Dementia is increasingly recognized as a life-course illness wherein a variety of lifestyle choices interact with genetic, vascular, and other risk factors to affect risk of disease. Given the rapid aging of developed and developing societies, it is projected that the prevalence of dementia will dramatically increase during the next 5 decades. Therefore, it is a public health priority to explore pathophysiological pathways underlying the development of dementia and its most common cause, Alzheimer disease (AD).

Novel risk factors investigated in the past decade include indices of midlife obesity (overall and central obesity), diabetes, the metabolic syndrome, and insulin resistance. Prospective studies have shown that overweight and obesity in mid-life are associated with poorer cognitive function in the general population and an increased risk of incident dementia. The mechanisms underlying these associations are not completely understood. Leptin, an adipokine that is produced in subcutaneous and visceral adipose tissue, is a plausible biological mediator; its physiological role appears to include signaling long-term caloric intake and fat stores to the hypothalamus, thereby modifying food consumption and energy expenditure. However, another emerging role for leptin, one that may permit survival under conditions of limited food availability, is the facilitation of memory processes. Recent evidence confirms that leptin exerts additional functions on the brain outside the hypothalamus, in particular in...
METHODS

Study Sample
Details of the recruitment and phenotyping of the Framingham Study original cohort have been described in detail elsewhere. The study was initiated in 1948 to identify risk factors for heart disease in the community. A total of 5209 participants were included and are seen in the Heart Study research clinic approximately every 2 years, in which a detailed medical history is obtained and all traditional cardiovascular risk factors are measured. Of 1060 dementia-free persons who attended the twenty-second examination cycle and were subsequently followed up for incident dementia until December 31, 2007, leptin levels were measured in 785 participants. In this study sample, participants who did not have leptin levels measured were significantly older than those with leptin measured. However, after adjustment for age, participants without and with leptin levels did not differ with respect to their baseline clinical or biochemical characteristics.

A subset of 198 individuals (33% of individuals surviving until 1999) underwent volumetric brain MRI between 1999 and 2005. The 406 individuals who survived until 1999 but did not undergo brain MRI either declined consent, had a contraindication to brain MRI (such as claustrophobia or a cardiac pacemaker), did not attend an on-site study examination between 1999 and 2005, or died before the MRI could be obtained. However, their clinical and biochemical characteristics did not differ substantially from those with available MRI (eTable 2). In addition, 16 persons were excluded from the analyses because they developed clinical stroke (n=11), dementia (n=4), or both (n=1) prior to the date of MRI and 4 persons were excluded with neurological conditions (such as a brain tumor) that would affect the assessment of brain MRI measures. At the 22nd examination cycle, the Center for Epidemiologic Studies for Depression scale was administered, which is an established tool to screen for depressive symptoms in community-based samples. The study protocol was approved by the institutional review board of the Boston University Medical Center and all participants provided written informed consent.

Leptin Assay
A commercial radioimmunoassay (Linco Research Inc, St Louis, Missouri) was used to determine leptin concentrations at the 22nd examination cycle (1990-1994) from nonfasting plasma samples. The interassay coefficient of variation ranged from 3.0% to 6.2%. The lower sensitivity limit of the assay was 0.5 ng/mL.

Brain Imaging
The imaging parameters, measurement protocols, and reproducibility of these measures have been described. The MRIs were obtained with a Siemens Magnetom 1-T field strength machine using a double spin-echo coronal imaging sequence of 4-mm contiguous slices from nasion to occiput. Imaging analyses were performed at a central location using a custom-designed image-analysis package QUANTA 6.2, operating on a Sun Microsystems (Santa Clara, California) Ultra 5 workstation. Brain images were evaluated by experienced clinicians who were blinded to the participants’ demographic, anthropometric, and clinical data, including leptin concentrations. Lobar and ventricular volumes were computed by rotating the images into anatomical standard space and subsequent operator-defined outlining of the individual lobes using standard anatomical landmarks. Because the hippocampus comprises the medial wall of the temporal horn, shrinkage of the hippocampus results in enlargement of the temporal horn. Intrarater and interrater correlations using this method were high.

Manual outlining of the intracranial vault and subsequent mathematical modeling was used to determine total brain parenchymal volume above the tentorium. The TCBV was calculated as the ratio of total brain parenchymal volume to total intracranial volume, thus adjusting for head size. The THV also was converted to a ratio over total intracranial volume and log-transformed to normalize the distribution. The THV of the lateral ventricles served as a surrogate marker inversely.
related to hippocampal volume.\textsuperscript{11,21} We used this surrogate marker because direct measures of hippocampal volume were not available in all participants.

**Clinical Definition of Dementia and AD**

All participants in the Framingham study are under periodic surveillance for impairment in cognitive function and dementia. The screening and surveillance methods for the detection of incident dementia in the Framingham original cohort have been outlined.\textsuperscript{22,23} Briefly, surviving cohort members who were deemed to be free of incident dementia based on a standardized neuropsychological test were followed up with a biennial history, physical examination, and administration of the Folstein Mini-Mental State Examination. Participants who were suspected to have possible cognitive decline based on the Mini-Mental State Examination score; self, physician, or family referral; telephone health status update; or records linkage underwent an in-depth evaluation that included neurological and neuropsychological evaluations.

We determined whether each person fulfilled criteria for a diagnosis of dementia. The probable date of onset and type of dementia was determined at a consensus review by a panel composed of at least 1 behavioral neurologist and 1 neuropsychologist. The panel reviewed all available records including examinations by the Framingham Heart Study investigators (Z.S.T., R.A., S.A., P.A.W., S.S.), hospital and nursing home records, data from structured family interviews, imaging, and when available, autopsy data. Participants with dementia met criteria outlined in the *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition),\textsuperscript{24} and were required to have symptoms for at least 6 months. Participants with AD met the criteria for definite, probable, or possible AD from the National Institute of Neurological Diseases and Stroke and the Alzheimer’s Disease and Related Disorders Association.\textsuperscript{25} For the present analyses, data for incident dementia obtained until December 31, 2007, were used.

**Statistical Analyses**

Circulating leptin levels were significantly higher in women and had a right-skewed distribution in each sex. Hence, leptin levels were first natural logarithmically transformed and then standardized within each sex (mean [SD], 0 [1]). Cox regression models were used to relate baseline sex-standardized log-leptin levels to the incidence of dementia and AD after confirming that the assumption of proportionality of hazards was met. Because the risk of dementia is more likely to change as a function of age than of calendar time, age was used as the time scale, except for the FIGURE, in which calendar time was displayed on the x-axis to facilitate interpretation. Initial analyses were adjusted for age and sex alone (model A). Model B additionally adjusted for 2 major risk factors for dementia in the cohort (ie, a high plasma homocysteine level [ie, level in the top age-specific quartile]) and presence or absence of an apolipoprotein E ε4 allele (APOE ε4). In model C, we additionally adjusted for other potential confounders, including waist-to-hip ratio and systolic blood pressure, which were treated as continuous variables, and presence or absence of antihypertensive treatment, diabetes, smoking, and atrial fibrillation at baseline. In additional analyses, waist-to-hip ratio was replaced with body mass index (BMI; calculated as weight in kilograms divided by height in meters squared) in the multivariable-adjusted models. We also tested for effect modification by obesity (BMI ≥30) of the association between leptin and dementia by including a leptin × obesity interaction term in the model. Because that interaction term was significant, we performed an analysis stratified by the presence or absence of obesity. We chose not to include both waist-to-hip ratio and BMI in the same model due to marked colinearity between these 2 variables. Waist-to-hip ratio was selected as the preferred metric of body fat content and distribution because it is more strongly correlated with plasma leptin levels,\textsuperscript{26} with midlife cognitive function in the Framingham Offspring cohort,\textsuperscript{3} and with the subsequent risk of AD in other cohorts.\textsuperscript{22} In secondary analyses, we also adjusted for change in waist-to-hip ratio from the twenty-first examination cycle to the twenty-second examination cycle (when leptin was assayed; model D) and also adjusted for baseline depression (defined as a Center for Epidemiologic Studies for Depression scale score ≥16; model E).\textsuperscript{28} Furthermore, we tested for effect modification by waist-to-hip ratio (relationship of leptin with incident dementia and AD) and by presence or absence of an APOE ε4 allele by including interaction terms in the statistical models. Because the interaction with waist-to-hip ratio was significant, the analyses were stratified by waist-to-hip ratio, evaluating the association between leptin and incident dementia and AD separately in participants in the fourth sex-specific waist-to-hip ratio quartile and in quartiles 1 through 3. In addition, the analyses were repeated after excluding participants who were diagnosed with dementia in the first 3 years after the baseline examination to exclude reverse causality (ie, the possi-
bility that weight loss or other neuroendocrine changes secondary to early undetected AD changes were altering baseline leptin levels. Linear regression models were used to relate sex-standardized log leptin to TCBV and to log THV. Covariate adjustments were the same as for the Cox regression models (models A-C). Statistical analyses were performed using SAS statistical software version 9.1 (SAS Institute Inc, Cary, North Carolina) and all statistical tests were 2-sided. A P value of less than .05 was used to indicate statistical significance.

### Power Estimates

The study had 90% power to detect hazard ratios of 0.55 or smaller for incident dementia comparing persons with leptin levels above those below the sex-specific median. For the regression analyses, the study had 90% power to detect partial R² values of 0.050 for TCBV and 0.055 for log THV.

### RESULTS

#### Baseline characteristics of the entire study sample and stratified by sex-specific leptin quartiles are displayed in Table 1 (baseline characteristics are stratified by sex in eTable 3). We observed a relatively high prevalence of cardiovascular disease in this elderly sample, which was expected, with an increasing burden of cardiovascular disease and risk factors across leptin quartiles. Leptin levels were significantly higher in women, as shown in prior studies. Mean (SD) TCBV in our sample was 0.73 (0.03) and mean (SD) log THV was −1.95 (0.71).

#### Association of Leptin With Incident Dementia and AD

During a median follow-up of 8.3 years (range, 0-15.5 years), 111 participants developed incident dementia; 89 of them were diagnosed with AD. Log-leptin levels showed a strong inverse relationship to the risk of incident all-cause dementia and AD in all models (Table 2), remaining statistically significant after the full covariate adjustment in model C. Additional adjustment for change in waist-to-hip ratio prior to the baseline examination and for depression (Table 2), as well as excluding participants who developed clinical dementia within 3 years after leptin was measured (eTable 4), did not alter the results. Replacing waist-to-hip ratio with BMI revealed comparable results in the multivariable-adjusted model (Table 2) except that the hazard ratio for all-cause dementia was not statistically significant.

The Figure demonstrates the cumulative incidence of AD over 15.5 years. In good agreement with our previous findings, the incidence of dementia decreases gradually across increasing sex-specific leptin quartiles; thus, a person with a baseline leptin level in the lowest quartile (Q1) had a 25% risk of developing AD after 12 years of follow-up, whereas the corresponding risk for...
a person in the top quartile (Q4) was only 6% (P < .001 for the overall test of equality [-2 log] among the 4 leptin quartiles). Cumulative incidence (as a measure of absolute risk) and hazard ratios for incident AD and allcause dementia comparing each quartile of baseline leptin concentration with the referent lowest quartile are shown in Table 3. We observed effect modification by waist-to-hip ratio of the association between leptin levels and incident dementia and AD (P = .003 for leptin × waist-to-hip ratio interaction term in the models predicting allcause dementia and P = .04 for AD). The stratified analyses revealed a strong inverse association between leptin and dementia and AD in participants in the first 3 sex-specific waist-to-hip ratio quartiles (Table 4). In the fourth waist-to-hip ratio quartile, no significant association between leptin and incident dementia was observed. When individuals were stratified by BMI of less than 30 or greater than or equal to 30, the association was significant only for BMI less than 30. However, we had only a limited number of events in the subgroups for the fourth waist-to-hip ratio quartile and BMI of 30 or greater. We observed no effect modification by APOE ε4 allele (P = .32 for interaction term APOE ε4 × leptin for the models predicting all-cause dementia and P = .79 for AD). We also observed no significant interaction between sex and leptin and their association with dementia or AD (eTable 5).

Association of Leptin Levels With MRI Measures of Brain Aging

The mean interval between leptin measurement and brain MRI was 7.7 years. Leptin levels were positively associated with TCBV in the models adjusting for age, sex, dementia risk factors, vascular risk factors, waist-to-hip ratio, and depression (Table 5). In addition, leptin was inversely associated with THV in age-adjusted and sex-adjusted models; however, additional adjustments for neurodegenerative or vascular risk factors rendered the association nonsignificant (Table 5).

Comment

In our moderately sized sample from the general population, higher leptin levels at baseline were prospectively associated with a lower incidence of AD and dementia. The association of high leptin levels with a lower incidence of all-cause dementia and AD remained significant after adjustment for traditional vascular risk factors and for waist-to-hip ratio. Although this association was not statistically significant in participants with higher waist-to-hip ratio and higher BMI, the numbers of participants in those subgroups were limited. The overall findings are intriguing given the emerging, if speculative, hypothesis that one reason for the observed association of midlife central obesity with subsequent risk of AD may be an acquired resistance to effects of leptin, including its neuroprotective effects. In addition, in a smaller subsample of survivors, higher leptin levels were associated with higher TCBV.

A growing body of evidence suggests that leptin has beneficial effects on brain development and function. Leptin-deficient mice have a lower brain weight, an immature expression pattern of synaptic and glial proteins, and disrupted projection pathways within the hypothalamus, indicating that leptin is necessary for normal brain development. Furthermore, leptin appears to mediate structural and functional changes in the hippocampus and to improve memory function. Leptin receptors are present in the CA1 region of the hippocampus and leptin-deficient or insensitive rats show reduced synaptic plasticity and poorer performance in spatial memory tasks. Leptin facilitates N-methyl-D-aspartate receptor-mediated conversion of short-term potentiation to long-term potentiation in the hippocampus and also improves neuronal survival.

Leptin also has been shown to increase apolipoprotein E–dependent β-amyloid uptake into the cell and reduce brain extracellular concentrations of β-amyloid, the major component of...
the neuritic plaques that are a histopathological hallmark of AD.37 Leptin and insulin act in a dose-dependent and synergistic manner to decrease hyperphosphorylation of tau, the primary component of the neurofibrillary tangle, the second major histopathological hallmark of AD.38 Most interesting is a recent observation that chronic leptin treatment improved memory performance in transgenic animal models of AD.39

Our epidemiological observations of an inverse association of baseline leptin concentrations with incident dementia in general and with AD in particular are consistent with these experimental results, as are our observations of a positive relationship of leptin with TCBV and (in age-adjusted and sex-adjusted models but not fully adjusted models) with THV. A 2008 small study of brain MRI in 34 elderly volunteers found that higher leptin levels were associated with larger hippocampal and parahippocampal gray matter volumes, but it did not observe any association of leptin with total cerebral brain volume and temporal horn volume.

### Table 3. Hazard Ratios (HRs) for Incident All-Cause Dementia and Alzheimer Disease According to Sex-Specific Quartiles of Baseline Leptin Levels

<table>
<thead>
<tr>
<th>Leptin Sex-Specific Quartiles</th>
<th>No. of Events/Persons at Risk</th>
<th>HR (95% CI)</th>
<th>P Value</th>
<th>No. of Events/Persons at Risk</th>
<th>HR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted for age and sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>37/165</td>
<td>1 [Reference]</td>
<td>.001</td>
<td>33/165</td>
<td>1 [Reference]</td>
<td>.001</td>
</tr>
<tr>
<td>Q2</td>
<td>27/158</td>
<td>0.86 (0.50-1.46)</td>
<td>.57</td>
<td>22/158</td>
<td>0.86 (0.48-1.54)</td>
<td>.61</td>
</tr>
<tr>
<td>Q3</td>
<td>19/164</td>
<td>0.48 (0.26-0.87)</td>
<td>.02</td>
<td>17/164</td>
<td>0.52 (0.27-0.99)</td>
<td>.045</td>
</tr>
<tr>
<td>Q4</td>
<td>12/154</td>
<td>0.42 (0.20-0.84)</td>
<td>.02</td>
<td>5/154</td>
<td>0.23 (0.08-0.61)</td>
<td>.003</td>
</tr>
</tbody>
</table>

### Table 4. Association of Sex-Standardized Log Leptin With Incident All-Cause Dementia and Incident Alzheimer Disease

<table>
<thead>
<tr>
<th>Modela</th>
<th>All-Cause Dementia</th>
<th>Alzheimer Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Cases/Total</td>
<td>HR (95% CI)b</td>
</tr>
<tr>
<td>Waist-to-hip ratio Quartiles 1-3</td>
<td>79/586</td>
<td>0.57 (0.45-0.73)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>31/195</td>
<td>0.92 (0.60-1.40)</td>
</tr>
<tr>
<td>Body mass indexc ≤30</td>
<td>95/561</td>
<td>0.69 (0.55-0.87)</td>
</tr>
<tr>
<td>≥30</td>
<td>16/221</td>
<td>1.87 (0.86-4.06)</td>
</tr>
</tbody>
</table>

### Table 5. Association of Sex-Standardized Log Leptin Levels With Magnetic Resonance Imaging Precursors of Subclinical Alzheimer Diseasea

<table>
<thead>
<tr>
<th>Total Cerebral Brain Volume</th>
<th>Temporal Horn Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>β (SE)b</td>
<td>P Value</td>
</tr>
<tr>
<td>Model A: age- and sex-adjusted</td>
<td>0.76 (0.26)</td>
</tr>
<tr>
<td>Model B: model A plus 2 risk factors for dementia</td>
<td>0.88 (0.28)</td>
</tr>
<tr>
<td>Model C: models A and B plus other vascular risk factors</td>
<td>0.88 (0.31)</td>
</tr>
<tr>
<td>Model D: model C plus CES-D scale</td>
<td>0.88 (0.31)</td>
</tr>
</tbody>
</table>

Abbreviations: CES-D: Center for Epidemiologic Studies Depression Scale; SE: standard error.

β indicates natural logarithmically transformed.

*Regression coefficient β indicates the increase in total cerebral brain volume and temporal horn volume per 1-SD increment in sex-standardized log leptin.

*Adjusted for model A, high plasma homocysteine level (ie, level in the top age-specific quartile), and presence or absence of an apolipoprotein E e4 allele.

*Adjusted for models A and B plus waist-to-hip ratio, systolic blood pressure, presence or absence of antihypertensive treatment, diabetes, smoking, and atrial fibrillation.
total brain volumes; thus, it is likely that the study was underpowered to detect an association such as we observed. Together, these data support the concept that leptin exerts multiple functions in the brain, beyond those involved in food consumption and energy expenditure. Interestingly, the association of leptin with AD and all-cause dementia is independent of classic neurodegenerative and vascular risk factors, suggesting that leptin might act along a new pathway relevant to cognitive function in humans. Although our study was observational, the biological plausibility of the findings, the temporal relationships (leptin measurements antedated dementia), and the consistency of results in multiple analyses (including an association with subclinical structural indices that have been correlated with cognitive function) suggest that the association may be a causal one, a premise that merits further investigation. The association of leptin with dementia was found in nonobese individuals but was not statistically significant in obese individuals. However, the number of obese participants and events in that group are substantially smaller compared with the nonobese participants, limiting the statistical power to detect modest associations.

The availability of prospective data on hard clinical end points (incident dementia and AD) as well as measures of subclinical disease (TCBV and THV), the comprehensive assessment of covariates, and the community-based sample strengthen this study. One potential explanation for the stronger association of TCBV might be that it is a lower inherent variability when compared with THV. One limitation is the restriction of this sample to older individuals with European ancestry; thus, it is unclear whether these findings are applicable to other ethnicities or age groups. Furthermore, we did not measure leptin in the cerebrospinal fluid or in the brain parenchyma. However, the correlation between plasma and cerebrospinal fluid leptin is high. Given our sample size, we had only modest power to exclude multivariable-adjusted associations of leptin with THV and we cannot rule out that the results relating leptin to MRI measures of brain aging (which were obtained almost 8 years after leptin was assayed) were biased through selective or conditional survival. In addition, no measures of physical activity, a potential confounder of the observed associations, were available at the baseline examination. Leptin levels were determined only once in each participant. This might have led to some random misclassification, likely biasing our results toward the null hypothesis, although the possibility of differential misclassification cannot be excluded. The literature indicates that leptin levels have intraclass correlation coefficients of 0.80 over 3 years. Leptin levels were not measured in our participants while they were middle-aged, so we are unable to address the relationship between mid-life leptin and cognitive outcomes. Furthermore, whereas published data suggest that leptin levels remain stable over time, the temporal stability of levels in obese individuals has not been specifically addressed.

In conclusion, in our community-based sample, higher baseline concentrations of leptin were associated with a reduced incidence of dementia and AD, even after adjustment for waist-to-hip ratio. Furthermore, higher leptin levels were associated with larger brain parenchymal and smaller ventricular volumes. These findings are consistent with recent experimental data indicating that leptin improves memory function in animals through direct effects on the hippocampus and strengthens the evidence that leptin is a hormone with a broad set of actions in the central nervous system. Due to the exploratory character of the present analyses, we did not adjust for multiple comparisons and acknowledge that our findings require confirmation in independent samples. If our findings are confirmed by others, leptin levels in older adults may serve as one of several possible biomarkers for healthy brain aging and, more importantly, may open new pathways for possible preventive and therapeutic intervention. Further exploration of the molecular and cellular basis for the observed association may expand our understanding of the pathophysiology underlying brain aging and the development of AD.

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Author Contributions: Dr Seshadri had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Study supervision: Seshadri.

Financial Disclosures: None reported.

Funding/Support: This work was supported by contracts N01-HE-25195, N01HV28178, RO1 DK080739, and DK045856 (awarded to Dr Vasan) from the National Institutes of Health and the National Heart, Lung, and Blood Institute; grants R01 AG16495, AG08122, AG033193, and AG031287 from the National Institute on Aging; and grant R01 NS17950 from the National Institute of Neurological Disorders and Stroke.

Role of the Sponsors: The sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Disclaimer: The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Neurological Disorders and Stroke, the National Heart, Lung, and Blood Institute, the National Institute on Aging, or the National Institutes of Health.

Additional Information: eTables 1 through 5 are available at http://www.jama.com.

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PLASMA LEPTIN LEVELS WITH INCIDENT ALZHEIMER DISEASE