala but not with other brain volumes.[36] Conversely, higher plasma leptin was associated with regional brain volume deficits in a set of 517 participants (mean age 75 years) from the Alzheimer's disease neuroimaging initiative (ADNI) study.[37] The variability surrounding these prior findings might be due to residual confounding introduced by the accumulation of neurodegenerative and cerebrovascular pathologies in older individuals,[38–40] which might in turn alter leptin signaling pathways.[41–43] To overcome this issue, along with potential power limitations of prior approaches, we studied the associations of leptin markers with volumetric brain indices in a relatively large sample of cognitively healthy middle-aged adults with < 1% prevalence of CVD. Additionally, taking into account that the development of central leptin resistance might lead to a state of relative leptin deficiency despite apparently high concentrations of circulating leptin,[13] we included leptin bioavailability in our analysis, considering it might better reflect underlying leptin signaling states. Nevertheless, no associations of leptin markers with total brain, cortical gray matter, or hippocampal volumes were observed in the younger FHS sample. This might be related to the relatively young age (brain volume changes usually appear later in life[44]) and overall good cognitive and cardiovascular health of study participants, leading to an overall preserved brain morphology.

4.3 | Leptin markers and white matter microstructural integrity

The "disconnected brain" hypothesis posits that aging-accompanying cognitive changes are the consequence of disrupted communication between different (mainly distant) cortical regions since higher-order cognitive functions rely on the speed and efficiency of communication among large-scale neural networks.[45,46] WM integrity seems to mediate this relationship between age and cognition through cortical disconnection[46]; therefore, WM integrity alterations might explain potential deviations from the normal aging pattern that lead to the development of dementia. This is evidenced by the loss of WM integrity in AD, starting in the prodromal stages,[47] and in individuals with VCID, during both the symptomatic[48] and pre-symptomatic[49] period. Consequently, alterations in WM integrity likely represent an early subclinical process in those at risk for cognitive impairment due to AD and/or vascular pathology, entities collectively accounting for the vast majority of dementia cases worldwide.[50] WM integrity can be evaluated using DTI-derived measures, with FA (expressing the degree to which a single diffusion orientation is dominant) and MD (expressing the average amount of apparent diffusion along each of the three

TABLE 5 Associations of leptin markers with MRI and cognitive outcomes in different sex subgroups.

			Men[b]			Women[b]		
Parameter	**Leptin marker**	***p* for interaction[a]**	***β***	**SE**	***p*-value**	***β***	**SE**	***p*-value**
MRI outcomes[c]			*n* = 958			*n* = 1070		
Total brain volume[d]	Leptin[e]	0.05	0.066	0.042	0.11	−0.045	0.040	0.26
Cognitive outcome[f]			*n* = 1076			*n* = 1186		
HVOT[e]	Leptin[e]	0.04	**0.112**	**0.047**	**0.02**	−0.016	0.040	0.70
	sOB-R[e]	0.04	−0.058	0.034	0.09	0.032	0.028	0.26
	FLI[e]	0.007	**0.123**	**0.044**	**0.006**	−0.036	0.038	0.35
TMT part B[e,g]	FLI[e]	0.1	**0.092**	**0.044**	**0.04**	−0.004	0.038	0.93

Note: Values represent beta coefficients (*β*), and their corresponding standard errors (SE) and *p*-values (*p*). Statistically significant findings at $p \leq 0.05$ are indicated in bold.
Abbreviations: FLI, free leptin index; HVOT, Hooper Visual Organization Test; MRI, magnetic resonance imaging; sOB-R, soluble leptin receptor.
[a]Linear regression models with the respective MRI marker or neuropsychological test score as the outcome and the respective leptin marker, sex, as well as their product term as the main predictors. Values represent the *p*-value of the respective product term (*p* for interaction). Only significant findings at $p \leq 0.1$ are presented.
[b]Linear regression models with the respective MRI marker or neuropsychological test score as the outcome and the respective leptin marker as the main predictor, conducted by sex subgroups.
[c]Models are adjusted for age, diabetes mellitus, body mass index, systolic blood pressure, antihypertensive medication use, current smoking, prevalent cardiovascular disease, and time interval between blood draw and MRI acquisition.
[d]as percentage of total cranial volume.
[e]These variables have been natural log-transformed to normalize their distributions.
[f]Models are adjusted for age, education, diabetes mellitus, body mass index, systolic blood pressure, antihypertensive medication use, current smoking, prevalent cardiovascular disease, and time interval between blood draw and neuropsychological assessment.
[g]Scores were multiplied by -1 so that higher scores reflect better cognitive performance.

TABLE 6 Associations of leptin markers with MRI and cognitive outcomes in different WHR subgroups.

			WHR Q_1-Q_3[b]			WHR Q_4[b]		
Parameter	**Leptin marker**	***p* for interaction[a]**	***β***	**SE**	***p*-value**	***β***	**SE**	***p*-value**
MRI outcomes[c]			*n* = 1543			*n* = 483		
PSMD[d,e]	sOB-R[d]	0.04	**0.054**	**0.023**	**0.02**	**0.156**	**0.048**	**0.001**
	FLI[d]	0.06	−0.059	0.031	0.06	**−0.188**	**0.068**	**0.006**

Note: Values represent beta coefficients (*β*), and their corresponding standard errors (SE) and *p*-values (*p*). Statistically significant findings at $p \leq 0.05$ are indicated in bold.
Abbreviations: FLI, free leptin index; HVOT, Hooper Visual Organization Test; MRI, magnetic resonance imaging; sOB-R, soluble leptin receptor; WHR, waist-to-hip ratio; WMH, white matter hyperintensity volume.
[a]Linear regression models with the respective MRI measure or neuropsychological test score as the outcome and the respective leptin marker, WHR, as well as their product term as the main predictors. Values represent the *p*-value of the respective product term (*p* for interaction). Only significant findings at $p \leq 0.1$ are presented.
[b]Linear regression models with the respective MRI measure or neuropsychological test score as the outcome and the respective leptin marker as the main predictor, conducted in different WHR subgroups.
[c]Models are adjusted for age, sex, diabetes mellitus, body mass index, systolic blood pressure, antihypertensive medication use, current smoking, prevalent cardiovascular disease, and time interval between blood draw and MRI acquisition.
[d]These variables have been natural log-transformed to normalize their distributions.
[e]Models are further adjusted for total cranial volume.

diffusion axes) being among the most commonly used ones; higher FA and lower MD indicate higher tissue integrity.[51] A more recent diffusion metric is the FW index, which measures the fraction of the diffusion signal explained by isotropically unrestricted water.[21] FW has been proposed as a sensitive biomarker of cognitive function and of subtle brain injury related to vascular risk factors.[51,52] It might also aid in the differentiation between healthy individuals and those with AD or MCI.[53] Although the precise biological mechanisms linking increases in WM FW signal to AD or vascular pathology remain unclear, these could, for example, involve WM inflammation in the setting of AD,[54] or vasogenic edema and intramyelinic vacuolization in the setting of small vessel disease.[55]

Herein, we report associations of leptin bioavailability with all markers of WM integrity considered in the present analysis. These findings

support the putative neuroprotective role of leptin,[13,56,57] and might account for its previously reported associations with lower dementia risk.[8] Specifically, the link of higher leptin bioavailability during midlife with better preservation of WM microstructure suggests a potential for better maintenance of cognitive functions and, consequently, lower dementia risk later in life by virtue of more efficient connectivity. Of note, the effect sizes of the associations between leptin bioavailability and PSMD were larger in participants with excess WHR, indicating that potential benefits might be even higher for individuals with visceral obesity.

Importantly, we replicated the association of increased leptin receptor concentrations with higher PSMD in an independent sample of dementia-free Mexican American older adults, expanding the generalizability and external validity of our findings. Associations with other DTI measures were not significant in our replication sample, likely due to the considerably smaller size ($n = 89$), limiting the power to detect associations compared to FHS. Nevertheless, these findings highlight the sensitivity of PSMD to capture associations between measures of interest and WM microstructural changes, even in small samples. This might be related to a variety of reasons. First, MD is one of the most robust metrics with respect to underlying WM tissue characteristics.[58] In contrast, FA has been criticized for its dependency on fiber geometry. Specifically, higher FA values might not necessarily reflect better tissue integrity (e.g., higher fiber density, lower membrane permeability, greater myelination, etc.), due to the presence of crossing fibers throughout the majority of cerebral WM.[58] Notably, PSMD performs even better than traditional MD by leveraging skeletonization to focus the analysis of MD on the main fiber tracts, eliminating CSF contamination.[22] Perhaps even more importantly for replication purposes, the peak width is calculated as the difference between the 95th and 5th percentiles of the voxel-based MD values within the skeleton and does not depend on absolute MD values, rendering PSMD less prone to inter-scanner and inter-study differences than other DTI parameters.[22]

4.4 | Leptin markers and subclinical neuropathology

Identifying associations with risk factors and health outcomes of interest during the preclinical dementia stages is challenging due to often normal cognitive measures. Biomarkers of amyloid-β and tau pathology [59–61] offer valuable alternatives for studying such associations during the preclinical stages of AD. However, for VCID such well-characterized biomarkers were, until recently, lacking.[62] Prior investigations have demonstrated that alterations in WM microstructure precede the development of cognitive decline in individuals who will later develop cognitive impairment due to either AD-driven[63–65] or vascular pathology,[49,66] highlighting their potential utility as susceptibility/risk biomarkers in these settings.[67] Capitalizing on these prior insights, we leveraged DTI-derived measures as sensitive surrogate markers of subclinical neuropathology in two independent samples of dementia-free individuals to study and identify associations with leptin bioavailability.

4.5 | Limitations

The mean delay of 8 years between exposure (leptin markers) and outcome (MRI and cognitive measures) assessment might have introduced noise to our data and led to false negative findings. Nevertheless, the detection of associations between leptin bioavailability and WM microstructural integrity despite a potentially increased noise-to-signal ratio, suggests that the underlying relationships might in fact be even stronger. We also replicated the association of higher leptin receptor concentration with worse WM microstructural integrity, as assessed with PSMD, in an older sample of different ethnicity with synchronous exposure and outcome assessments. Nevertheless, the cross-sectional design of the present analysis does not allow for causal or temporal inferences. Longitudinal studies are needed to examine associations of leptin bioavailability in middle age with the risk for dementia or cognitive decline later in life. Studies exploring associations with biomarkers of amyloid-β and/or tau pathology would also be important to elucidate whether lower leptin bioavailability is associated with the presence of core AD neuropathology in cognitively intact middle-aged adults.

5 | CONCLUSION

In summary, the present findings support the putative role of leptin perturbations in late-life dementia risk by relating relative leptin deficiency (i.e., lower leptin bioavailability) with alterations in WM microstructure, an early event in the pathogenetic processes of cognitive impairment due to AD-driven and/or vascular pathology.

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clinical trials aimed at identifying disease-modifying therapies for VCID. For up-to-date information, see www.markvcid.org.

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CONFLICT OF INTEREST STATEMENT

Dr. DeCarli reports consulting for Novartis Pharmaceuticals. Dr. Seshadri reports consulting for Eisai and Biogen. The rest of the authors have nothing to disclose. Author disclosures are available in the supporting information.

CONSENT STATEMENT

All study participants have provided written informed consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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