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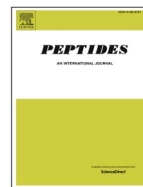
Interplay of circulating leptin and obesity in cognition and cerebral volumes in older adults

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ABSTRACT

We aimed to investigate whether circulating leptin and body mass index (BMI) associate independently with cognitive function (decline) and brain volumes using magnetic resonance imaging (MRI) in older individuals at risk of cardiovascular disease. We studied the cross-sectional and longitudinal associations in participants enrolled in the PROSPER study (Prospective Study of Pravastatin in the Elderly at Risk). Cognitive function was tested at baseline and repeated during a mean follow-up time of 3.2 years. Analyses were performed with multivariable (repeated) linear regression models and adjusted for demographics, cardiovascular risk-factors, and stratified by sex. We included 5623 dementia-free participants (52 % female, mean age 75 years) with a mean BMI of 26.9 (SD = 4.1). In a sub-study, 527 participants underwent brain MRI. At baseline, individuals with a BMI > 30 had a worse performance on the Stroop test (β 5.0 s, 95 %CI 2.6;7.5) and larger volumes of the amygdala (β 234 mm^3, 95 %CI 3;464) and hippocampus (β 590 mm^3, 95 %CI 181;999), independent of intra-cranial volume and serum leptin levels, compared with individuals with the reference BMI (BMI 18−25 kg/m^2). Per log ng/mL higher serum leptin, independent of BMI, a 135 mm^3 (95 %CI 2;268) higher volume of the amygdala was found, but no association was observed with cognitive tests nor with other brain volumes. Stratification for sex did not materially change the results. Whereas higher BMI associated with worse cognitive function independent of leptin levels, our study provided evidence that leptin and BMI independently associate with amygdala volume suggesting potential distinct biological associations.

1. Introduction

Obesity, defined as a Body Mass Index (BMI) greater than 30 kg/m^2, is reaching epidemic proportions in developing countries [1]. According to the World Health Organization, over 650 million people worldwide are obese [2]. A high BMI is a modifiable risk-factor for several adverse health outcomes, such as cardiovascular disease, type 2 diabetes mellitus and dementia [4–8]. The prevalence of dementia, including Alzheimer's disease (AD), has also increased in the last decades as a consequence of the growing number of older individuals [3]. The

biological mechanisms by which body composition can have an influence on the brain and cognitive function are not well understood. As adipose tissue is the largest endocrine organ in the human body, secretion of cell-signaling peptides represents one of the potential mechanisms. White adipose tissue (WAT) secretes various adipokines that can be transported across the blood-brain barrier (BBB) and bind to receptors in the brain, including leptin [9]. The regulatory function of leptin has been widely studied, and it is known that leptin is highly correlated to the amount of body fat [4]. Indeed, obesity may attenuate leptin signaling leading to leptin resistance, indirectly amplifying the

extent of weight gain [10]. As a consequence, the body may also further increase serum leptin in attempt to compensate the resistance. Via hypothalamic signaling, leptin inhibits appetite and energy consumption, and contributes to regulation of bone metabolism, and immune and reproductive function [9,11,12]. In addition to the hypothalamus, leptin receptors are also present in two major areas affected in AD: the cerebral cortex and hippocampus [13]. Improved memory performance, reduced tau pathologies as well as neuron and synapse formation has been shown in the hippocampus of transgenic mice following chronic exogenous leptin administration via subcutaneous mini-osmotic pumps [14,15].

In humans, leptin and BMI have been found to be lower in older patients with AD compared with patients with mild neurocognitive deficits [11] and healthy counterparts [16]. The obesity-associated (*FTO*) risk allele, as an instrumental variable for higher BMI, has been associated with lower volumes of the nucleus accumbens [17]. Other preliminary studies have also hinted toward a possible association between BMI and leptin with cognitive decline and dementia [18–21]. Furthermore, we have previously shown in our study population (Prospective Study of Pravastatin in the Elderly at Risk study) that individuals with obesity have larger amygdalar and left hippocampal volumes than normal weight individuals [22]. However, whether this association is dependent on circulating leptin levels, has not yet been clarified.

In this study, we assessed the associations between BMI, serum leptin levels, cognition and various brain structural volumes at baseline, and cognitive decline during follow-up in a cohort of older individuals at increased risk for cardiovascular disease. More specifically, we aimed to assess the mutual independence of effects of BMI and circulating leptin on cognitive function and brain volumes.

2. Methods

2.1. Study design and participants

The data used for this study was obtained from the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) study. This large, prospective multicenter randomized clinical trial assessed whether treatment with pravastatin diminishes the risk of major vascular events in older individuals from three countries (the Netherlands, Scotland, Ireland). Between December 1997 and May 1999, 5804 men and women aged 70–82 years were enrolled if they had pre-existing vascular disease or increased risk due to smoking, hypertension, or diabetes. They were randomly assigned to either pravastatin or placebo for an average 3.5-year intervention period. Both participants from pravastatin and placebo groups were included, as it was shown previously that pravastatin does not affect cognitive function [23]. Participants with the following conditions were not recruited in the PROSPER study: congestive heart failure; significant arrhythmia; cognitive impairment (Mini-Mental Score Examination score <24). The inclusion and exclusion criteria of PROSPER have been described in more detail elsewhere [24,25]. In the present study, participants were excluded if they had BMI < 18 or missing leptin measurements. The original PROSPER study was approved by the Medical Ethics Committees of the three collaborating centers and complied with the Declaration of Helsinki. All participants gave written informed consent.

2.2. Data collection

2.2.1. BMI measurements
BMI was measured at baseline and is reported in kg/m^2. On the basis of BMI, participants were divided into the following groups: 18−25; 25−30; ≥30 kg/m^2 [26].

2.2.2. Serum leptin measurements
Fasting morning baseline leptin concentration (ng/mL) was measured at baseline, before participants received study medication by an in-house RIA validated thoroughly against the commercially available Linco Research Co. (St. Charles, MO) assay [27]. The intra- and interassay coefficients of variation were below 7 % and below 10 %, respectively. The lower detection limit of the assay was 0.5 ng/mL [28]. For presentation purposes, we stratified the study population into three equally sized groups based on their leptin levels. Due to substantial baseline differences between men and women in serum leptin concentration, the thirds of leptin levels were standardized for sex.

2.2.3. Cognitive function measurements
The Mini-Mental State Examination (MMSE) was used to measure global cognitive function. The MMSE scores range from zero points (very severe cognitive impairment) to 30 points (optimal cognitive function). In the original PROSPER study, participants with poor cognitive function (MMSE < 24) were excluded. Four neuropsychological performance tests were used to measure various cognitive domains: the Stroop test, the Letter-Digit Coding test (LDCT), the 15-Picture Learning test immediate, and the 15-Picture Learning Test delayed. The Stroop test for attention and the Letter-Digit Coding Test (LDCT) for processing speed were used to measure executive functioning. The outcome parameter for the Stroop test was the total number of seconds to complete the third Stroop card containing 40 items. The outcome variable for the LDT was the total number of correct entries in 60 s. Memory was assessed using two versions of the 15-Picture Learning test (PLT), testing immediate and delayed recall. The main outcome parameters were the accumulated number of recalled pictures over the three learning trials and the number of pictures recalled after 20 min.

2.2.4. Brain volumes – MRI acquisition and processing
From a random subgroup (n = 527) of the Dutch participants, structural magnetic resonance imaging (MRI) brain scans were obtained at baseline and used for assessment of the following brain volumes: amygdala, hippocampus, nucleus accumbens, foramen putamen, caudate nucleus, thalamus, white matter volume, gray matter volume. All analyses in this study were based on a high resolution three-dimensional (3D) T1-weighted (T1-w) gradient echo MRI scans obtained at 1.5 T (Philips Medical Systems, Best, the Netherlands). Acquisition parameters were: repetition time (TR) =30 ms; echo time (TE) = 4.6 ms; flip angle = 30°; slice thickness = 1.5 mm; 120 slices; no interslice gap; field of view (FOV) = 220 × 220 mm, and a matrix size of 256 × 256 [17,29,30].

All MRI scans were analyzed using different tools of FSL (FMRIB Software Library) [31,32]. Gray and white matter volumes were calculated using the FSL- SIENAX tool (Structural Image Evaluation, using Normalization, of Atrophy). Tissue-type segmentation using FAST4 (FMRIB's Automated Segmentation Tool) with partial volume estimation was performed to calculate the total volume of brain tissue, including separate estimates of gray matter and white matter volumes.

To determine the volumes of the amygdala, hippocampus, nucleus accumbens, foramen putamen, caudate nucleus, and thalamus, the integrated registration and segmentation tool of the Oxford Center for Functional MRI of the Brain was used [33].

The characteristics of participants in the Dutch MRI sub-study in comparison with nonparticipants have been studied previously [34]. Participants in this subgroup were more frequently men and less frequently current smoker.

2.2.5. Covariates
For each participant, an extensive medical history was obtained during a 10-week screening period using routine care data. Using the medical history, medication use, years of education, smoking status, alcohol intake were evaluated [25]. A fasting venous blood sample was drawn at baseline to measure lipid and lipoprotein profiling [25]. History of diabetes mellitus was defined as fasting blood glucose ≥ 7 mmol/L or self-reported. Data on history of cardiovascular disease, diabetes mellitus, transient ischemic attack, stroke or myocardial

infarction were provided by the participant's general practitioner.

2.3. Statistical analysis

Baseline characteristics of the study participants are reported as mean (standard deviation) for continuous variables and number (percentage) for categorical variables. The distribution of serum leptin was positively skewed; therefore, a logarithmic transformation on a natural scale was used. The associations between BMI and leptin with measures of cognitive function and brain volumes were studied using multivariable linear regression and linear mixed models' analyses. The multivariable linear regression was used to evaluate cross-sectional associations between baseline BMI and leptin and cognitive function measures, whereas the linear mixed models' analyses was used to evaluate prospective associations between baseline BMI and leptin and cognitive decline during follow-up.

Results are presented as either an estimate (beta, β) for the continuous variables (per BMI point or per ng/mL serum leptin), per BMI category, or per sex-standardized leptin third, together with the accompanying 95 % confidence interval. The reference category for BMI is between 18 and 25 kg/m², the reference category for leptin is the lower third. Moreover, the analyses (per BMI point, per ng/mL serum leptin or per BMI category) were stratified for sex.

The analyses were performed using a three-step approach. At first, the multivariable linear regression analyses were only adjusted for age, sex, country, years of education, and intercranial volume (analyses on brain volumes only; denoted as the minimally adjusted model). In the second step, the analyses were further adjusted for: alcohol intake (units per week); smoking status; serum cholesterol; diabetes mellitus; history of cardiovascular disease; history of myocardial infarction (denoted as the fully adjusted model) (26). In the third model, depending on the determinant, we either performed an additional adjustment for BMI or serum leptin. These procedures were repeated when performing the multivariable linear mixed models' analyses.

An interaction analysis was performed using 4 groups: low BMI and low serum leptin; low BMI and high serum leptin; high BMI and low serum leptin; high BMI and high serum leptin. All analyses were performed using SPSS Windows version 26 (IBM Corp., 2019).

3. Results

3.1. Baseline characteristics

The PROSPER study included a total of 5804 participants. After excluding participants with a BMI lower than 18 (n = 49, 0.8 %) and missing leptin measurements (n = 132, 2.3 %), 5623 participants remained and were included in the analysis (see Figure S1 in the supplement for patient inclusion flow diagram). Of the 1046 participants from the Netherlands, 527 had undergone an MRI scan of the brain (50.4 %).

Table 1 reports characteristics of the study population at baseline including cognitive function and brain structural volumes. The mean age of all individuals was 75.3 years (SD = 3.3), and over half of the participants were female (n = 2900, 51.6 %). Most participants had a history of hypertension (n = 3495, 62.2 %), and almost a third were current smokers (n = 1484, 26.4 %). Female participants had a median serum leptin of 23.5 ng/mL (IQR = 14.6–36.9) and a median BMI of 27.2 kg/m² (SD = 4.6), whereas male participants had a median serum leptin of 8.1 ng/mL (IQR = 5.0–12.8) and median BMI of 26.6 kg/m² (SD = 3.5). Moreover, the demographics of participants included in follow-up (Supplementary Table 3) did not differ substantially from participants at baseline.

3.2. Associations of BMI with cognitive function and cerebral volumes

In the fully adjusted model without leptin as an additional covariate

Table 1
Demographics and clinical characteristics of study population.

Sociodemographics	All (N = 5623)	Female (N = 2900)	Male (N = 2723)
Age, y, mean (SD)	75.3 (3.3)	75.7 (3.4)	75.0 (3.3)
Female, n (%)	2900 (51.6)	2900 (51.6)	2723 (48.4)
Age left school, y, mean (SD)	15.1 (2.0)	15.1 (1.9)	15.1 (2.2)
Current smoker, n (%)	1484 (26.4)	606 (20.9)	878 (32.2)
Alcohol intake, unit intake per week, mean (SD)	5.2 (9.2)	2.2 (4.7)	8.3 (11.5)
Cardiovascular risk factors			
History of CVD, n (%)	2486 (44.2)	1068 (36.8)	1418 (52.1)
History of hypertension, n (%)	3495 (62.2)	2106 (72.6)	1389 (51.0)
History of stroke or TIA, n (%)	622 (11.1)	273 (9.4)	349 (12.8)
History of myocardial infarction, n (%)	758 (13.5)	231 (8.0)	527 (19.4)
Serum cholesterol, mmol/L, mean (SD)	5.7 (0.9)	6.0 (0.9)	5.3 (0.8)
Body mass index, kg/m², mean (SD)	26.9 (4.1)	27.2 (4.6)	26.6 (3.5)
Serum leptin, ng/mL, median (IQR)	13.9 (7.2; 25.9)	23.5 (14.6; 36.9)	8.1 (5.0; 12.8)
Serum glucose, ng/mL, mean (SD)	5.5 (1.4)	5.4 (1.4)	5.6 (1.5)
Diabetes mellitus, n (%)	601 (10.7)	263 (9.1)	338 (12.4)
Pravastatin treatment, n (%)	2801 (49.8)	1441 (49.7)	1360 (49.9)
Cognitive function			
Stroop test, seconds, mean (SD)	66.5 (27.0)	65.9 (26.0)	67.2 (28.1)
LDCT, digits coded, mean (SD)	23.1 (7.8)	23.2 (7.8)	22.9 (7.9)
PLTi, pictures remembered, mean (SD)	9.3 (1.9)	9.6 (1.9)	9.0 (1.8)
PLTd, pictures remembered, mean (SD)	10.1 (2.6)	10.5 (2.6)	9.8 (2.5)
Cerebral volumes based on MRI			
Intracranial volume, cm³, mean (SD)[1]	1405 (145.6)	1298 (108.2)	1489 (112.1)
Amygdala volume, mm³, mean (SD)[2]	4015 (629.8)	3923 (592.6)	4096 (651.2)
Hippocampus volume, mm³, mean (SD)[3]	9305 (1121.6)	9103 (1057.4)	9487 (1148.9)
Nucleus accumbens volume, mm³, mean (SD)[4]	1173 (308.2)	1090 (287.9)	1255 (306.6)
Foramen putamen volume, mm³, mean (SD)[5]	10,439 (1143.9)	9974 (1058.2)	10,861 (1053.3)
Caudate nucleus volume, mm³, mean (SD)[6]	7497 (1024.0)	7151 (977.9)	7808 (964.9)
Thalamus volume, mm³, mean (SD)[7]	16,235 (1286.5)	15,711 (1219.3)	16,712 (1155.9)
White matter volume, ml, mean (SD)[8]	30 (4.8)	30 (4.7)	31 (4.8)
Gray matter volume, ml, mean (SD)[8]	25 (4.6)	24 (4.5)	25 (4.7)

Abbreviations: MRI = magnetic resonance imaging; SD = standard deviation; IQR = interquartile range; LDCT = Letter-Digit Coding Test; PLTi = Picture-Word Learning test immediate; PLTd = Picture-Word Learning Test delayed. Measured in: [1]=513 participants, [2]=411 participants, [3]=401 participants, [4]=204 participants, [5]=400 participants, [6]=411 participants, [7]=416 participants, [8]=252 participants.

(Table 2), in comparison to the reference category (BMI 18–25 kg/m²), individuals with a BMI between 25 and 30 kg/m² performed 0.95 s slower (95 % CI -0.67; 2.58) on the Stroop test, and individuals with a BMI above 30 kg/m² 3.32 s slower (95 % CI 1.29; 5.36). After adjusting for leptin, the effect size in individuals with a BMI between 25 and 30 kg/m² changed to 1.94 slower (95 % CI 0.14; 3.74), and individuals with a BMI above 30 kg/m² changed to 5.04 s (95 % CI 2.56; 7.53) slower performance on the Stroop test. A similar trend was observed in performance on the Letter-Digit Coding test in the group with BMI above 30 kg/m², where fully adjusting without leptin resulted in -1.11 digits coded (95 % CI -1.69; -0.52), and adjusting with leptin in -1.57 digits coded (95 % CI -2.27; -0.86). We did not find evidence of an association

Table 2

Cross-sectional associations of body mass index (BMI) and cognitive function.

| Cognitive test | Body mass index (BMI), kg/m² | | | | | |
	18−25 (N = 1906) β (95 % CI)	25−30 (N = 2557) β (95 % CI)	>30 (N = 1160) β (95 % CI)	Continuous All (N = 5623) β (95 % CI)	Continuous Female (N = 2900) β (95 % CI)	Continuous Male (N = 2723) β (95 % CI)
Minimally adjusted[1]						
Stroop, seconds	Ref	0.29 (−1.33; 1.91)	2.54 (0.53; 4.54) *	0.18 (0.01; 0.35) *	0.05 (−0.16; 0.26)	0.47 (0.17; 0.77) *
LDCT, digits coded	Ref	0.43 (−0.03; 0.89)	−0.82 (−1.40; −0.25) *	−0.05 (−0.10; −0.004) *	−0.001 (−0.06; 0.06)	−0.16 (−0.24; −0.08) *
PLTi, pictures remembered	Ref	0.06 (−0.05; 0.17)	−0.07 (−0.20; 0.07)	−0.01 (−0.02; 0.01)	−0.01 (−0.02; 0.01)	−0.003 (−0.02; 0.02)
PLTd, pictures remembered	Ref	0.08 (−0.08; 0.23)	−0.23 (−0.41; −0.03) *	−0.02 (−0.03; 0.001)	−0.01 (−0.03; 0.01)	−0.02 (−0.05; 0.00)
Fully adjusted without leptin[2]						
Stroop, seconds	Ref	0.95 (−0.67; 2.58)	3.32 (1.29; 5.36) *	0.25 (0.07; 0.42) *	0.11 (−0.10; 0.33)	0.56 (0.25; 0.87) *
LDCT, digits coded	Ref	0.21 (−0.25; 0.68)	−1.11 (−1.69; −0.52) *	−0.08 (−0.13; −0.03) *	−0.02 (−0.08; 0.05)	−0.21 (−0.29; 0.12)
PLTi, pictures remembered	Ref	0.07 (−0.05; 0.18)	−0.05 (−0.19; 0.09)	−0.001 (−0.01; 0.01)	0.00 (−0.02; 0.01)	0.00 (−0.02; 0.02)
PLTd, pictures remembered	Ref	0.08 (−0.08; 0.23)	−0.22 (−0.41; −0.02) *	−0.02 (−0.03; 0.002)	−0.02 (−0.04; 0.01)	−0.02 (−0.05; 0.01)
Fully adjusted with leptin[3]						
Stroop, seconds	Ref	1.94 (0.14; 3.74) *	5.04 (2.56; 7.53) *	0.40 (0.17; 0.64) *	0.32 (0.03; 0.61) *	0.78 (0.38; 1.17) *
LDCT, digits coded	Ref	−0.05 (−0.56; 0.47)	−1.57 (−2.27; −0.86) *	−0.13 (−0.20; −0.06) *	−0.07 (−0.16; 0.02)	−0.30 (−0.41; −0.20) *
PLTi, pictures remembered	Ref	0.06 (−0.06; 0.18)	−0.06 (−0.23; 0.11)	−0.002 (−0.02; 0.01)	−0.01 (−0.03; 0.01)	0.00 (−0.03; 0.02)
PLTd, pictures remembered	Ref	0.10 (−0.08; 0.27)	−0.20 (−0.43; 0.04)	−0.01 (−0.04; 0.01)	−0.03 (−0.06; 0.00)	−0.01 (−0.05; 0.02)

Abbreviations: LDCT = Letter-Digit Coding Test; PLTi = Picture-Word Learning test immediate; PLTd = Picture-Word Learning Test delayed. [1]=sex, age, country, years of education. [2]=sex, age, country, years of education, history of cardiovascular disease, history of diabetes, history of myocardial infarct, smoking, alcohol intake. [3]=sex, age, country, years of education, history of cardiovascular disease, history of diabetes, history of myocardial infarct, smoking, alcohol intake, serum glucose, leptin. *= P-value<0.05.

between BMI and performance on the Picture-Word learning tests.

When stratifying our continuous analyses of BMI for sex (Table 2), a slightly larger effect size was seen in men, where a higher BMI was associated with 0.78 slower performance on the Stroop test (95 % CI 0.38; 1.17) compared to 0.32 slower performance in women (95 % CI 0.03; 0.61). After performing a sensitivity analysis with full adjustments including leptin, we did not find evidence of effect modification (P-value = 0.187). Stratifying the BMI categories for sex did not substantially

Table 3

Cross-sectional associations of body mass index (BMI) and various brain volumes.

| Brain part volumes | Body mass index (BMI), kg/m² | | | | | |
	18−25 (N = 169) β (95 % CI)	25−30 (N = 276) β (95 % CI)	>30 (N = 82) β (95 % CI)	Continuous All (N = 527) β (95 % CI)	Continuous Female (N = 231) β (95 % CI)	Continuous Male (N = 296) β (95 % CI)
Minimally adjusted[1]						
Amygdala, mm³	Ref	179.5 (48; 311) *	388.5 (205; 572) *	36.9 (21; 53) *	31.2 (11; 51) *	47.4 (20; 75) *
Hippocampus, mm³	Ref	130.2 (−106; 367)	424.3 (95; 753) *	34.5 (5; 64) *	31.3 (−4; 66)	43.1 (−7; 93)
Nucleus accumbens, mm³	Ref	69.7 (−26; 165)	107.8 (−34; 250)	6.9 (−6; 20)	8.1 (−9; 25)	7.9 (−13; 28)
Foramen putamen, mm³	Ref	−15.3 (−244; 213)	−12.1 (−322; 298)	−5.0 (−33; 23)	−20.8 (−57; 15)	22.0 (−23; 67)
Caudate nucleus, mm³	Ref	−25.9 (−234; 183)	−115.7 (−399; 168)	−6.3 (−32; 19)	−25.6 (−57; 5)	27.8 (−13; 69)
Thalamus, mm³	Ref	241.9 (14; 470) *	321.9 (11; 633) *	26.8 (−1; 55)	6.9 (−28; 42)	63.0 (18; 107) *
White matter volume, ml	Ref	0.47 (−0.79; 1.72)	1.02 (−0.96; 2.99)	0.11 (−0.06; 0.29)	0.08 (−0.14; 0.29)	0.20 (−0.10; 0.51)
Gray matter volume, ml	Ref	0.42 (−0.88; 1.72)	1.21 (−0.83; 3.25)	0.11 (−0.07; 0.29)	0.13 (−0.09; 0.35)	0.11 (−0.21; 0.42)
Fully adjusted without leptin[2]						
Amygdala, mm³	Ref	182.5 (49; 316) *	397.6 (210; 585) *	39.0 (22; 56) *	35.9 (14; 57) *	44.4 (16; 73) *
Hippocampus, mm³	Ref	153.3 (−87; 394)	459.3 (123; 795) *	38.7 (8; 69) *	35.7 (−1; 73)	37 (−15; 89)
Nucleus accumbens, mm³	Ref	44.0 (−52; 140)	92.7 (−50; 235)	4.6 (−9; 18)	6.3 (−12; 25)	5.7 (−15; 26)
Foramen putamen, mm³	Ref	−1.0 (−230; 228)	11.2 (−302; 324)	−2.4 (−31; 26)	−10.4 (−48; 28)	14.5 (−32; 61)
Caudate nucleus, mm³	Ref	−15.6 (−225; 193)	−57.9 (−344; 228)	−3.5 (−29; 22)	−12.8 (−46; 20)	15.4 (−27; 58)
Thalamus, mm³	Ref	236.6 (7; 466) *	317.8 (4; 632) *	26.7 (−2; 55)	17.0 (−20; 54)	51.9 (6; 98) *
White matter volume, ml	Ref	0.45 (−0.86; 1.75)	0.99 (−1.08; 3.06)	0.11 (−0.08; 0.30)	0.04 (−0.19; 0.28)	0.22 (−0.12; 0.55)
Gray matter volume, ml	Ref	0.44 (−0.91; 1.79)	1.28 (−0.86; 3.43)	0.12 (−0.08; 0.31)	0.11 (−0.14; 0.35)	0.14 (−0.20; 0.48)
Fully adjusted with leptin[3]						
Amygdala, mm³	Ref	102.3 (−46; 251)	233.6 (3; 464) *	23.9 (1; 47) *	27.4 (−4; 59)	26.3 (−10; 63)
Hippocampus, mm³	Ref	218.2 (−49; 485)	590.0 (181; 999) *	60.7 (19; 102) *	72.5 (19; 126) *	48.3 (−19; 116)
Nucleus accumbens, mm³	Ref	56.3 (−47; 159)	119.5 (−42; 281)	7.6 (−10; 25)	23.1 (−0.4; 47)	−4.0 (−30; 22)
Foramen putamen, mm³	Ref	50.2 (−205; 306)	111.6 (−273; 496)	8.1 (−31; 47)	8.9 (−45; 62)	17.0 (−46; 80)
Caudate nucleus, mm³	Ref	19.7 (−212; 252)	3.3 (−348; 355)	4.6 (−31; 40)	15.8 (−31; 63)	9.2 (−47; 65)
Thalamus, mm³	Ref	220.4 (−36; 476)	273.9 (−114; 662)	19.7 (−19; 59)	34.1 (−19; 87)	31.0 (−30; 92)
White matter volume, ml	Ref	0.92 (−0.56; 2.39)	1.82 (−0.58; 4.22)	0.26 (0.01; 0.50) *	0.35 (0.03; 0.66) *	0.26 (−0.17; 0.68)
Gray matter volume, ml	Ref	0.89 (−0.64; 2.41)	2.06 (−0.43; 4.54)	0.26 (0.01; 0.51) *	0.35 (0.01; 0.68) *	0.24 (−0.20; 0.67)

[1]=sex, age, intercranial volume, years of education. [2]=sex, age, intercranial volume, years of education, history of cardiovascular disease, history of diabetes, history of myocardial infarct, smoking, alcohol intake. [3]=sex, age, country, intercranial volume, years of education, history of cardiovascular disease, history of diabetes, history of myocardial infarct, smoking, alcohol intake, serum glucose, leptin. *= P-value<0.05.

change the results (Supplementary Table 1). Moreover, during follow-up (Supplementary Table 4), all three models showed that a higher BMI at baseline was not associated with a faster decline in cognition over time.

The associations of BMI and brain volumes are displayed in Table 3. The largest effect sizes are seen in the amygdala and hippocampal volumes. A 1-point higher BMI results in hippocampal volume change from 39 mm^3 (95 % CI 8; 69) to 61 mm^3 (95 % CI 19; 102), after adjusting for leptin. With respect to the amygdala, a 1-point higher BMI resulted in a volume change from 39 mm^3 (95 % CI 22; 56) to 24 mm^3 (95 % CI 1; 47), also after adjusting for leptin. Overall, per BMI point increase, a larger volume of the amygdala, hippocampus, gray and white matter were observed. We did not find evidence for associations between BMI and the nucleus accumbens, foramen putamen, caudate nucleus nor thalamus. When stratifying for sex, a higher continuous BMI is associated with a higher hippocampal volume in men (β 48 mm^3, 95 % CI -19; 116) as well as women (β 73 mm^3, 95 % CI 19; 126), after adjusting for leptin. Last, stratifying the BMI categories for sex did not materially change the results (Supplementary Table 2).

3.3. Associations of serum leptin with cognitive function and cerebral volumes

In our study population (Table 4), we observed no consistent association between leptin and cognitive function using the sex-standardized thirds, and no association was observed in the continuous analysis. In addition, during follow-up (Supplementary Table 7), we observed no associations between baseline leptin concentration and faster decline in cognition over time. Stratification for sex did not substantially change the results (Table 4 and Supplementary Table 5).

Table 5 displays the associations between measures of sex-standardized thirds of leptin and brain part volumes. A 1 log ng/mL higher leptin concentration was associated with a larger amygdala volume, which attenuated slightly after adjusting for BMI (β 219 mm^3, 95 % CI 122; 316, to β 135 mm^3, 95 % CI 2; 268). We did not find associations for the other brain volumes. After adjusting for BMI, the upper third of leptin in comparison to the lower third, was associated with a lower white matter volume (β -2.18 mL, 95 % CI -4.07; -0.28).

After stratifying for sex (Table 5), a higher serum leptin in men was associated with an increased volume of the thalamus (β 277 mm^3, 95 % CI 39; 525). This association did not remain after adjusting for BMI. In the fully adjusted model, a higher serum leptin was associated with a lower white matter volume (β -2.67 mL, 95 % CI -4.52; -0.83) and lower

gray matter volume (β -2.14, 95 % CI -4.12; -0.17) in females. This was not found in males. Stratifying the thirds of leptin for sex as opposed to sex-standardizing the thirds did not materially change the results (Supplementary Table 6).

Last, we did not find evidence of interaction between low BMI and high serum leptin nor high BMI and low serum leptin and the outcomes (see Supplementary Tables 8 and 9).

4. Discussion

This follow-up study assessed the independent associations of BMI and circulating leptin levels with various domains of cognitive functioning and brain MRI volumes in a cohort of older individuals at increased risk for cardiovascular disease. At baseline, we found that a BMI above 30 was associated with a worse performance in executive functioning, as well as with higher volumes of the amygdala and the hippocampus. The association remained after adjusting for multiple possible confounders and leptin, excluding the possibility of leptin fulfilling the role of mediator in the present study. A higher circulating leptin concentration was associated with a larger amygdala volume, even after adjusting for BMI. There was no association between either BMI and leptin levels at baseline and cognitive decline during follow-up. Furthermore, stratifying for sex did not significantly change the results. Thus, BMI associates independent of leptin with cognition, amygdala and hippocampal volumes, and leptin associates with amygdalar volumes independent of BMI. These results specifically suggest that BMI and circulating leptin associate with amygdalar volume through distinct mechanisms.

Results from previous studies report mixed associations between BMI and cognition. Marioni et al found a phenotypic correlation that indicated better cognitive function to be associated with lower BMI [35]. This was corroborated by Gunstad et al., where longitudinal mixed-effects regression models showed multiple obesity indices, including BMI, to be associated with poorer performance in a variety of cognitive domains in the Baltimore Longitudinal Study of Ageing [36]. On the other hand, studies report a lower BMI was associated with faster rate of decline in global cognition in old age [11,37]. Losing weight in a secondary prevention setting has shown to be disadvantageous and increase health risks such as systemic inflammation and total CVD mortality. Thus, timing of weight loss may in part explain why individuals with lower BMI have found to be associated with faster cognitive decline [38].

Table 4
Cross-sectional associations of sex-standardized log-transformed thirds of leptin and cognitive function.

| Cognitive test | Leptin (log-transformed), ng/mL | | | | | |
	Low third (N = 1852) β (95 % CI)	Intermediate third (N = 1877) β (95 % CI)	Upper third (N = 1894) β (95 % CI)	Continuous All (N = 5623) β (95 % CI)	Continuous Female (N = 2900) β (95 % CI)	Continuous Male (N = 2723) β (95 % CI)
Minimally adjusted[1]						
Stroop, seconds	Ref	−0.77 (−2.51; 0.97)	0.72 (−1.02; 2.47)	−0.33 (−1.33; 0.68)	−0.59 (−1.90; 0.72)	0.30 (−1.24; 1.85)
LDCT, digits coded	Ref	0.74 (−1.01; 2.49)	0.11 (−0.39; 0.61)	0.12 (−0.17; 0.40)	0.17 (−0.23; 0.56)	0.01 (−0.40; 0.43)
PLTi, pictures remembered	Ref	0.52 (0.02; 1.01) *	−0.06 (−0.19; 0.06)	−0.01 (−0.08; 0.06)	−0.02 (−0.11; 0.08)	−0.01 (−0.11; 0.09)
PLTd, pictures remembered	Ref	0.11 (−0.39; 0.61)	−0.18 (−0.35; −0.01)	−0.07 (−0.16; 0.03)	−0.02 (−0.15; 0.11)	−0.13 (−0.27; 0.01)
Fully adjusted without BMI[2]						
Stroop, seconds	Ref	−0.16 (−1.92; 1.60)	1.54 (−0.24; 3.32)	0.57 (−0.44; 1.58)	0.15 (−1.20; 1.50)	1.34 (−0.25; 2.94)
LDCT, digits coded	Ref	0.33 (−0.18; 0.83)	−0.17 (−0.67; 0.34)	−0.19 (−0.47; 0.10)	−0.05 (−0.45; 0.36)	−0.36 (−0.79; 0.07)
PLTi, pictures remembered	Ref	−0.02 (−0.14; 0.10)	−0.07 (−0.19; 0.05)	−0.01 (−0.08; 0.06)	−0.03 (−0.13; 0.07)	−0.02 (−0.12; 0.09)
PLTd, pictures remembered	Ref	−0.05 (−0.21; 0.12)	−0.20 (−0.37; −0.03) *	−0.07 (−0.17; 0.03)	−0.05 (−0.19; 0.09)	−0.13 (−0.27; 0.02)
Fully adjusted with BMI[3]						
Stroop, seconds	Ref	−0.64 (−2.50; 1.23)	0.15 (−2.05; 2.34)	−1.11 (−2.45; 0.23)	−1.03 (−2.84; 0.77)	−1.23 (−3.16; 0.93)
LDCT, digits coded	Ref	0.58 (0.05; 1.11) *	0.47 (−0.16; 1.09)	0.34 (−0.05; 0.72)	0.19 (−0.35; 0.73)	0.58 (0.03; 1.12) *
PLTi, pictures remembered	Ref	−0.03 (−0.16; 0.10)	−0.09 (−0.24; 0.06)	−0.01 (−0.10; 0.09)	0.00 (−0.13; 0.13)	−0.01 (−0.14; 0.12)
PLTd, pictures remembered	Ref	−0.03 (−0.21; 0.15)	−0.16 (−0.37; 0.05)	−0.02 (−0.15; 0.11)	0.04 (−0.14; 0.23)	−0.09 (−0.28; 0.10)

Abbreviations: LDCT = Letter-Digit Coding Test; PLTi = Picture-Word Learning test immediate; PLTd = Picture-Word Learning Test delayed. [1]=sex, age, country, years of education. [2]=sex, age, country, years of education, history of cardiovascular disease, history of diabetes, history of myocardial infarct, smoking, serum cholesterol, serum glucose, alcohol intake. [3]=sex, age, country, years of education, history of cardiovascular disease, history of diabetes, history of myocardial infarct, smoking, serum cholesterol, serum glucose, alcohol, BMI. *= P-value<0.05.

Table 5

Cross-sectional associations of sex-standardized log-transformed leptin and various brain volumes.

Brain part volume	Leptin (log-transformed), ng/mL					
	Lower third (N = 174) β (95 % CI)	Intermediate third (N = 177) β (95 % CI)	Upper third (N = 176) β (95 % CI)	Continuous All (N = 527) β (95 % CI)	Continuous Female (N = 231) β (95 % CI)	Continuous Male (N = 296) β (95 % CI)
Minimally adjusted[1]						
Amygdala, mm^3	Ref	98.7 (−45; 242)	273.5 (127; 420) *	220.1 (125; 316)*	190.4 (60; 321) *	251.1 (111; 392) *
Hippocampus, mm^3	Ref	−94.9 (−352; 163)	150.9 (−113; 414)	55.3 (−118; 229)	−6.2 (−235; 223)	116.4 (−144; 377)
Nucleus accumbens, mm^3	Ref	85.4 (−16; 187)	33.2 (−74; 141)	12.7 (−62; 87)	−39.5 (−137; 58)	87.5 (−30; 204)
Foramen putamen, mm^3	Ref	27.0 (−220; 274)	−67.5 (−317; 182)	−47.4 (−211; 116)	−162.2 (−391; 66)	74.0 (−161; 309)
Caudate nucleus, mm^3	Ref	12.4 (−214; 239)	−37.9 (−265; 189)	−49.7 (−199; 99)	−220.7 (−421; −21)	134.3 (−83; 352)
Thalamus, mm^3	Ref	33.8 (−213; 281)	289.8 (40; 539) *	156.4 (−8; 320)	−8.3 (−232; 216)	328.3 (92; 564) *
White matter volume, ml	Ref	0.41 (−0.96; 1.77)	−0.72 (−2.15; 0.72)	−0.27 (−1.24; 0.70)	−0.98 (−2.30; 0.35)	0.50 (−0.93; 1.93)
Gray matter volume, ml	Ref	0.50 (−0.91; 1.91)	−0.70 (−2.19; 0.79)	−0.28 (−1.28; 0.73)	−0.48 (−1.88; 0.92)	−0.03 (−1.50; 1.44)
Fully adjusted without BMI[2]						
Amygdala, mm^3	Ref	114.8 (−32; 262)	280.8 (132; 430) *	219.2 (122; 316) *	195.3 (61; 330) *	230.9 (88; 374) *
Hippocampus, mm^3	Ref	−79.1 (−343; 185)	155.5 (−114; 425)	55.1 (−121; 232)	6.6 (−227; 240)	78.9 (−186; 343)
Nucleus accumbens, mm^3	Ref	66.4 (−38; 171)	6.6 (−103; 116)	−0.4 (−76; 75)	−70.2 (−173; 33)	72.5 (−43; 188)
Foramen putamen, mm^3	Ref	34.2 (−216; 284)	−65.1 (−317; 187)	−56.1 (−220; 108)	−129.6 (−363; 104)	37.4 (−200; 275)
Caudate nucleus, mm^3	Ref	49.2 (−181; 280)	−23.7 (−253; 206)	−50.5 (−201; 98)	−176.6 (−380; 27)	84.5 (−135; 304)
Thalamus, mm^3	Ref	40.3 (−210; 291)	275.1 (23; 528) *	138.6 (−26; 34)	7.7 (−220; 235)	277.1 (39; 516) *
White matter volume, ml	Ref	0.34 (−1.08; 1.77)	−0.79 (−2.30; 0.71)	−0.32 (−1.33; 0.68)	−1.25 (−2.62; 0.12)	0.44 (−1.08; 1.96)
Gray matter volume, ml	Ref	0.37 (−1.11; 1.84)	−0.77 (−2.33; 0.80)	−0.29 (−1.33; 0.75)	−0.72 (−2.17; 0.74)	−0.03 (−1.58; 1.53)
Fully adjusted with BMI[3]						
Amygdala, mm^3	Ref	39.0 (−115; 193)	107.2 (−82; 296)	134.8 (2; 268) *	72.1 (−124; 268)	169.1 (−18; 356)
Hippocampus, mm^3	Ref	−179.3 (−456; 97)	−80.5 (−418; 257)	−176.1 (−417; 65)	−327.1 (−659; 5)	−23.2 (−369; 323)
Nucleus accumbens, mm^3	Ref	52.7 (−57; 162)	−22.4 (−154; 109)	−28.5 (−125; 68)	−155.2 (−286; −24)	89.1 (−61; 239)
Foramen putamen, mm^3	Ref	21.6 (−242; 285)	−94.8 (−414; 224)	−85.2 (−312; 142)	−166.3 (−497; 165)	−5.1 (−328; 318)
Caudate nucleus, mm^3	Ref	49.4 (−193; 292)	−23.4 (−316; 269)	−60.9 (−268; 146)	−240.5 (−533; 52)	75.7 (−216; 367)
Thalamus, mm^3	Ref	14.6 (−249; 278)	212.4 (−109; 534)	65.9 (−162; 294)	−135.7 (−464; 192)	191.3 (−127; 510)
White matter volume, ml	Ref	−0.23 (−1.72; 1.26)	−2.18 (−4.07; −0.28) *	−1.25 (−2.57; 0.07)	−2.67 (−4.52; −0.83) *	−0.32 (−2.28; 1.64)
Gray matter volume, ml	Ref	−0.20 (−1.74; 1.35)	−2.12 (−4.09; 0.15)	−1.27 (−2.64; 0.10)	−2.14 (−4.12; −0.17)	−0.81 (−2.80; 1.19)

[1] =Age, sex, intracranial volume, years of education. [2] =Age, sex, intracranial volume, years of education, history of diabetes, history of myocardial infarction, history of cardiovascular disease, smoking, alcohol intake, serum glucose. [3] =Age, sex, intracranial volume, years of education, history of diabetes, history of myocardial infarction, history of cardiovascular disease, smoking, alcohol intake, serum glucose, BMI. *= P-value<0.05.

We previously showed that amygdala and hippocampal volumes are larger in obesity [22], and results from the present study suggest that this association is independent of circulating leptin. Other studies exploring associations between BMI and cerebral volumes present mixed results [39], however they did not correct for leptin, thus differing from the present study. On the contrary, data from the UK Biobank showed obesity to be negatively associated with hippocampal volume, and found no association with amygdala volume [40]. However, individuals in the UK Biobank are younger and arguably healthier, thereby perhaps explaining the difference in observations.

We also found increased amygdala volumes in individuals with higher fasting circulating levels of leptin and higher BMI. Of note, circulating leptin may not be representative of leptin concentrations present in the brain due to the selectivity of the blood brain barrier (BBB). It is possible that in obesity, high levels of circulating leptin may go hand-in-hand with what appears to be leptin resistance, due to the inability of leptin to cross the BBB resulting in less leptin signaling in the brain. Leptin is known to have pleiotropic metabolic effects including modulation of brain reward centers in the amygdala, and is able to bind to its receptor-expressing (LepRb) neuron population. LepRB is found mostly in the lateral hypothalamic area and the dopaminergic ventral tegmental area of the amygdala [41]. Thus, our finding may be explained by the fact that with an increased volume of the amygdala, more LepRb could be present, whereas the enlargement of the amygdala with higher BMI may be due the contribution of cerebral blood flow and higher glucose metabolism, increased activity in relating pleasure in the amygdala to memory in the hippocampus, or the abnormal functioning of the reward system [22].

In relation to cognition, leptin may exert neuroprotective effects by inhibiting neural nitric oxide synthase (NOS), and modulating amyloid-beta levels in hippocampal neurons through increasing Apo-E dependent removal [41]. Higher leptin levels have been associated with gray matter volumes of the hippocampus in older adults, suggesting a protective effect against age-related atrophy [42]. This finding is in line with the findings of the present study. Overall levels of hippocampal NOS have been found to be higher in rats with slower learning due to more memory errors [43]. In humans with congenital leptin deficiency, it has also been shown that leptin has the capacity to reverse neuro-cognitive deficits and increase gray matter tissue concentration in the hippocampus [44]. Furthermore, deficient NOS has been linked to Alzheimer's Disease [45], however the exact physiological mechanism remains unknown. It must also be emphasized that participants in the present study did not have severe cognitive impairment, and as this study was cross-sectional, statements about order of events cannot be made. Despite the fact that leptin receptors are found in various areas of the brain, including the hippocampus and amygdala [41], we were unable to explain the relationship between obesity and cognitive function through increased circulating leptin levels based on the present findings.

Unexpectedly, we found that obesity was associated with both worsened cognitive performance as well as an increased hippocampal volume. Interestingly, neurogenesis in the dentate gyrus of the hippocampus continues throughout adulthood [46]. This contributes significantly to hippocampal plasticity across the lifespan. As new neurons are generated, they compete with existing cells for synaptic connections, resulting in the remodeling of connections [47]. This may lead to losing information already stored in those circuits [48]. In line, it was demonstrated that high levels of neurogenesis disrupted established hippocampus-dependent memories [47]. Although the specific mechanism remains unknown, it can be argued that the reconfiguration of hippocampal circuits may reduce the likelihood that a given retrieval cue will trigger a previously stored pattern. With this, the artificial induction of neurogenesis after learning may be sufficient to induce forgetting [46,47]. However, due to the cross-sectional design of this study, it is difficult to draw conclusions from these contradictory findings.

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