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# Association of cognition with leptin and vascular endothelial growth factor in individuals with type 2 diabetes mellitus

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## Abstract

**Objective:** The 10-year intensive lifestyle intervention (ILI) of the Look AHEAD study left a legacy of relative deficits in cognitive function among participants who entered the clinical trial with obesity or a history of cardiovascular disease. We hypothesized that altered levels of two weight-sensitive proangiogenic cytokines, leptin and vascular endothelial growth factor (VEGF), accounted for this concerning finding.

**Methods:** Serum leptin and VEGF concentrations were determined in 1,279 Look AHEAD participants at baseline, proximal to cessation of the interventions (Epoch 1), and an average of 4 years later (Epoch 2). Up to four standardized assessments of attention, executive function, and memory were collected during follow-up. Mixed effects models were used to assess relative differences in leptin and VEGF concentrations between intervention groups and whether these accounted for changes in cognitive composite scores.

**Results:** ILI and diabetes support and education differences in VEGF, but not leptin, concentrations varied depending on baseline history of cardiovascular disease and obesity, but neither leptin nor VEGF concentrations accounted for the relative decrements in cognitive function in participants assigned to ILI.

**Conclusions:** Alterations in two weight-sensitive proangiogenic cytokines did not account for the long-term adverse effects of ILI on cognitive function among adults with diabetes and either obesity or cardiovascular disease.

## INTRODUCTION

Multidomain lifestyle interventions hold promise to benefit cognitive function through many mechanisms, and there is considerable interest in assessing their effectiveness across diverse cohorts [1]. It is unlikely, however, that all individuals benefit equally from these interventions, and trials have reported that relative intervention effects on cognition vary across clinical subgroups, with potential benefits limited to adherent participants with untreated hypertension or no history of cardiovascular disease [2] or who were amyloid positive [3]. This heterogeneity is also supported by post hoc analyses from the Action for Health in Diabetes (Look AHEAD) randomized controlled clinical trial. Although there was some evidence that the intensive weight-loss intervention tested by the trial prevented the development of self-reported cognitive deficits [4], there was no overall long-term benefit for cognitive function [5]. Of concern, there was some evidence that the lifestyle intervention led long term to relative deficits in cognitive abilities and function among individuals with obesity and with history of cardiovascular disease [4–7]. This manuscript is based on exploratory analyses to examine whether two weight-sensitive biomarkers related to angiogenesis may provide information about the possible mechanisms underlying these concerning findings.

Leptin is an adipokine that is transported into the brain and linked to vascular repair and angiogenesis [8, 9]. Reports showed that higher concentrations of plasma leptin were associated with better cognitive functioning in individuals with diabetes [10]. Levels of leptin may be substantially lowered by weight loss, and this effect may be much greater among individuals with obesity [11, 12], the group with the greatest weight losses in Look AHEAD [13]. One speculation is that the greater weight loss among participants with obesity reduced levels of leptin, blunting any neurovascular response (e.g., angiogenesis, increased cerebral blood flow) and leading to neurodegeneration and cognitive inefficiency. However, other reports showed that higher levels of leptin may adversely affect cognition: they were associated with increased levels of atherosclerosis and inflammation and, in a cohort free of obesity, poorer cognitive function [14].

Vascular endothelial growth factor (VEGF) is a signaling protein that promotes angiogenesis and glucose uptake in the brain and it may protect the brain from hypoglycemia [15–17]. Substantial weight loss associated with bariatric surgery in Class 3 obesity led to marked reductions in VEGF concentrations [18]. Diet- and physical activity-induced weight losses in less heavy individuals may also result in decreases in VEGF concentrations, albeit more modest than those from surgery [19]. We speculated that the greater weight loss among Look AHEAD participants with obesity resulted in reduced levels of VEGF, blunting any neurovascular response and leading long term to cognitive deficits.

We therefore hypothesized that the Look AHEAD multidomain lifestyle intervention would lead to a legacy of relatively lower levels of leptin and VEGF and that this would result in lower cognitive function among individuals at increased risk for neurodegeneration due to obesity and history of cardiovascular disease.

### Study Importance

#### What is already known?

- Relative to diabetes support and education, the intensive lifestyle intervention of the Look AHEAD clinical trial among individuals with type 2 diabetes left a legacy of cognitive deficits among participants with obesity or cardiovascular disease history at baseline.
- Two weight-sensitive proangiogenic cytokines, leptin and vascular endothelial growth factor, are known to be associated with cognitive function.

#### What does this study add?

- Although the intensive lifestyle intervention was associated with alterations in serum leptin and vascular endothelial growth factor concentrations, concentrations of these cytokines had little association with cognitive function.
- Alterations in leptin and vascular endothelial growth factor levels did not account for the long-term adverse relative effects that the intensive lifestyle intervention had on cognitive function among those with obesity or a history of cardiovascular disease.

#### How might these results change the direction of research?

- Other mechanisms should be explored to understand how intensive lifestyle intervention may adversely affect cognitive function in important clinical subgroups.

## METHODS

The Look AHEAD design, methods, and CONSORT (Consolidated Standards for Reporting Trials) diagram have been published previously [20, 21]. Look AHEAD was a multisite, single-blind randomized controlled clinical trial that enrolled 5,145 individuals (during 2001 to 2004) from 16 US centers. All participants had type 2 diabetes and met the following criteria: 45 to 76 years of age, BMI > 25 kg/m$^2$ (> 27 kg/m$^2$ if on insulin), glycated hemoglobin ($HbA_{1c}$) < 97 mmol/mol (11%), systolic/diastolic blood pressure < 160/< 100 mmHg, triglycerides < 600 mg/dL, and successful passing of a maximum graded exercise test. The study interventions were stopped in September 2012, and participants continued to be monitored with observational follow-up studies. Protocols and consent forms were approved by local Institutional Review Boards.

The Look AHEAD MIND ancillary study administered standardized cognitive assessments to the cohort. It also performed analyses of stored samples from a subset of individuals, oversampling those who were enrolled in six clinical sites that participated in prior ancillary studies involving cognitive assessments [22, 23] or who had prior study-based

**TABLE 1** Baseline characteristics of cohort by intervention assignment

| | DSE ($N = 634$) | ILI ($N = 645$) | *p* value |
|---|---|---|---|
| Sex | | | |
| Female | 257 (40.5%) | 265 (41.1%) | 0.84 |
| Male | 377 (59.5%) | 380 (58.9%) | |
| Current age, y, mean ± SD | 58.5 ± 6.6 | 58.6 ± 6.6 | 0.87 |
| Race/ethnicity | | | |
| African American/Black (not Hispanic) | 110 (17.4%) | 110 (17.1%) | 0.88 |
| American Indian/Native American/Alaskan Native | 10 (1.6%) | 8 (1.2%) | |
| Asian/Pacific Islander | 4 (0.6%) | 7 (1.1%) | |
| White | 397 (62.6%) | 395 (61.2%) | |
| Hispanic | 104 (16.4%) | 117 (18.1%) | |
| Other/multiple | 9 (1.4%) | 8 (1.2%) | |
| Years of education, y | | | |
| <13 | 144 (23.4%) | 186 (29.5%) | 0.05 |
| 13–16 | 228 (37.1%) | 210 (33.3%) | |
| >16 | 243 (39.5%) | 235 (37.2%) | |
| BMI, kg/m$^2$ | | | |
| Mean ± SD | 35.6 ± 5.7 | 35.3 ± 5.7 | 0.39 |
| 25–29 | 100 (15.8%) | 114 (17.7%) | |
| 30–39 | 408 (64.4%) | 409 (63.4%) | |
| ≥40 | 126 (19.9%) | 122 (18.9%) | |
| Diabetes duration, y | | | |
| <5 | 298 (47.4%) | 294 (45.9%) | 0.59 |
| ≥5 | 331 (52.6%) | 347 (54.1%) | |
| CVD history[a] | 77 (12.1%) | 86 (13.13%) | 0.52 |
| Smoking status | | | |
| Never | 341 (53.9%) | 337 (52.4%) | 0.83 |
| Former | 266 (42.0%) | 281 (43.7%) | |
| Current | 26 (4.1%) | 25 (3.9%) | |
| $HbA_{1c}$, %, mean ± SD | 7.2 ± 1.2 | 7.3 ± 1.2 | 0.24 |
| Analyte, mean ± SD | | | |
| Leptin, ng/mL | 36.57 ± 26.0 | 34.99 ± 28.0 | 0.30 |
| VEGF, pg/mL | 289.2 ± 253.5 | 272.0 ± 224.1 | 0.21 |

Abbreviations: CVD, cardiovascular disease; DSE, diabetes support and education; $HbA_{1c}$, hemoglobin $A_{1c}$; ILI, intensive lifestyle intervention; VEGF, vascular endothelial growth factor.

[a]CVD history: self-report of prior myocardial infarction, coronary artery bypass, angioplasty/stent procedures, peripheral vascular disease, stroke, stable angina, and class I/II heart failure.

classification of mild cognitive impairment or dementia when these were done, approximately 4 years earlier [7].

## Interventions

Participants were randomly assigned to intensive lifestyle intervention (ILI) or diabetes support and education (DSE). The multidomain ILI targeted reducing caloric intake and increasing physical activity to induce weight loss to average > 7% at year 1 and to maintain this over time [24, 25]. Caloric consumption goals of 1,200 to 1,800 kcal/d were based on initial weight. Physical activity of >175 min/wk through activities similar in intensity to brisk walking was also targeted, as was improved diet quality (< 30% calories from fat, < 10% calories from saturated fat, > 15% calories from protein). Cardiometabolic risk factors (lipids; hemoglobin $A_{1C}$; blood pressure) were monitored, and participants were provided the results, which (with their consent) were shared with their clinicians. During the first 6 months, ILI participants attended three group meetings and one individual session per month. For the remainder of the first year, they were provided two group meetings and one individual meeting per month. The intensity of the intervention gradually decreased thereafter [24].

**TABLE 2** Mean changes from baseline in leptin, VEGF, and BMI (at leptin and VEGF assessments), by intervention group from mixed-effects model

| | Mean [95% CI] change from baseline | | |
|---|---|---|---|
| | Epoch 1 | Epoch 2 | *p* value |
| BMI, kg/m$^2$ | | | |
| DSE | −1.38 [−1.68, −1.08] | −1.63 [−1.94, −1.32] | DSE vs. ILI: 0.01 |
| ILI | −1.99 [−2.29, −1.69] | −2.05 [−2.36, −1.75] | Epoch 1 vs. 2: 0.07 |
| ILI-DSE | −0.61 [−1.03, −0.18] | −0.42 [−0.86, 0.01] | Interaction: 0.29 |
| Leptin, ng/mL | | | |
| DSE | 4.79 [2.70, 6.88] | 5.96 [3.80, 8.12] | DSE vs. ILI: 0.19 |
| ILI | 1.75 [−0.30, 3.82] | 5.43 [3.30, 7.55] | Epoch 1 vs. 2: <0.001 |
| ILI-DSE | −3.03 [−5.96, −0.09] | −0.54 [−3.57, 2.50] | Interaction[a]: 0.06 |
| VEGF, pg/mL | | | |
| DSE | −5.70 [−17.72, 6.32] | −20.72 [−33.18, −8.27] | DSE vs. ILI: 0.46 |
| ILI | 8.78 [−3.10, 20.66] | −23.85 [−36.14, −11.57] | Epoch 1 vs. 2: <0.001 |
| ILI-DSE | 14.48 [−2.42, 31.38] | −3.12 [−20.62, 14.37] | Interaction: 0.04 |

Abbreviations: DSE, diabetes support and education; ILI, intensive lifestyle intervention; VEGF, vascular endothelial growth factor.
[a]Epoch × intervention assignment interaction.

**TABLE 3** Mean domain-specific *z* scores at Epochs 1 and 2 by intervention assignment

| Cognitive domain (*z* scores[a]) | DSE, *N* = 628 | ILI, *N* = 641 | Difference: ILI-DSE, mean [95% CI] |
|---|---|---|---|
| Attention | | | |
| Epoch 1 | −0.25 | −0.35 | −0.10 [−0.22, 0.02] |
| Epoch 2 | −0.65 | −0.68 | −0.04 [−0.16, 0.09] |
| Change: mean [95% CI] | −0.40 [−0.45, −0.35] | −0.33 [−0.38, −0.29] | |
| Executive function | | | |
| Epoch 1 | −0.24 | −0.38 | −0.14 [−0.26, −0.02] |
| Epoch 2 | −0.51 | −0.63 | −0.12 [−0.24, 0.01] |
| Change: mean [95% CI] | −0.27 [−0.33, −0.21] | −0.25 [−0.31, −0.19] | |
| Memory | | | |
| Epoch 1 | −0.17 | −0.21 | −0.04 [−0.16, 0.06] |
| Epoch 2 | −0.28 | −0.31 | −0.02 [−0.15, 0.09] |
| Change: mean [95% CI] | −0.12 [−0.17, −0.06] | −0.09 [−0.15, −0.03] | |
| Global function | | | |
| Epoch 1 | −0.42 | −0.48 | −0.06 [−0.20, 0.09] |
| Epoch 2 | −0.61 | −0.73 | −0.11 [−0.27, 0.04] |
| Change: mean [95% CI] | −0.19 [−0.26, −0.12] | −0.25 [−0.32, −0.18] | |
| Composite | | | |
| Epoch 1 | −0.26 | −0.36 | −0.11 [−0.21, −0.00] |
| Epoch 2 | −0.53 | −0.61 | −0.08 [−0.19, 0.03] |
| Change: mean [95% CI] | −0.27 [−0.31, −0.24] | −0.25 [−0.28, −0.21] | |

*Note:* Epochs include all cognitive test scores in analytical cohort for years 8–13 and years 14–18.
Abbreviations: DSE, diabetes support and education; ILI, intensive lifestyle intervention.
[a]Lower scores reflect poorer performance.

DSE participants were invited to attend three group sessions each year, which focused on diet, physical activity, and social support. They did not receive specific diet, activity, or weight goals or information on behavioral strategies but they had similar cardiometabolic risk factor monitoring as ILI participants [25].

**TABLE 4** Regression slopes [95% CI] from mixed effects models across epochs

| | Slope with log-leptin (log ng/mL/SD) | Slope with log-VEGF (log pg/mL/SD) |
|---|---|---|
| Attention | | |
| DSE | −0.029 [−0.100, 0.042] | 0.018 [−0.061, 0.096] |
| ILI | −0.060 [−0.128, 0.008] | 0.022 [−0.057, 0.102] |
| Difference | $p = 0.49$ | $p = 0.92$ |
| Executive function | | |
| DSE | 0.055 [−0.026, 0.136] | −0.016 [−0.106, 0.075] |
| ILI | 0.014 [−0.064, 0.093] | −0.002 [−0.092, 0.088] |
| Difference | $p = 0.41$ | $p = 0.79$ |
| Memory | | |
| DSE | 0.052 [−0.027, 0.130] | 0.035 [−0.054, 0.124] |
| ILI | 0.009 [−0.068, 0.085] | −0.004 [−0.093, 0.085] |
| Difference | $p = 0.38$ | $p = 0.44$ |
| Global function | | |
| DSE | 0.106 [0.009, 0.203] | 0.021 [−0.088, 0.130] |
| ILI | −0.029 [−0.124, 0.066] | 0.062 [−0.047, 0.171] |
| Difference | $p = 0.03$ | $p = 0.51$ |
| Composite | | |
| DSE | 0.058 [−0.001, 0.116] | 0.029 [−0.035, 0.094] |
| ILI | 0.005 [−0.051, 0.061] | 0.012 [−0.052, 0.077] |
| Difference | $p = 0.16$ | $p = 0.66$ |

*Note:* Covariates (at baseline) are age, sex, education, race/ethnicity, BMI, and log analyte; separate models for each analyte.
Abbreviations: DSE, diabetes support and education; ILI, intensive lifestyle intervention; VEGF, vascular endothelial growth factor.

## Cognitive assessment

Centrally trained, certified, and masked staff conducted standardized assessments of cognitive function in the full Look AHEAD cohort in years 11 to 13 from baseline [6] and again at the end of follow-up in years 14 to 18 [26]. Other assessments for those participating in ancillary studies occurred during years 8 to 13 from baseline. The cognitive battery included the Rey Auditory Verbal Learning Test, the Digit Symbol Coding test, the Modified Stroop Color and Word Test, the Trail Making Test-Part B, and the Modified Mini-Mental Status Exam. Test scores were standardized, using *z* scores, by subtracting the overall cohort-wide mean of the initial assessments and dividing this by the SD and then ordering them so that higher scores reflected better performance [26].

To condense our presentation, we focused on the following loosely defined domains. Verbal memory was defined as the average of the Rey immediate and delayed tasks *z* scores. Executive function was defined as the average of the Stroop (time + errors of Part C) and Trails-B *z* scores. Attention was based on the Digit Symbol *z* score. Global cognitive function was based on *z* scores from the Modified Mini-Mental Status Exam. We report results for these four domains and the cognitive composite score, i.e., an average of all test *z* scores, adopted by Look AHEAD MIND for its primary outcome [26].

## Leptin and VEGF assays

Leptin and VEGF assays were conducted centrally at Medpace, Inc., Cincinnati, Ohio, based on samples collected at three times: (1) baseline; (2) at follow-up years 8, 10, or 12; and (3) at follow-up years 14, 16, or 18. Serum leptin concentrations were measured by a monoclonal antibody-based quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Inc., Minneapolis, MN) fully validated in the laboratory. The assay low level of quantification was 1.56 ng/mL, and the reference range was 1.0 to 50.0 ng/mL. Three quality control samples with low, medium, and high levels of leptin were analyzed in each plate, and the interassay coefficients of variation were typically < 6.0% for all three control samples.

Serum VEGF concentrations were measured by a monoclonal antibody-based quantitative sandwich ELISA kit (R&D Systems) that was fully validated in the laboratory. The assay low limit of quantification was 50.0 pg/mL, and the reference range was 62 to 707 pg/mL. Three quality control samples with low, medium, and high levels of VEGF were analyzed in each plate and the interassay coefficients of variation were typically 12.4%, 9.5%, and 10.6%, respectively.

## Collateral data

Certified clinic staff, masked to intervention assignment, collected collateral data. Sex was based on self-report. Participants were given the option to report ethnicity as Latino/Hispanic/Spanish Origin (yes/no) and race as African American/Black, American Indian/Native American/Alaskan Native, Asian/Pacific Islander, White, or other. Digital scales were used throughout follow-up to obtain annual measures of weight [20], and obesity was defined by BMI ≥ 30 kg/m$^2$. History of cardiovascular disease was defined by a self-report of prior myocardial infarction, coronary artery bypass, angioplasty/stent procedures, peripheral vascular disease, stroke, stable angina, or class I/II heart failure. Blood specimens were collected after a ≥ 12-hour fast and analyzed centrally for $HbA_{1c}$.

## Statistical analysis

Differences in baseline characteristics between intervention groups were assessed with *t* and $\chi 2$ tests. Follow-up times were grouped based on the date when biospecimens were collected for assays: as Epoch 1 (follow-up years 8 to 13) and Epoch 2 (follow-up years 14 to 18). Changes from baseline in BMI and leptin and VEGF concentrations during these epochs were assessed with mixed-effects models, and interaction terms were used to assess whether differences between intervention groups varied across epochs. Cognitive function scores by intervention group at Epochs 1 and 2 were described using

**TABLE 5** Mean changes from baseline in leptin, VEGF, and BMI across Epochs 1 and 2, by intervention group, from mixed-effects model

| | Mean [95% CI] change from baseline | | |
|---|---|---|---|
| | No CVD history at baseline, $N = 1094$ | CVD history at baseline, $N = 163$ | Intervention group × CVD interaction ($p$ value) |
| BMI, kg/m$^2$ | | | |
| DSE | −1.52 [−1.82, −1.22] | −1.35 [−2.16, −0.54] | 0.43 |
| ILI | −1.98 [−2.28, −1.69] | −2.29 [−3.05, −1.53] | |
| ILI-DSE | −0.46 [−0.89, −0.04] | −0.94 [−2.05, 0.17] | |
| Leptin, ng/mL | | | |
| DSE | 5.05 [3.02, 7.08] | 8.22 [2.72, 13.71] | 0.61 |
| ILI | 3.40 [1.38, 5.42] | 4.47 [−0.71, 9.64] | |
| ILI-DSE | −1.65 [−4.51, 1.22] | −3.75 [−11.30, 3.80] | |
| VEGF, pg/mL | | | |
| DSE | −12.40 [−23.66, −1.13] | −22.00 [−52.85, 8.85] | 0.03 |
| ILI | −12.63 [−23.86, −1.39] | 28.00 [−0.72, 56.73] | |
| ILI-DSE | −0.23 [−16.13, 15.68] | 50.01 [7.87, 92.14] | |
| | **Mean [95% CI] change from baseline** | | |
| | **No baseline obesity, BMI < 30 kg/m$^2$, $N = 211$** | **Baseline obesity, BMI ≥ 30 kg/m$^2$, $N = 1046$** | **Intervention group × obesity interaction ($p$ value)** |
| BMI, kg/m$^2$ | | | |
| DSE | −0.75 [−1.44, −0.05] | −1.64 [−1.95, −1.34] | 0.39 |
| ILI | −0.90 [−1.56, −0.25] | −2.26 [−2.57, −1.96] | |
| ILI-DSE | −0.16 [−1.12, 0.80] | −0.62 [−1.05, −0.19] | |
| Leptin, ng/mL | | | |
| DSE | 5.99 [1.22, 10.75] | 5.32 [3.24, 7.41] | |
| ILI | 4.96 [0.48, 9.44] | 3.24 [1.17, 5.31] | |
| ILI-DSE | −1.03 [−7.57, 5.51] | −2.09 [−5.02, 0.85] | 0.77 |
| VEGF, pg/mL | | | |
| DSE | 16.44 [−9.90, 42.86] | −19.25 [−30.80, −7.70] | 0.04 |
| ILI | −12.39 [−37.42, 12.63] | −6.15 [−17.67, 5.37] | |
| ILI-DSE | −28.83 [−65.22, 7.56] | 13.11 [−3.20, 29.41] | |

Abbreviations: CVD, cardiovascular disease; DSE, diabetes support and education; ILI, intensive lifestyle intervention; VEGF, vascular endothelial growth factor.

mixed-effects models, and 95% CIs were used to describe differences between intervention groups.

Mixed-effects models were also used to assess associations that log-transformed leptin and VEGF levels had with $z$-transformed cognitive scores across epochs. Fitted slopes and 95% CIs were calculated for the two intervention groups, and interaction tests were used to compare associations between groups. Race/ethnicity was included as a covariate in these analyses to account for potential biases in cognitive assessment methods. Baseline age, sex, education, BMI, and the log of the analyte concentration were also included as covariates. Differences in cognitive test scores between intervention groups were assessed without and with covariate adjustment for log-transformed leptin and VEGF levels. This was done to assess for mediation of any intervention effects within subgroups defined by baseline obesity and cardiovascular disease.

## RESULTS

Our analyses included the 1,279 participants described in Table 1. Balance provided by the original randomization was largely preserved in this subsample, with slightly greater formal education in the DSE compared with the ILI group. Importantly, the groups did not differ according to sex, age, race/ethnicity, body size, and diabetes duration and there were comparable mean baseline levels of leptin and VEGF in the two intervention groups.

Leptin and VEGF levels were assayed from samples collected (1) at baseline, (2) at Epoch 1, a mean (SD) of 10.4 (1.6) years after baseline for DSE participants and 10.3 (1.6) years after baseline for ILI participants ($p = 0.49$), and (3) at Epoch 2, a mean (SD) of 14.5 (0.9) years after baseline for DSE participants and 14.6 (0.9) years after baseline for ILI participants ($p = 0.52$). The mean age at the time of

**TABLE 6** Mean composite cognitive function scores by intervention assignment for participants grouped by baseline CVD history, with covariate adjustment for age, education, race/ethnicity, sex, and number of prior cognitive tests (for learning effects)

| No covariate adjustment for leptin and VEGF | | | |
|---|---|---|---|
| **Baseline CVD history** | **Intervention** | **Epoch 1, mean (SE)** | **Epoch 2, mean (SE)** |
| No CVD | DSE | −0.462 (0.045) | −0.775 (0.043) |
| | ILI | −0.491 (0.045) | −0.764 (0.042) |
| | Difference (ILI − DSE) | −0.029 (0.045) | 0.011 (0.046) |
| | *p* value | $p = 0.52$ | $p = 0.82$ |
| CVD | DSE | −0.568 (0.093) | −0.810 (0.098) |
| | ILI | −0.785 (0.088) | −1.166 (0.094) |
| | Difference | −0.217 (0.119) | −0.356 (0.130) |
| | *p* value | $p = 0.07$ | $p = 0.006$ |
| **With covariate adjustment for leptin and VEGF** | | | |
| **Baseline CVD history** | **Intervention** | **Epoch 1, mean (SE)** | **Epoch 2, mean (SE)** |
| No CVD | DSE | −0.439 (0.047) | −0.743 (0.044) |
| | ILI | −0.466 (0.046) | −0.736 (0.044) |
| | Difference | −0.027 (0.046) | 0.007 (0.047) |
| | *p* value | $p = 0.56$ | $p = 0.87$ |
| CVD | DSE | −0.508 (0.096) | −0.735 (0.101) |
| | ILI | −0.746 (0.091) | −1.120 (0.096) |
| | Difference | −0.239 (0.122) | −0.386 (0.133) |
| | *p* value | $p = 0.06$ | $p = 0.004$ |

Abbreviations: CVD, cardiovascular disease; DSE, diabetes support and education; ILI, intensive lifestyle intervention; VEGF, vascular endothelial growth factor.

sampling for Epoch 1 was 68.9 (6.4) years in both intervention groups; at Epoch 2, these were 72.3 (6.1) years for DSE participants and 72.4 (6.1) years for ILI participants ($p = 0.77$).

As seen in Table 2, during these epochs, ILI participants had larger mean decreases in BMI from baseline compared with DSE participants (overall, $p = 0.01$); however, only at Epoch 1 did the 95% CI for differences between intervention groups exclude zero. Overall, weight losses were larger in both intervention groups at Epoch 2 compared with Epoch 1 ($p = 0.05$). Leptin levels tended to increase from baseline, more among DSE compared with ILI participants at Epoch 1, with continued increases to Epoch 2 ($p < 0.001$) and an attenuation of differences between groups. At Epoch 2, VEGF levels were markedly decreased from baseline in both groups and the decline from Epoch 1 was greater among ILI than DSE participants (interaction $p = 0.04$).

Changes in BMI from baseline were strongly correlated with changes in leptin levels at both epochs and for both intervention groups ($r = 0.51$ to $0.54$, $p < 0.001$). However, changes in BMI were not correlated with changes in VEGF ($r = 0.03$ to $r = 0.06$, $p \geq 0.09$), and changes in leptin and VEGF were not correlated ($r = 0.00$ to $r = 0.07$, $p \geq 0.11$).

Table 3 portrays mean standardized cognitive domain scores by intervention assignment and epoch. Performance tended to decline from Epoch 1 to Epoch 2 for all domains within both intervention groups. ILI participants, compared with DSE participants, had lower mean scores for executive function at Epoch 1 (mean [95% CI] of −0.14 [−0.26, −0.02] SD) with the difference attenuated by Epoch 2. In a similar fashion, ILI participants had lower mean composite cognitive function scores at Epoch 1 as compared with DSE participants (−0.11 [−0.21, −0.00] SD), which also attenuated by Epoch 2.

Because distributions of leptin and VEGF concentrations were right-skewed, data were log-transformed before being included as predictors in mixed effects models. As shown in Table 4, with adjustment for baseline risk factors and analyte levels, there was some evidence that global cognitive function scores tended to be higher among participants with greater log-leptin levels among DSE participants but not ILI participants (interaction $p = 0.03$). There was little evidence for associations with any other domain or the composite scores within either intervention group. No associations were observed between cognitive function and VEGF levels for any cognitive domain in either intervention group.

As indicated in Table 5, averaged across the two epochs, differences between intervention groups in changes from baseline in body mass indices and leptin concentrations were comparable for participants with and without a history of cardiovascular disease history and with and without obesity at baseline. However, differences in VEGF concentrations between intervention groups did vary among these subgroups (interaction $p = 0.03$ for cardiovascular disease history and $p = 0.04$ for obesity). Among DSE participants, VEGF concentrations tended to decrease from baseline for both participants with and

**TABLE 7** Mean composite cognitive function scores by intervention assignment for participants grouped by baseline obesity status, with covariate adjustment for age, education, race/ethnicity, sex, and number of prior cognitive tests (for learning effect)

| No covariate adjustment for leptin and VEGF | | | |
|---|---|---|---|
| **Baseline obesity** | **Intervention** | **Epoch 1, mean (SE)** | **Epoch 2, mean (SE)** |
| Nonobesity | DSE | −0.604 (0.082) | −0.971 (0.084) |
| | ILI | −0.395 (0.075) | −0.675 (0.075) |
| | Difference | 0.209 (0.102) | 0.296 (0.107) |
| | *p* value | $p = 0.04$ | $p = 0.006$ |
| Obesity | DSE | −0.455 (0.045) | −0.752 (0.043) |
| | ILI | −0.565 (0.046) | −0.848 (0.043) |
| | Difference | −0.110 (0.046) | −0.096 (0.048) |
| | *p* value | $p = 0.02$ | $p = 0.04$ |
| **Covariate adjustment for leptin and VEGF** | | | |
| **Baseline obesity** | **Intervention** | **Epoch 1, mean (SE)** | **Epoch 2, mean (SE)** |
| Nonobesity | DSE | −0.613 (0.087) | −0.978 (0.089) |
| | ILI | −0.350 (0.083) | −0.623 (0.083) |
| | Difference | 0.263 (0.104) | 0.355 (0.110) |
| | *p* value | $p = 0.01$ | $p = 0.001$ |
| Obesity | DSE | −0.416 (0.047) | −0.703 (0.044) |
| | ILI | −0.537 (0.047) | −0.819 (0.045) |
| | Difference | −0.121 (0.047) | −0.117 (0.049) |
| | *p* value | $p = 0.01$ | $p = 0.017$ |

Abbreviations: CVD, cardiovascular disease; DSE, diabetes support and education; ILI, intensive lifestyle intervention; VEGF. vascular endothelial growth factor.

without cardiovascular disease histories. Among ILI participants, however, VEGF concentrations tended to decrease among those with no cardiovascular disease history but tended to increase among those with a history of cardiovascular disease. Among DSE participants without obesity at baseline, VEGF concentrations tended to increase from baseline, whereas for DSE participants with obesity, the concentrations tended to have decreased. In contrast, among ILI participants, VEGF concentrations had decreased (but not markedly) in both groups.

As listed in Table 6, assignment to ILI was associated with a relative deficit in composite cognitive function at Epoch 2 among participants with a history of cardiovascular disease but not for those without such a history. Inclusion of current leptin and VEGF resulted in no material impact on findings, and neither their current levels or changes in levels from baseline were significantly associated with composite cognitive function among those without and with a history of cardiovascular disease. Table 7 provides results from similar analyses for participants grouped by baseline obesity status. Among those without obesity at baseline, assignment to ILI compared with DSE was associated with a relative benefit in composite cognitive function at both epochs. However, among those with obesity at baseline, assignment to ILI was associated with a relative decrement in composite cognitive function. Inclusion of leptin and VEGF again had no material impact on these findings, and their levels and changes from baseline in their levels were not significantly associated with cognitive function within either subgroup. Parallel analyses for the individual cognitive domains also found no alteration of intervention effects related to leptin and VEGF.

## DISCUSSION

We report three main findings that we will discuss in turn. First, in a cohort of adults with type 2 diabetes and overweight or obesity who were initially ages 45 to 76 years, 10 years of ILI targeting weight loss left a legacy of modestly lower BMI levels. However, this did not markedly influence subsequent leptin levels, which—although lower among ILI participants proximal to the end of the intervention—tended to have increased from baseline in both intervention groups by Epoch 2. VEGF concentrations were slightly increased from baseline in the ILI group, but by Epoch 2, there were marked decreases in VEGF levels from baseline in both groups. Second, current levels of leptin and VEGF had little overall association with cognitive functioning, and this finding did not vary between intervention groups (except for modest evidence that greater leptin levels were associated with better global cognitive function only among DSE participants). Third, although assignment to ILI compared with DSE resulted in poorer long-term cognitive functioning among participants who at baseline had a history of cardiovascular disease or obesity, these associations were not linked to levels of leptin or VEGF.

Look AHEAD has previously reported that, for the full cohort, the 10-year ILI left an enduring legacy of modest differences (1% to 2%) in mean changes in weight from baseline [27]. This finding holds for the subsample included in our analyses. Overall changes in weight were positively associated with leptin levels but had little association with VEGF levels. Compared with DSE, ILI participants had lost a mean of 0.61 kg/m$^2$ of BMI at Epoch 1, and at this time, leptin levels were slightly lower among ILI participants and VEGF levels were slightly higher (although the 95% CI included 0). By Epoch 2, differences between groups in weight, leptin, and VEGF changes from baseline had all attenuated. Abbenhardt et al. examined the impact of a caloric restriction and/or physical activity (based on a modification of the Look AHEAD intervention) on leptin levels over 1 year [12]. They found that weight loss was strongly associated with decreases in leptin concentrations and that an average relative weight loss of 8% to 11% was associated with average decreases in leptin levels of 6 to 10 ng/mL in a cohort with mean baseline BMI of 31 kg/m$^2$ and age of 58 years. Others have found similar marked associations with weight loss in relatively short-term weight-loss trials [28, 29] and even modest weight changes of 2% to 3% may be associated with significant 1-year reductions in leptin levels [30]. Thus, it is likely that ILI participants, who lost an average of 8.6% of their initial body weight at 1 year [31], had short-term reductions in leptin levels. However, these initial weight losses were not maintained across the subsequent follow-up, and leptin levels tended to increase with age, as expected [32].

Others have also reported that weight-loss interventions may lower VEGF levels in shorter-term studies. Duggan et al. found that across 30 months, behavioral dietary interventions leading to 7% to 8% weight losses were associated with 14% lower VEGF levels in postmenopausal women aged 50 to 75 years [33]. Bariatric surgery may lead to even greater decreases in VEGF [18]. We observed a greater decline in VEGF levels from Epoch 1 to Epoch 2 among ILI compared with DSE participants, which was driven by the slight increase in VEGF levels among ILI participants at Epoch 1 attenuating to the level of decreases among DSE participants at Epoch 2. It is possible that the greater VEGF levels among ILI participants at Epoch 1 were linked to greater increases in adipose tissue in this cohort as the intervention intensity waned [34].

Greater levels of serum leptin are reported to be associated with lower risk for Alzheimer disease and lower levels are a hallmark of Alzheimer disease [35, 36]. Leptin may play an important role in repairing cerebral ischemia, restoring perfusion, and enhancing neurogenesis and angiogenesis [8, 9]. However, obesity is known to blunt neuroprotective leptin actions in the brain and increase leptin resistance [32, 37], which may have muted any association between leptin levels and cognitive functioning in the Look AHEAD cohort.

Diabetes and insulin resistance are associated with increased levels of VEGF, and the VEGF-A protein (the most common VEGF isoform), through increased perfusion and enhanced insulin delivery, may protect against the metabolic consequences of diabetes and obesity [38]. However, increased VEGF levels also have adverse consequences for individuals with diabetes, including increased retinopathy and other microvascular conditions [39]. Greater circulating levels of VEGF are associated with greater blood–brain permeability and increased brain angiogenesis. There are conflicting reports on whether VEGF is increased or decreased in the brain with diabetes, which may depend on the duration of disease [40]. Greater VEGF levels within cerebral spinal fluid are linked to greater hippocampal volume and cortical thickenss and less hippocampal atrophy, and associations may be stronger for individuals with hallmarks of Alzheimer disease [41, 42]. Although increased brain angiogenesis may be thought to promote brain perfusion, increased VEGF signaling is associated with cerebral neovascularization that is immature and at greater risk for injury [40, 43]. Thus, the lack of evidence for an association between VEGF and cognitive function in our cohort may derive from a complex web of potentially conflicting mechanisms.

Differences between intervention groups in changes in BMI and leptin levels from baseline across Epochs 1 and 2 did not vary depending on baseline history of cardiovascular disease or obesity. VEGF concentrations, however, tended to be increased from baseline when averaged across Epochs 1 and 2 among ILI participants with cardiovascular disease history but to be decreased among such DSE participants (interaction $p = 0.03$). VEGF declines from baseline were similar among ILI individuals without and with obesity at baseline, but VEGF was slightly increased from baseline among DSE participants without baseline obesity and decreased from baseline among DSE participants with baseline obesity (interaction $p = 0.04$). Thus, it appears that the ILI intervention may have a legacy on VEGF levels that varies depending on cardiovascular disease and obesity status.

Nevertheless, and contrary to our motivating hypothesis, leptin and VEGF concentrations did not account for different ILI effects on cognition among participants grouped by baseline cardiovascular disease history or obesity. We have speculated that other weight-related mechanisms may account for these troubling findings. There is evidence that obesity may provide some protection against age-related neurodegeneration [44], which may be accelerated among those with cardiovascular disease and obesity; however, this remains controversial [45].

Our study has a number of strengths to note. Because of randomization, demographic and health characteristics were comparable across intervention groups at baseline. Intervention groups retained this comparability among participants selected for the current study. Participants have been followed closely for nearly 20 years since randomization using rigorous methods that were part of the randomized controlled clinical trial and provide rich phenotypic data. Limitations to this work include the lack of baseline cognitive assessments; however, because the balance afforded by randomization has held for this subsample for other characteristics, there is no reason to believe there would have been baseline differences in cognitive function. Beause our sample is composed of individuals with type 2 diabetes and overweight or obesity, the generalizeability of findings may be limited. Serum levels of leptin and VEGF may not accurately reflect levels in the brain and relations may be altered by increased permeability of the blood–brain barrier assocated with diabetes [9, 40].

## CONCLUSION

In a cohort of older individuals with type 2 diabetes and overweight or obesity, serum leptin and VEGF were not associated with cognitive function and they did not account for the potential that a 10-year ILI focused on weight loss may lead to relative deficits in cognitive function among individuals with cardiovascular disease or obesity at baseline.O

## AUTHOR CONTRIBUTIONS

MAE, JAL, and KMH conceived the project and obtained funding. MAE, JKE, and RN developed analysis plans and carried out statistical analyses. SMM oversaw laboratory analyses. All authors were involved in writing the paper and gave final approval of the submitted version.

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## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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## REFERENCES

1. Kivipelto M, Mangialasche F, Snyder HM, et al. World-wide FINGERS network: a global approach to risk reduction and prevention of dementia. *Alzheimers Dement*. 2020;16:1078-1094.
2. Moll van Charante EP, Richard E, Eurelings LS, et al. Effectiveness of a 6-year multidomain vascular care intervention to prevent dementia (preDIVA): a cluster-randomised controlled trial. *Lancet*. 2016;388:797-805.
3. Andrieu S, Guyonnet S, Coley N, Cantet C, et al. Effect of long-term omega 3 polyunsaturated fatty acid supplementation with or without multidomain intervention on cognitive function in elderly adults with memory complaints (MAPT): a randomised, placebo-controlled trial. *Lancet Neurol*. 2017;16:377-389.
4. Espeland MA, Dutton GR, Neiberg RH, et al.; Action for Health in Diabetes (Look AHEAD) Research Group. Impact of a multidomain intensive lifestyle intervention on self-reported memory, problem-solving, and decision-making: the action for health in diabetes randomized controlled clinical trial. *J Gerontol A Biol Sci Med Sci*. 2018;73:1560-1567.
5. Rapp SR, Luchsinger JA, Baker LD, et al.; the Look AHEAD Research Group. Effect of a long-term intensive lifestyle intervention on cognitive function: action for health in diabetes study. *J Am Geriatr Soc*. 2017;65;966-972.
6. Espeland MA, Carmichael O, Hayden K, et al.; Action for Health In Diabetes Brain Magnetic Resonance Imaging (Look AHEAD Brain) and Action for Health Movement and Memory Ancillary Study Research Groups. Long-term impact of weight loss intervention on changes in cognitive function: exploratory analyses from the action for health in diabetes randomized controlled clinical trial. *J Gerontol A Biol Sci Med Sci*. 2018;73:484-491.
7. Espeland MA, Luchsinger JA, Baker LD, et al.; Look AHEAD Study Group. Effect of a long-term intensive lifestyle intervention on prevalence of cognitive impairment. *Neurology*. 2017;88:2026-2035.
8. Zhang WF, Jin YC, Li XM, Yang Z, Wang D, Cui JJ. Protective effects of leptin against cerebral ischemia/reperfusion injury. *Exp Ther Med*. 2019;17:3282-3290.
9. Calió ML, Mosini AC, Marinho DS, et al. Leptin enhances adult neurogenesis and reduces pathological features in a transgenic mouse model of Alzheimer's disease. *Neurobiol Dis*. 2021;148:105219. doi: 10.1016/j.nbd.2020.105219
10. Yin H, Sai AT, Rong H, et al. Low plasma leptin and high soluble leptin receptor levels are associated with mild cognitive impairment in type 2 diabetic patients. *Front Aging Neurosci*. 2018;10:132. doi:10.3389/fnagi.2018.00132

11. Rolland C, Hession M, Broom I. Effect of weight loss on adipokine levels in obese patients. *Diabetes Metab Syndr Obes*. 2011;4:315-323.
12. Abbenhardt C, McTiernan A, Alfano CM, et al. Effects of individual and combined dietary weight loss and exercise interventions in postmenopausal women on adiponectin and leptin levels. *J Intern Med*. 2013;274:163-175.
13. Unick JL, Beavers D, Jakicic JM, et al.; Look AHEAD Research Group. Effectiveness of lifestyle interventions for individuals with severe obesity and type 2 diabetes: results from the Look AHEAD trial. *Diabetes Care*. 2011;34:2152-2157.
14. Feinkohl I, Janke J, Slooter AJC, Winterer G, Spies C, Pischon T. Plasma leptin, but not adiponectin, is associated with cognitive impairment in older adults. *Psychoneuroendocrinology*. 2020;120:104783. doi:10.1016/j.psyneuen.2020.104783
15. Jais A, Solas M, Backes H, et al. Myeloid-cell-derived VEGF maintains brain glucose uptake and limits cognitive impairment in obesity. *Cell*. 2016;165:882-895.
16. Kristensen PL, Hoi-Hansen T, Boomsma F, Pedersen-Bjergaard U, Thorsteinsson B. Vascular endothelial growth factor during hypoglycemia in patients with type 1 diabetes mellitus: relation to cognitive function and renin-angiotensin system activity. *Metabolism*. 2009;58:1430-1438.
17. Rosenstein JM, Mani N, Silverman WF, Krum JM. Patterns of brain angiogenesis after vascular endothelial growth factor administration in vitro and in vivo. *Proc Natl Acad Sci U S A*. 1998;95:7086-7091.
18. de la Torre NG, Rubio MA, Bordiú E, et al. Effects of weight loss after bariatric surgery for morbid obesity on vascular endothelial growth factor-A, adipocytokines, and insulin. *J Clin Endocrinol Metab*. 2008;93:4276-4281.
19. Cullberg KB, Christiansen T, Paulsen SK, Bruun JM, Pedersen SB, Richelsen B. Effect of weight loss and exercise on angiogenic factors in the circulation and in adipose tissue in obese subjects. *Obesity (Silver Spring)*. 2013;21:454-460.
20. Ryan DH, Espeland MA, Foster GD, et al; Look AHEAD Research Group. Look AHEAD (Section for Health in Diabetes): design and methods for a clinical trial of weight loss for the prevention of cardiovascular disease in type 2 diabetes. *Control Clin Trials*. 2003;24:610-628.
21. Wing RR, Bolin P, Brancati FL, et al.; Look AHEAD Research Group. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *New Eng J Med*. 2013;369:145-154.
22. Espeland MA, Rapp SR, Bray GA, et al.; Action for Health In Diabetes (Look AHEAD) Movement and Memory Subgroup; Look AHEAD Research Group. Long-term impact of behavioral weight loss intervention on cognitive function: the action for health in diabetes movement and memory study. *J Gerontol A Biol Sci Med Sci*. 2014;69;1101-1118.
23. Espeland MA, Erickson K, Neiberg RH, et al.; Action for Health in Diabetes Brain Magnetic Resonance Imaging (Look AHEAD Brain) Ancillary Study Research Group. Brain and white matter hyperintensity volumes after ten years of random assignment to lifestyle intervention. *Diabetes Care*. 2016;39:764-771.
24. Wadden TA, West DS, Delahanty L, et al.; Look AHEAD Research Group. The Look AHEAD Study: a description of the lifestyle intervention and the evidence supporting it. *Obesity (Silver Spring)*. 2006;14:737-752.
25. Look AHEAD Research Group. The development and description of the diabetes support and education (comparison group) intervention for the action for health in diabetes (Look AHEAD) trial. *Clin Trials*. 2011;8:320-329.
26. Hayden KM, Neiberg RH, Evans JK, et al.; Action for Health in Diabetes (Look AHEAD) Research Group. Legacy of a 10-year multidomain lifestyle intervention on the cognitive trajectories of individuals with overweight/obesity and type 2 diabetes mellitus. *Dement Geriatr Cogn Disord*. 2021;50:237-249.
27. Houston DK, Neiberg RH, Miller ME, et al. Physical function following a long-term lifestyle intervention among middle aged and older adults with type 2 diabetes: the Look AHEAD Study. *J Gerontol A Biol Sci Med Sci*. 2018;73:1552-1559.
28. Herrick JE, Panza GS, Gollie JM. Leptin, leptin soluble receptor, and the free leptin index following a diet and physical activity lifestyle intervention in obese males and females. *J Obes*. 2016;2016:8375828. doi:10.1155/2016/8375828
29. Abd El-Kader SM, Al-Jiffri OH. Impact of weight reduction on insulin resistance, adhesive molecules and adipokines dysregulation among obese type 2 diabetic patients. *Afr Health Sci*. 2018;18:873-883.
30. Salas-Salvadó J, Díaz-López A, Ruiz-Canela M, et al. Effect of a lifestyle intervention program with energy-restricted mediterranean diet and exercise on weight loss and cardiovascular risk factors: one-year results of the PREDIMED-plus trial. *Diabetes Care*. 2019;42:777-788.
31. Pi-Sunyer X, Blackburn G, Brancati FL , et al.; Look AHEAD Research Group. Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: one-year results of the Look AHEAD trial. *Diabetes Care*. 2007;30:1374-1383.
32. Lee EB. Obesity, leptin, and Alzheimer's disease. *Ann N Y Acad Sci*. 2011;243:15-29.
33. Duggan C, de Dieu Tapsoba J, Wang CY, Schubert KEF, McTiernan A. Long-term effects of weight loss and exercise on biomarkers associated with angiogenesis. *Cancer Epidemiol Biomarkers Prev*. 2017;26:1788-1794.
34. Pownall HJ, Schwartz AV, Bray GA, et al.; Look AHEAD Research Group. Changes in regional body composition over 8 years in a randomized lifestyle trial: the Look AHEAD Study. *Obesity (Silver Spring)*. 2016;24:1899-1905.
35. Lieb W, Beiser AS, Vasan RS, et al. Association of plasma leptin levels with incident Alzheimer disease and MRI measures of brain aging. *JAMA*. 2009;302:2565-2572.
36. Khemka VK, Bagchi D, Bandyopadhyay K, et al. Altered serum levels of adipokines and insulin in probable Alzheimer's disease. *J Alzheimers Dis*. 2014;41:525-533.
37. Ha J, Kwak S, Kim KY, et al. Relationship between adipokines, cognition, and brain structures in old age depending on obesity [published online February 8, 2022]. *J Gerontol A Biol Sci Med Sci*. doi:10.1093/gerona/glac021
38. Zafar MI, Mills K, Ye X, et al. Association between expression of vascular endothelial growth factors and metabolic syndrome or its components: a systematic review and meta-analysis. *Diabetol Metabol Syndrome*. 2018;10:62. doi:10.1186/s13098-018-0363-0
39. Bonnefond A, Saulnier PJ, Stathopoulou MG, et al. What is the contribution of two genetic variants regulating VEGF levels to type 2 diabetes risk and to microvascular complications?. *PLoS ONE*. 2013;8:e55921. doi:10.1371/journal.pone.0055921
40. Li X, Yin Q, Han X, et al. Dynamic expression of vascular endothelial growth factor (VEGF) and platelet-derived growth factor receptor beta (PDGFRβ) in diabeteic brain contributes to cognitive dysfunction. *Brain Res Bull*. 2021;175:99-106.
41. Hohman TJ, Bell SP, Jefferson Alzheimer's Disease Neuroimaging Initiative. The role of vascular endothelial growth factor in neurodegeneration and cognitive decline: exploring interactions with biomarkers of Alzheimer's disease. *JAMA Neurol*. 2015;72:520-529.
42. Tubi MA, Kothapalli D, Hapenney M, et al. for Alzheimer's Disease Neuroimaging Initiative. Regional relationships between CSF VEGF levels and Alzheimer's disease brain biomarkers and cognition. *Neurobiol Aging*. 2021;105:241-251.
43. Abdelsaid M, Coucha M, Hafez S, Yasir A, Johnson MH, Ergul A. Enhanced VEGF signalling mediates cerebral neovascularisation via downregulation of guidance protein ROBO4 in a rat model of diabetes. *Diabetologia*. 2017;60:740-750.
44. Lan YT, Blacker D, Yuan C, Chibnik LB, Hofman A, Ma Y. Longitudinal body weight change, visit-to-visit body weight fluctuation, and cognitive decline among older adults. *J Alzheimers Dis*. 2021;84:777-786.

45. Kronschnabl JM, Knelp T, Weiss LM, Bergmann M. Bodyweight change and cognitive performance in the older population. *PLoS ONE*. 2021;16:e0249651. doi:10.1371/journal.pone.0249651

## SUPPORTING INFORMATION

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

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