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Research Article

Plasma Leptin Is Associated With Amyloid CSF Biomarkers and Alzheimer's Disease Diagnosis in Cognitively Impaired Patients

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Abstract

Background: Metabolic dysfunction and dysregulation of leptin signaling have been linked to Alzheimer's disease (AD)'s pathophysiology. The objectives of this study were to examine the associations between plasma leptin, cerebrospinal fluid (CSF), beta-amyloid (Aβ), and tau biomarkers (AT[N] status) and with the stage of cognitive impairment.

Methods: Cross-sectional analysis of data from cognitively impaired patients from a tertiary memory clinic. Plasma leptin levels were compared according to the stage of cognitive impairment and biomarker profiles, using the AT(N) classification. Linear regression models were performed to examine the relationship between leptin and CSF biomarkers. Results were adjusted for age, gender, body mass index (BMI), and *APOE* ε4. In a subgroup of A+T+ individuals, we compared the 2-year evolution of Mini-Mental State Examination scores, according to the participants' tertile of plasma leptin levels.

Results: We included 1 036 participants (age 68.7 ± 9.1, females = 54.1%). A+T+ and A+T− patients had significantly lower plasma leptin levels than amyloid negative participants ($p < .01$). CSF Aβ concentration was significantly associated with lower plasma leptin β = −4.3 (1.5), $p = .005$ unadjusted; and β = −3.4 (1.6), $p = .03$ after adjustment for age, female gender, BMI, and *APOE* ε4. Patients with major neurocognitive disorder due to AD had a difference of leptin of −7.3 ng/mL 95% confidence interval (CI; −11.8; −2.8), $p = .0002$, compared to individuals with other causes of cognitive impairment. Leptin was not associated with the slope of cognitive decline.

Conclusion: Plasma leptin levels were associated with CSF Aβ and with the diagnosis of AD confirmed by CSF biomarkers, suggesting a molecular interplay between leptin metabolism and brain amyloid deposition.

Keywords: Alzheimer's disease, Biomarkers, Leptin, Metabolic dysfunction

Abnormal protein deposits, including beta-amyloid (Aβ) plaques and neurofibrillary tangles, define Alzheimer's disease (AD) as a unique neurodegenerative disease among those leading to cognitive impairment (1,2). The novel research definition of AD is based on 3 biomarkers, Aß42, total-tau (T-tau), and phosphorylated-tau (P-tau), reflecting the core pathological features of AD. Thus, this Amyloid Tau (Neurodegeneration), that is, AT(N) classification suggests the presence of *Alzheimer's continuum* as follows (1). First, participants with biomarker evidence of Aβ deposition without pathological tau biomarker experience *Alzheimer's pathological*

change. Second, both pathological Aβ and tau biomarkers (also referred as A+T+ profiles) denote AD. Furthermore, cognitive impairment and neurodegenerative markers are less specific for AD and should rather be used to characterize the stage of the disease than the presence of the disease. The main purpose of this biological definition was to better identify and stage patients with AD, to facilitate their inclusion in clinical trials for disease-modifying therapies. Nevertheless, despite recent efforts to treat AD using antiamyloid therapies, the failure of phase III trials has shed the light on alternative pathophysiological hypotheses, underlining the potential role of inflammation, nutrition or hormonal imbalance influencing the onset of AD lesions (2,3). Insulin resistance and dysfunction of leptin signaling have been suggested to be key factors associated with the pathophysiology of type 2 diabetes and obesity, as well as AD (4,5). Midlife obesity and type 2 diabetes, which are associated with elevated plasma leptin concentrations and leptin resistance, are also established risk factors for the major neurocognitive disorder (NCD) and AD (6).

Leptin is a 16 kDa adipokine secreted primarily by adipocytes, acting as a key regulator of body weight and fat stores by modulating food intake and metabolism (7). Leptin crosses the blood–brain barrier using selective transporters and acts primarily on arcuate nuclei in the hypothalamus, to regulate energy balance, satiety, and body weight (8). Leptin receptors (LepR) are also expressed in the hippocampus, one of the earliest areas affected in AD (9). Thus, leptin can regulate hippocampal neuron excitability via both synaptic and nonsynaptic mechanisms and can influence hippocampal-dependent learning and memory (10). Numerous metabolic dysfunctions have been reported in AD. Malnutrition and weight loss have been associated with a higher incidence of AD (11,12). Conversely, higher body mass index (BMI) in late life was identified as a protective factor against cognitive decline (13,14)). Impaired leptin signaling could play a role in the pathophysiology of AD, more specifically at the onset of brain amyloid deposition (5,14,15). Several studies have underlined the relationship between low plasma leptin circulating levels and cognitive impairment, either in paticipants with mild or major NCD (16–18). The lowest leptin concentrations were measured in individuals with advanced-stage dementia (16,17). Finally, despite a growing body of evidence indicating that leptin plays a central role in the pathophysiology of AD, solid evidence of a specific relationship between leptin and biomarkers of AD is still missing in clinical studies. In the following investigation, we aimed to study the association between plasma leptin and amyloid and tau CSF biomarkers in individuals from a tertiary memory clinic. We also examined the associations between plasma leptin levels and the diagnostic groups, with regard to the stage of cognitive impairment and to confounding factors such as gender or BMI that modify circulating leptin concentrations (19). Finally, we analyzed the longitudinal evolution of cognitive function from individuals with biological evidence of brain amyloid and tau deposition, depending on their plasma leptin levels.

Method

Study Design and Participants

This is an observational monocentric study conducted in the Cognitive Neurological Center (Tertiary Memory Clinic of Paris North, Assistance Publique-Hôpitaux de Paris. University of Paris Cité, France), which included data recorded from November 2008 to June 2018. All patients (or their legal guardian) signed an informed consent, and the protocol was approved by the Medical Ethic Committee of the University of Paris (Bichat Hospital).

Patients were referred to the memory clinic by their general practitioner or their primary care neurologist, to investigate a memory complaint or any other cognitive symptoms, reported either by the patient or his/her caregiver. All the patients were assessed by a multidisciplinary team of dementia experts: 7 neurologists, 1 geriatrician, 7 neuropsychologists, 1 biochemist, 1 neuroradiologist, and a nuclear medicine physician. In this study, cognitively impaired patients with mild or major NCD were referred to our day-care department for comprehensive assessment, including cerebrospinal fluid (CSF) collection to measure Aβ, T-tau, and P-tau concentrations.

Neurological Diagnosis: Cognitive Impairment Stage, CSF Biomarkers, AT(N) Profile

First, the stage of cognitive impairment (mild or major NCD) was determined by the attending physician after a comprehensive clinical and neuropsychological assessment. Mild NCD was defined, according to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) criteria, as an objective decline in cognitive abilities with preserved independence in functional abilities (20). Likewise, major NCD was defined as a decline in mental ability interfering with independence and daily life (20). The clinical diagnosis of AD was suspected according to the consensual McKhann's diagnostic criteria and subsequently confirmed or excluded after CSF biomarkers assessment (21).

Amyloid and tau CSF measurements were performed in the biochemistry unit of the Lariboisière Hospital, using enzyme-linked immunosorbent assay (ELISA) Innotest® (Fujirebio) kits until May 2018, then ECLIA Elecsys® (Roche Diagnostics, Basel, Switzerland). According to the AT(N) classification, patients were considered as A+ if their CSF Aβ-42/Aβ-40 ratio or Aβ-42 level was below the specified cutoff and as T+ and N+, P-tau level, and T-tau levels, respectively, were above the determined cutoff (see Supplementary Table 1). All patients finally diagnosed with AD were on the *AD continuum*, either at the stage of mild or major NCD and had an A+ biomarker profile (1). The participants who did not meet AD criteria after comprehensive examination, including CSF biomarkers (eg, vascular cognitive impairment, Lewy body disease, and frontotemporal lobar degeneration) were all grouped as "other mild NDC" or "other major NCD," respectively. It is noteworthy the clinicians remained blinded from plasma leptin results, all along the diagnostic process. Leptin results were only considered for research purpose.

Blood Sampling for Leptin

Plasma leptin measurement was also performed in our day-care department. A peripheral venous puncture was performed between 8:00 AM and 9:00 AM, after a 12-hour overnight fast in all participants. Plasma leptin levels were measured by ELISA using TECO® leptin kits (TecoMedical Group, Sissach, Switzerland).

Other Variables of Interest

In our population, data were collected for age, gender, education level, Apolipoprotein E ε4 (*APOE* ε4) status, and cardiovascular risk factors (diagnosis of hypercholesterolemia, hypertension, and diabetes mellitus). Weight and height were measured, and BMI was calculated as the weight in kilograms divided by the square of height

in meters. Cognitive performance at baseline was evaluated with the Mini-Mental State Examination (MMSE) (22). When available, the successive MMSE scores measured over the first 2 years of follow-up were used for the longitudinal analysis of cognitive impairment.

Statistical Analysis

The baseline participants' characteristics were presented as means (standard deviation) for quantitative variables or as frequencies and numbers for categorical variables. First, univariate analyses were performed to compare the characteristics of the participants according to their tertile of plasma leptin. Quantitative variables were compared using analysis of variance (ANOVA) or Kruskal–Wallis tests, depending on their parametric or nonparametric distribution, and qualitative variables were compared using the homogeneity Chi-squared test. ANOVA models were adjusted for age, gender and BMI, given their established influence on leptin concentration (19)).

Second, linear regression models were performed to examine the relationship between leptin levels and the 3 AT(N) core CSF biomarkers, taking into account the variables associated with leptin in univariate analyses ($p < .10$), after checking the linearity assumption of the models. Among the variables associated with leptin concentrations in univariate analyses, we selected the variables related to AD (eg, age, gender, and *APOE* ε4 carriership) and removed collinear variables. Interactions between age, gender, BMI, and AT(N) CSF biomarkers were assessed to understand the relative importance of individual predictors of leptin levels.

Third, plasma leptin levels were compared according to the stage of cognitive impairment (mild or major NCD) and biological signature of AD (A+T+ vs other profiles) with an ANOVA model followed by post hoc multiple pairwise comparisons, using Tuckey's tests. Given the established influence of age, gender, and BMI on leptin concentrations, all analyses were adjusted for these variables (19).

Finally, a repeated-measures ANOVA model was performed to compare the evolution of MMSE scores over a 2-year follow-up among the 3 tertiles of plasma leptin, in individuals with biological AD (A+T+ profile). Deviations from sphericity were addressed by the Gasser-Greenhouse correction. All statistical analyses and graphs were performed using R software (v 4-1-0) and Graphpad Prism (v 9-2-0). Statistical analysis was conducted assuming a 2-sided 5% level of significance.

Ethical Considerations

All the participants were provided oral and written information about the opportunity to collect additional blood and CSF samples for further research analyses, in the BioCogBank© protocol. Written informed consent was obtained from all the patients or their legal guardians, when applicable. All the latter participants assented to participate after information, even if their legal guardians consented on their behalf. All the participants also consented for the anonymous use of their clinical data and the results of their CSF analyses. This study was approved by the local Ethic Committee (*Comité d'Evaluation et d'Ethique pour la Recherche Paris Nord*, Institutional Review Board 00006477 ref 16-004) and the *Commission Nationale Informatique et Libertés*.

Results

Over the screening period, 1 036 patients were included in the analyses; mean age was 68.7 (standard deviation = 9.1) years old, and 45.9% were males. The mean plasma level of leptin was 17.7 (20.5) ng/mL. A typical CSF AD profile (ie, A+T+ profile) was found in 41.5 % of our participants. The main characteristics of the study population are presented in Table 1.

Cross-Sectional Analysis

Univariate analyses

Female gender, higher weight, and BMI were associated with the highest tertile of plasma leptin. Higher education level was also associated with lower plasma leptin concentrations ($p = .03$). It should be noted that higher education was also significantly associated with lower BMI (ANOVA $F = 5.0$, $df = 2$, $p = .007$), which was, in turn, associated with lower leptin concentrations. *APOE* ε4 carriers were more represented in the lowest tertile of leptin group ($p = .04$). Participants with A+T+ and A+T– profiles showed lower leptin levels than their A–T– counterparts (ANOVA $F = 4.4$, $df = 3$, $p = .005$; see Figure 1). Likewise, participants with major or mild NCD due to AD showed significantly lower leptin levels than those this other diagnoses (ANOVA $F = 9.2$, $df = 3$, $p < .0001$; see Figure 2). Tukey's pairwise comparisons showed that patients with major NCD due to AD had a mean difference of plasma leptin of –7.3 ng/mL, 95% confidence interval (CI; –11.8; –2.8), $p = .0002$, compared to those with other causes of dementia. Similarly, patients with mild NCD due to AD had a mean difference of plasma leptin of –6.9 ng/mL, 95% CI (–12.7; –1.0), $p = .01$, when compared to those with other causes of mild NCD. However, there was no significant difference according to the stage of cognitive impairment (mild vs major NCD) among individuals with AD or those without AD.

Multivariate analyses

In a multivariate model adjusted for age, gender and BMI, an A+T+ CSF profile remained significantly associated with lower plasma leptin levels (β = –3.5 [2.0], $p = .01$). Leptin plasma levels were also significantly lower in patients with major NCD due to AD, compared with those with other causes of mild or major NCD (multiple regression model adjusted for age, gender, and BMI β = –3.8 [1.5], $p < .01$).

In a multivariate model including A(amyloid), T(phosphorylated-tau), and N(total-tau) biomarkers to explain plasma leptin variation, A+ was associated with lower plasma leptin β = –4.3 (1.5), $p = .005$, whereas T+ and N+ were not (Table 2). After controlling for the female gender, which was significantly associated with higher leptin (β = 14.7 [1.2], $p \leq .0001$), and age, A+ remained independently associated with leptin (β = –4.9 [1.5], $p < .001$). The association between A+ and lower leptin remained significant in models also adjusted for BMI and *APOE* ε4 carriers: β = –3.6 (1.5), $p = .02$ and β = –3.4 (1.6), $p = .03$, respectively. Given the marked collinearity between weight and BMI, we selected BMI as the preferred anthropometric measurement in the regression models. In Model 2, there was no significant interaction between gender and A+ ($p = .07$) nor between age and A+ ($p = .17$). In Model 3, BMI had significant interactions with age ($p < .01$) and gender ($p < .0001$) as well as with CSF biomarkers: A+ ($p < .001$) and T+ ($p < .05$) but not N+ ($p = .52$). In women, A+ and N+ were associated with lower plasma leptin β = –7.3 (2.3), $p = .002$, and β = –6.5 (3.2), $p < .05$, whereas T+ was not. In men, A+ was not associated with plasma leptin (β = –1.8 [1.3], $p = .16$).

Longitudinal Analysis

Among the population described earlier, 276 participants completed a 2-year follow-up with 3 MMSE scores measured in our memory clinic at 6 months, 12 months, and 24 months. Among them, 140 had an A+T+ CSF profile and were considered for the following analysis. In

Table 1. Characteristics of Our Population, According to the Plasma Leptin Level of the Participants

Characteristics *M* (*SD*)	Total *N* = 1 036	Tertile of Plasma Leptin (ng/mL)			
		Lowest < 7.1 *N* = 352	Middle *N* = 335	Highest > 19.0 *N* = 349	*p*
Age (years)	68.7 (9.1)	68.4 (8.9)	69.9 (8.9)	68.0 (9.5)	.01*
Male gender % (*N*)	45.9 (476)	72.7 (252)	47.8 (160)	17.2 (60)	<.0001****
Education level % (*N*)					
Under primary school certificate	29.4 (275)	24.6 (80)	31.1 (94)	32.5 (101)	.03*
Secondary to high school	35.1 (328)	37.5 (122)	30.1 (91)	37.0 (115)	
University degree	35.5 (332)	37.8 (123)	38.4 (116)	29.9 (93)	
Weight (kg)	69.0 (14)	65.6 (12.1)	69.2 (14.2)	72.2 (14.7)	<.0001****
Body mass index	24.8 (4.7)	22.7 (3.2)	24.7 (4.6)	27.1 (5.1)	<.0001****
Hypertension % (*N*)	44.1 (419)	41.9 (183)	40.8 (187)	49.5 (161)	.05
Diabetes mellitus % (*N*)	15.1 (143)	14.3 (45)	15.9 (50)	15.1 (48)	.86
Dyslipidemia % (*N*)	31.0 (293)	26.5 (83)	32.5 (102)	34.1 (108)	.1
MMSE score (/30)	23.0 (5.3)	23.1 (5.2)	23.1 (5.3)	22.8 (5.4)	.82
APOE ε4 carrier* % (*N*)	42.1 (421)	46.6 (158/339)	42.6 (138/324)	37.0 (125/338)	.04*
CSF biomarkers AT(N) profile					
A+ % (*N*)	55.1 (571)	57.1 (201)	58.2 (195)	50.1 (175)	.07
T+ % (*N*)	50.6 (524)	52 (183)	52.5 (176)	47.3 (165)	.31
N+ % (*N*)	56.8 (588)	59.4 (209)	58.8 (197)	52.1 (182)	.1
A+T+ % (*N*)	41.5 (430)	43.2 (152)	45.4 (152)	36.1 (126)	.21
A+T− % (*N*)	13.6 (141)	13.9 (49)	12.8 (43)	14 (49)	
A−T+ % (*N*)	9.1 (94)	8.8 (31)	7.2 (24)	11.2 (39)	
A−T− % (*N*)	35.8 (371)	34.1 (120)	34.6 (116)	38.7 (135)	
Diagnosis (*N* = 987†)					
Major NCD due to AD % (*N*)	36.2 (358)	39.8 (137)	37.3 (124)	28.0 (97)	<.0001****
Mild NCD due to AD % (*N*)	10.2 (101)	11.6 (40)	11.7 (39)	6.3 (22)	
Other major NCD % (*N*)	21.7 (214)	18.6 (64)	19.3 (64)	24.8 (86)	
Other mild NCD % (*N*)	35.5 (350)	30.0 (103)	31.6 (105)	40.9 (142)	

Notes: A+ = β-amyloid +; AD = Alzheimer's disease; *APOE* ε4 = apolipoprotein E ε4 allele; BMI = body mass index; CSF = cerebrospinal fluid; MMSE = Mini-Mental State Examination; T+ = p181-tau +; N+ = neurodegeneration +; NCD = neurocognitive disorder; *SD* = standard deviation.

*_APOE_ ε4 carrier: missing values = 35.

†Missing diagnoses = 49.

p-Values: *$p < .05$; ****$p < .0001$.

comparison with the other A+T+ participants included in the former cross-sectional analysis, but excluded from the longitudinal analysis ($n = 290$), these 140 A+T+ individuals showed better cognitive performance at baseline (mean difference in MMSE score = +1.7 points) and were more likely to go to high school ($p < .05$), but there was no difference regarding their plasma leptin levels. In comparison with all the other participants included in the cross-sectional analysis ($N = 896$), the A+T+ individuals with longitudinal follow-up data were older but also more likely to be *APOE* ε4 carriers or diagnosed with AD. However, these differences may be due to their A+T+ CSF profile. There was also a difference regarding their weight and BMI that could also explained by their exclusive A+T+ profile. Finally, we observed a nonsignificant 2-point mean difference in plasma leptin levels, between these 2 groups (see detailed characteristics in Supplementary Table 2).

We performed a repeated-measures ANOVA model to assess the role of leptin (in tertiles) on the slope of cognitive decline (see Figure 3). Leptin was not associated with the change of MMSE score ($F = 0.08$, $df = 2$, $p = .92$), whereas time was ($F = 31.7$, $df = 3$, $p < .0001$; Geisser-Greenhouse's epsilon 0.91).

Discussion

This study highlighted a specific association between lower plasma leptin levels and CSF amyloid biomarker positivity, indicating brain amyloid deposition. Likewise, individuals with AD showed significantly lower plasma leptin concentrations than those with other causes of cognitive impairment.

To the best of our knowledge, this study is the first one reporting a relationship between leptin and the diagnosis of AD confirmed by CSF biomarkers, whereas most published studies in this area considered the diagnosis of AD according to the DSM criteria and MRI abnormalities. The NIA-AA research framework defined AD by the presence of neuropathologic changes identified by biomarkers, whereas cognitive symptoms should be regarded as consequences of neurodegeneration, rather than part of the core definition of AD (1). Recent clinicopathological evidence emphasized the accuracy of CSF biomarkers (such as Aβ-40/Aβ-42 ratio that we used to define an A+ status) to discriminate absent or very low stages of neuropathologic changes due to AD lesions from intermediate-high stages (23). The study of Khemka et al. (16). included 120 participants, of whom 60 patients with end-stage dementia (mean MMSE score = 10.3 ± 3.8) diagnosed as AD. The patients with dementia reported very low leptin concentrations (4.6 ± 2.8 ng/mL) compared to age-matched control participants (10.1 ± 2.6 ng/mL). However, the certainty of AD diagnosis in patients with severe dementia only assessed by clinical judgment is questionable. Some of these patients could have been affected by other neurodegenerative conditions or vascular cognitive impairment. Besides, malnutrition in advanced-stage

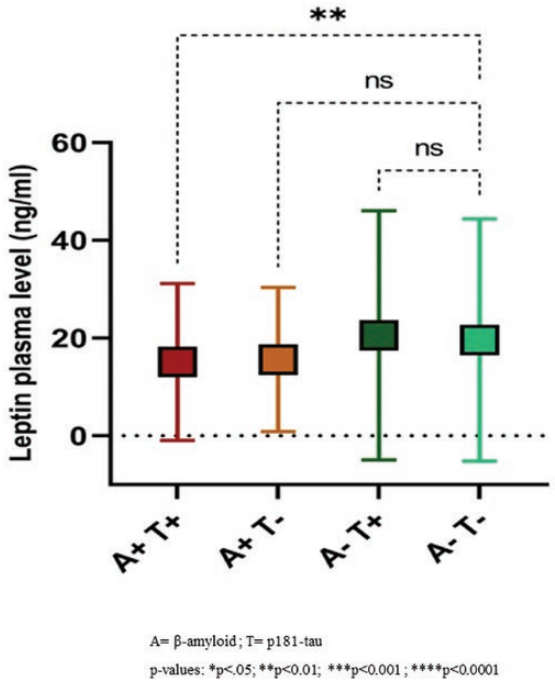

Figure 1. Leptin plasma levels according to AT(N) classification.

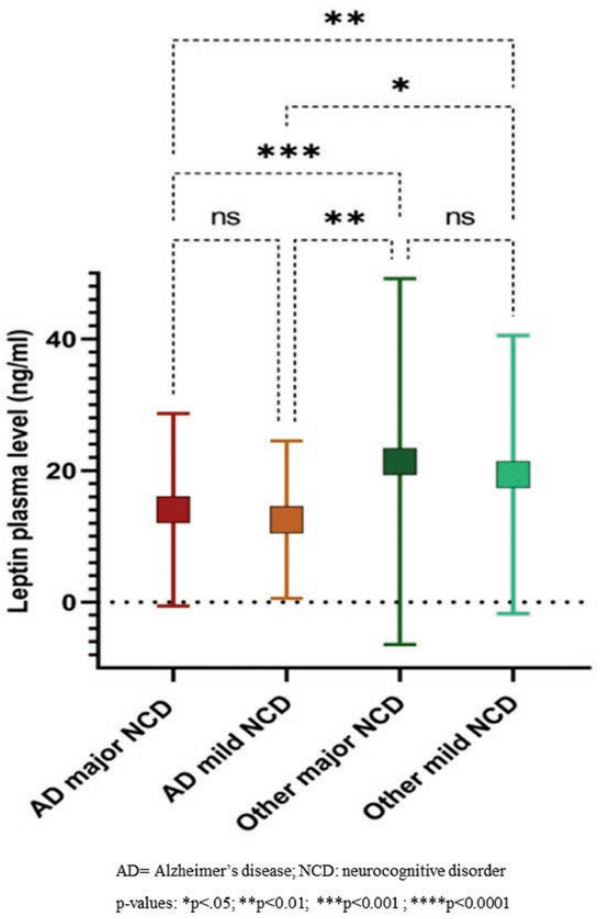

Figure 2. Leptin plasma levels in the different diagnostic groups defined according to CSF biomarkers. CSF = cerebrospinal fluid.

dementia and low body fat mass may, in turn, be responsible for low plasma leptin concentrations. In a large-sampled longitudinal analysis from the Framingham Original Cohort study, leptin levels showed a strong inverse relation with the risk of incident AD dementia, independent from age, gender, or BMI. Even though the authors made a distinction between AD and other causes of dementia, their diagnostic assumptions were also solely based on the same DSM-IV criteria. Higher leptin levels appeared as a protective factor against dementia, associated with preserved temporal or cerebral brain volumes (24). Likewise, in a recent case-cohort study embedded in the Rotterdam Study, higher plasma leptin was associated with a decreased risk of clinically-diagnosed AD dementia (as well as all-cause dementia). Interestingly the authors reported a protective effect of leptin in participants with a BMI at least 25 kg/m^2 and suggested that the weight loss preceding dementia onset might lead to a reduced protective action of leptin (25). Another small-sampled study analyzed the role of leptin, LepR, and free leptin index in advanced-stage AD, early-stage AD, and control participants (17). Alterations in leptin metabolism were highlighted in advanced AD. Although consistent with our results, these conclusions should be again mitigated by the absence of biological evidence of AD in cognitively impaired participants. The same remarks should apply to the results of a meta-analysis reporting the relationship between severe dementia symptoms and low plasma leptin levels (26). None of the 23 analyzed studies provided evidence of amyloid or tau lesions, either by CSF or positron emission tomography (PET) evaluations, in patients diagnosed with AD.

Our results extend the previous findings from preclinical models of brain amyloidopathy or AD and support the central role of leptin in the pathophysiology of AD (27,28). The amyloid cascade hypothesis, suggests that AD results from an imbalance between Aβ production and elimination (29). Our findings indicated a robust and specific association between low plasma leptin and brain Aβ deposition, reflected by lower CSF Aβ levels. This relationship was consistent with the results of previously published studies, including preclinical animal models of AD or in vitro investigations. For example, Greco et al. previously investigated the signaling pathways activated by leptin to reduce Aβ deposition in neuronal cultures (30). They observed that AMPK could mediate the effect of leptin on Aβ release. Niedowicz et al. observed plasma leptin was strongly and negatively correlated with total brain Aβ in a knocked-in AD mouse model (31). Presenilin 1 protein expression was inversely correlated with plasma leptin, suggesting that leptin would regulate γ-secretase activity in vivo. Moreover, in neuroglioma cells treated by leptin, the authors demonstrated reduced expression of 4 components of γ-secretase (presenilin, nicastrin, PEN2, and APH1), which may explain the reduced amyloid precursor protein cleavage and, in turn less production of Aβ. In another cell culture study, leptin was also shown to decrease aspartyl protease β-site Aβ-cleaving enzyme (BACE1, also known as β-secretase) expression via the activation of sirtuin 1, leading to a reduced intracellular Aβ production (15). Another study brought evidence of altered intraneuronal expression of leptin and impaired transport into the brain by its receptor: LepR, in aged 5XFAD mice (27). LepR, which is widely expressed in the cortex and the hippocampus is also involved in the activation of various intracellular signaling molecules regulating body weight and energy balance. Conversely, AD may also disturb leptin central action, because Aβ oligomers could bind directly to LepR and act as negative allosteric modulators of LepR (32). Therefore,

Table 2. Association Between Plasma Leptin, AT(N) Biomarkers and Confounding Factors

	Model 1*			Model 2†			Model 3‡			Model 4§		
Variable	β	*SD*	*p*	β	*SD*	*p*	β	*SD*	*p*	β	*SD*	*p*
A+	−4.3	1.5	.005**	−4.9	1.5	<.001***	−3.6	1.5	.02*	−3.4	1.6	.03*
T+	2.0	2.1	.34	1.7	2.0	.38	1.7	2.0	.41	1.6	2.1	.46
N+	−2.7	2.1	.18	−4.1	1.9	.03*	−2.2	2.0	.28	−1.8	2.1	.38
Age				0.0	0.1	.76	−0.0	0.1	.66	−0.0	0.1	.57
Gender (female)				14.7	1.2	<.0001****	15.2	1.2	<.0001****	15.2	1.3	<.0001****
BMI							1.7	0.1	<.001***	1.7	0.1	<.0001****
APOE ε4 (carrier)										−1.7	1.3	.21

Notes: Multiple regression analyses. A+ = β-amyloid +; *APOE* ε4 = apolipoprotein E ε4 allele; BMI = body mass index; T+ = p181-tau +; N+ = neurodegeneration +; *SD* = standard deviation.

Multiple linear regression models:

*Model 1: Plasma leptin explained by A, T, and N biomarkers.

†Model 1 + age and gender.

‡Model 2 + body mass index.

§Model 3 + *APOE* ε4 carriership.

β coefficient = change in plasma leptin for a 1-unit increase in the respective independent variable.

p-Values: **p* < .05; ***p* < .01; ****p* < .001; *****p* < .0001.

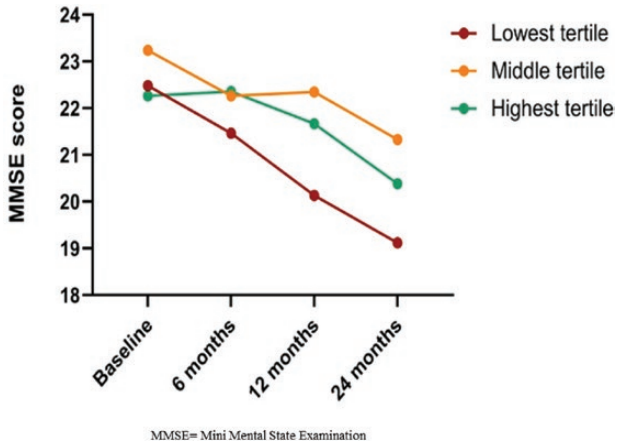

Figure 3. Evolution of the MMSE score over 2 years of follow-up in A+T+ individuals (*N* = 140), according to their plasma leptin concentration (tertiles).

leptin-induced intracellular signaling would be inefficient, increasing the risk of Aβ accumulation.

On the other hand, we did not advocate a specific relationship between leptin and tau pathology. Although plasma leptin was lower in participants with AD than in other causes of cognitive impairment, we did not find any specific relationship in our multivariate analyses between leptin and CSF phosphorylated-tau (T+). Thus, the link between AD and leptin seemed to be driven by brain amyloid deposition. A couple of preclinical studies suggested a specific effect of leptin on the regulation of tau phosphorylation, through Wnt signaling pathway or in AD mice supplemented with geniposide (33,34). One study involving CRND8 transgenic mice demonstrated that leptin treatment resulted in significantly lower levels of phosphorylated-tau, as well as a significant reduction in amyloid burden (35). As a result, evidence regarding a specific link between leptin and tau lesions seems scarce.

Just as we did not find a specific relationship between leptin and CSF T+, leptin was not associated with cognitive performance in our overall sample. It is noteworthy that tau pathology was shown to be a good predictor of hippocampal volume change and cognitive decline, whereas amyloid biomarkers were not (36). In our longitudinal analysis, including a subgroup of patients with a CSF biological profile of AD, leptin concentration was neither associated with cognitive performance at baseline, nor with the slope of cognitive decline. Our results are consistent with those from Gilbert et al. who did not show any significant relationship between leptin plasma levels and evolution of the MMSE score over 5 years, in a population of cognitively impaired older adults (18). Similarly, plasma leptin did not predict conversion from mild to major NCD in 352 participants followed-up over 3 years (37). A longitudinal analysis from the Framingham Heart Study in young adults did not find any statistically significant association between leptin and any of the MRI brain volume measures, or cognitive performance, after adjusting for BMI (38). However, higher leptin concentration in a large-sampled prospective study of community-dwelling older adults, was associated with slower cognitive decline, over 4 years, even after adjusting for gender, BMI, or total percent body fat (39).

In conclusion, leptin could be related to the onset of biological lesions of AD, whereas the evolution of cognitive performance may be mediated by individual characteristics such as age, *APOE* ε4, or cerebrovascular lesions (40). Therefore, leptin should neither be regarded as a progression biomarker, nor as a specific diagnostic biomarker of AD, as many other factors, such as gender or BMI, would influence leptin concentration. For instance, the proportion of leptin variance explained by the female gender was fivefold higher than by A+ status. However, the consistent and independent relationship between CSF amyloid biomarkers and leptin levels suggests considering this adipokine as a potential therapeutic target. In an animal model of AD, leptin treatment over 8 weeks was associated with a significant reduction of brain amyloid deposition, but also reduced levels of phosphorylated-tau in the brain (35). Interestingly, these benefits were associated with clinical and behavioral improvements: better novel object recognition performance and improved fear conditioning tests. In another AD mice model (2xTgAD), leptin treatment increased neurogenesis and modulated microglial activation as well as amyloid deposition in the hippocampus, suggesting leptin as a potential modifying agent to slow down the course of AD (41).

As advocated by Tezapsidis et al. preclinical evidence regarding AD and safety data from studies of leptin replacement therapy, in individuals with congenital leptin deficiency, support the design of a pilot trial assessing the therapeutic role of leptin supplementation in AD (42). Johnston et al. also suggested leptin replacement therapy for individuals with mild NCD at risk of AD (eg, *APOE* ε4 carriers), with low plasma leptin concentration (43). CSF biomarkers have enabled the early identification of such participants with early AD who could be screened in for interventional studies based on leptin therapy. Yet, there is currently no ongoing trial in this field, registered on Clinicaltrials.gov.

The present study has several limitations that should be acknowledged. We only included cognitively impaired individuals, and cannot extend our findings to preclinical AD. Biomarkers were rarely prescribed in the clinical setting for individuals with subjective cognitive complaints. The plasma leptin levels may not perfectly reflect its brain concentration and central action; further studies, including measures of CSF leptin concentrations, are warranted to address this issue. Other adipokines (eg, adiponectin) that were not measured in this study might also play a role on brain amyloid deposition and deserve further investigation. Finally, leptin levels were strongly influenced by gender and body composition, and we observed gender differences in the associations between A+ biomarker and leptin. Further investigations on plasma leptin levels in male are warranted to better understand the role of this adipokine in the pathophysiology of AD.

The main strengths of the study are (a) the large-sampled population of individuals diagnosed in a tertiary memory clinic, with expertise in AD biomarkers, (b) the adjusted analyses for the main factors known as related to plasma leptin concentrations: gender, BMI, and *APOE* ε4. In conclusion, our findings brought additional insights to target leptin signaling, in future clinical trials, including amyloid-positive participants, with the objective of mitigating the accumulation of brain lesions leading to cognitive impairment.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None declared.

Author Contributions

M.L., E.B.-A., J.D., J.H., and C.P. conceptualized and designed the present study. M.L., J.D., E.C., C.H., J.H., and C.P. participated to the clinical assessments and the data collection of the patients in the study. M.L. and J.D. analyzed data and performed statistical analyses. M.L. and E.B.-A. wrote the first draft of the manuscript. F.M.-L., J.D., E.C., M.M., M.S., A.-C.T., C.H., J.H., and C.P. analyzed data and revised the manuscript critically for intellectual content. E.B.-A. played a major role in data acquisition. All authors have read and approved the final version of the manuscript before publication.

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