Impact of Leptin on Memory Function and Hippocampal Structure in Mild Cognitive Impairment

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Abstract: Metabolic changes have been suggested to contribute to dementia and its precursor mild cognitive impairment (MCI), yet previous results particularly for the “satiety hormone” leptin are mixed. Therefore, we aimed to determine if MCI patients show systematic differences in leptin, independent of sex, adipose mass, age, and glucose and lipid metabolism, and whether leptin levels correlated with memory performance and hippocampal integrity. Forty MCI patients (20 females, aged 67 years $\pm$ 7 SD) were compared to 40 healthy controls (HC) that were pair-wise matched for sex, age, and body fat. Memory performance was assessed using the auditory verbal learning test. Volume and microstructure of the hippocampus were determined using 3T-neuroimaging. Fasting serum markers of leptin, glucose and lipid metabolism, and other confounding factors were assayed. MCI patients, compared with HC, showed lower serum leptin, independent of sex, age, and body fat ($P < 0.001$). Glucose and lipid markers did not attenuate these results. Moreover, MCI patients exhibited poorer memory and lower volume and microstructural integrity within hippocampal subfields. While leptin and memory were not significantly correlated, mediation analyses indicated that lower leptin contributed to poorer memory through its negative effect on right hippocampus volume and left hippocampus microstructure. We demonstrated that MCI is associated with lower serum leptin independent of

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sex, age, body fat, glucose, and lipid metabolism. Our data further suggest that inefficient leptin signaling could partly contribute to decreases in memory performance through changes in hippocampus structure, a hypothesis that should now be verified in longitudinal studies. *Hum Brain Mapp* 37:4539–4549, 2016. © 2016 Wiley Periodicals, Inc.

Key words: hippocampus structure; leptin; mediation model; memory performance; MCI

INTRODUCTION

The rising incidence of neurodegenerative diseases such as Alzheimer's disease (AD) is a major social and economic challenge of our aging societies [Thies and Bleiler, 2013]. As causal therapies are lacking, reductions of modifiable risk factors for AD, such as type 2 diabetes and metabolic syndrome, become increasingly recognized as key preventive strategies [Barnes and Yaffe, 2011].

Several studies in the last decade linked metabolic changes, including elevated glucose, hyperlipidemia and low levels of the adipose tissue-derived hormone leptin, which is involved in appetite and weight regulation, to accelerated brain aging and a higher risk to develop AD [reviewed in Biessels et al., 2008; Fadel et al., 2013; Reitz and Mayeux, 2014]. For example, higher glucose and lipid levels have been associated with reduced volume and microstructural integrity of the hippocampus [Cherbuin et al., 2012; Kerti et al., 2013; Wolf et al., 2004], a key area implicated in memory processing and AD pathology. Also, both conditions were independent risk factors for AD in two large prospective cohort studies [e.g., Crane et al., 2013; Whitmer et al., 2005]. In addition, lower serum leptin has been linked to reduced gray matter volume, including the hippocampus [Lieb et al., 2009; Narita et al., 2009]. Lower leptin has also been associated with a higher risk for AD [Holden et al., 2009; Johnston et al., 2014; Lieb et al., 2009; Power et al., 2001], however, others could not support these findings [Kamogawa et al., 2010; Maioli et al., 2015; Oania and McEvoy, 2015; Rajagopalan et al., 2013]. Mixed results also emerged when looking at lean men and women [Zeki Al Hazzouri et al., 2012] compared with a cohort of male patients with type 2 diabetes mellitus (T2DM) [Labad et al., 2012], or individuals at younger age [Gustafson et al., 2012; Pannacciulli et al., 2007], suggesting potential confounding effects of adipose mass, sex, age, and concurrent changes in other metabolic pathways.

In sum, specific metabolic changes are thought to exert negative effects on the brain, yet with regard to leptin, observational human studies yielded in part controversial results. In addition, the neuronal and cognitive correlates of changes in peripheral leptin, specifically in relation to individual adipose mass, other metabolic conditions, and age remain poorly understood. We therefore aimed to determine if patients with prodromal AD exhibit alterations in leptin, using a homogenous sample of 40 patients with mild cognitive impairment (MCI) and 40 healthy

controls (HC) that were carefully matched for sex, age, and body fat. Glucose and lipid metabolism were determined to avoid confounding effects of concurrent changes in other metabolic markers. Using high-resolution magnetic resonance imaging (MRI) and sensitive neuropsychological testing, we further examined if changes in leptin correlated with hippocampal volume and microstructure, and if this would mediate interindividual differences in memory performance.

METHODS

Study Design

All participants were enrolled in ongoing longitudinal studies at the Department of Neurology, Charité University Hospital Berlin, Germany (NCT01219244, NCT00996229). For the current analyses, baseline assessments of MCI patients and healthy participants were considered. Forty MCI patients were recruited via the memory clinics of the Department of Neurology, Charité University Hospital Berlin, medical practitioners and advertisements in Berlin, Germany. Inclusion criteria included diagnosis of amnesic MCI within 12 months before baseline visits, diagnosed according to Mayo criteria based on a subjective cognitive complaint and an objective memory impairment in a standardized test (performing at least 1 SD below age- and education-specific norm in relevant subtest of the CERAD-Plus test or the AVLT test battery (Total Word List, Delayed Recall Word List/Figures), no impairment in activities of daily living, and no dementia [Petersen et al., 1999]. On average, 80% of patients had a deficit in the verbal domain, and 50% in the visuoconstructive domain. Exclusion criteria comprised MMSE ≤ 24 at baseline visits and history of severe untreated medical, neurological, and psychiatric diseases, diabetes mellitus type 2, stroke or pathological MR-findings, specifically, stroke and tumors, intake of cholinesterase-inhibitors or memantine, and daily consumption of more than 50 g of alcohol, more than 10 cigarettes, or more than 6 cups of coffee. Medical examination at baseline visits ensured absence of peripheral edema; serum analyses at baseline normal-range creatinine levels. MCI-patients were pair-wise matched to 40 healthy older adults for sex, age, and body fat (Table I). Additional inclusion criteria for healthy controls included an MMSE [Folstein et al., 1975] score of 26 points or higher. MCI and

TABLE I. Baseline characteristics of mild cognitive impairment (MCI) patients and healthy controls (HC)

Parameter	MCI	HC	<i>P</i>
<i>n</i> (female/male)	40 (20/20)	40 (20/20)	
Age (years)	67.4 ± 7.3 (51–77)	66.9 ± 6.8 (51–75)	0.29 ^a
Body fat (%)	29.7 ± 7.6 (16–48)	28.9 ± 7.6 (12–40)	0.43 ^b
Education (years)	15.2 ± 3.4 (6–23)	16.4 ± 3.1 (10–24)	0.12 ^b
Body mass index (BMI) (kg/m ²)	26.4 ± 3.5 (21–37)	26.9 ± 2.1 (24–35)	0.15 ^a
Mini mental state examination (MMSE) (score)	28.4 ± 1.5 (24–30)	28.9 ± 1.0 (26–30)	0.10 ^a
Systolic blood pressure (mm Hg)	141.7 ± 17.9 (109–181)	136.7 ± 13.1 (101–162)	0.17 ^b
APOE ε4 (carriers/non-carriers)	18/22	10/20 ^c	0.32 ^d

Data given as mean ± SD and range (min–max).

^aWilcoxon signed-rank test.

^bPaired *t*-test.

^c*n* = 30.

^dChi-square-test.

HC did not differ significantly with regard to age, body fat, education, BMI, MMSE scores, and systolic blood pressure (Table I).

During baseline visits, subjects underwent a standardized medical interview and a neurological examination. We assessed neuropsychological test performance, collected blood samples after an overnight fast of at least 10 hours, and magnetic resonance imaging (MRI) at 3 Tesla (see below for details). All subjects provided written informed consent and received reimbursement for participation. The research protocol was approved by the Ethics Committee of the Charité Universitätsmedizin Berlin, Germany and was in accordance with the declaration of Helsinki.

Neuropsychological Testing

Subjects were tested for memory performance using the German version of the Auditory Verbal Learning Test (AVLT) [Lezak, 2004]. Participants had to remember and recall a list of 15 words within five immediate recall trials (=“learning ability”), followed by a 30 min “delayed recall” and “recognition” trial. “Retention of words” was defined as the number of correct words recalled after the fifth trial subtracted by those correctly recalled after the 30 min delay, multiplied by –1 to create positive relations. Further testing included tasks on working memory and executive functions including trail making test (TMT) A and B [Lezak, 2004].

Blood Parameters and Anthropometric Data

Fasting blood donations were collected to assess levels of the adipokine leptin, of markers of glucose metabolism [glucose, glycated hemoglobin A1c (HbA1c), insulin], and lipid metabolism [total cholesterol, high-to-low-density lipoprotein (HDL-to-LDL)-ratio, triacylglycerides]. Leptin was assessed using RayBio[®] enzyme-linked immunosorbent assay (ELISA) Kits, with a maximal detectable leptin level of 40.1 ng/mL (intra-assay coefficients of variation,

CV: <10%, inter-assay CV <12%). All parameters were analyzed by trained personnel at the IMD laboratory, Berlin, Germany according to standardized procedures. Anthropometry included body fat measured using bioelectrical impedance analysis, weight, and height. Also, blood pressure was measured twice in a seating position after at least 5 min rest, and averaged.

Magnetic Resonance Imaging (MRI)

MRI was performed on two Siemens Trio systems operating at 3 Tesla, using 12-channel head coils. All subjects underwent the same 3D structural scanning protocol using high-resolution T1-weighted Magnetization Prepared Rapid Gradient Echo (MPRAGE) imaging (TR = 1,900 ms, TE = 2.52 ms, 192 sagittal slices, voxel-size of 1.0 × 1.0 × 1.0 mm³, flip angle = 9°), and the same diffusion-weighted spin-echo echo-planar imaging (EPI) sequence (TR = 7,500 ms, TE = 86 ms, 61 axial slices, voxel size of 2.3 × 2.3 × 2.3 mm³; 64 directions with a b-value of 1,000 s/mm² and one b0). All MCI patients and 16 HC were scanned at scanner #1, 24 HC were scanned at scanner #2. To avoid a potential confounding, we adjusted for scanner site (1 or 2) in the statistical analyses (see below). MRI processings were done using the software packages FSL 4.1 (www.fmrib.ox.ac.uk/fsl), AFNI (<http://afni.nimh.nih.gov/afni>), and FreeSurfer 5.1.0 (<http://surfer.nmr.mgh.harvard.edu/>).

Hippocampal Volume

To estimate hippocampal volume, high-resolution T1-weighted images were first registered to MNI-space by rigid body transformation. Cortical and subcortical reconstruction and volumetric segmentation, including the left and right hippocampus, was performed by FreeSurfer. Briefly, this process included motion correction, intensity normalization, and skull stripping using a watershed algorithm [more technical details are described in Dale et al., 1999; Fischl et al., 2002]. FreeSurfer-based automated

TABLE II. Cognitive performance of mild cognitive impairment (MCI) patients and healthy controls (HC)

		MCI (<i>n</i> = 40)	HC (<i>n</i> = 40)	<i>P</i>
Verbal memory (# words)	Learning ability	45.4 ± 9.6 (28–68)	50.9 ± 9.0 (33–67)	0.009^a
	Delayed recall	7.4 ± 3.7 (0–14)	10.7 ± 3.1 (1–15)	<0.001^a
	Retention of words	−3.8 ± 2.5 (−9 to 1)	−1.6 ± 2.3 (−8 to 4)	<0.001^a
	Recognition	8.3 ± 5.8 (−7 to 15)	12.1 ± 2.6 (6–15)	<0.001^b
Processing speed	Trail making test (TMT), part A (sec)	43.3 ± 22 (20–127)	33.8 ± 10 (20–62)	0.019^b
Executive functions	Trail making test (TMT), part B (sec)	108 ± 60 (41–300)	68.3 ± 40 (18–156)	0.001^b

Significant differences are indicated by bolding the number. Data given as mean ± SD and range (min–max)

^aPaired *t*-test.

^bWilcoxon signed-rank test.

subfield segmentation was carried out according to Van Leemput et al. [2009] in order to extract individual volumes of cornu ammonis (CA) fields 1–4 and dentate gyrus (DG). Individual hippocampal volumes were adjusted for intracranial volume (ICV) according to previous studies [den Heijer et al., 2012; Kerti et al., 2013; Raz et al., 2004] using the following formula: adjusted volume = raw volume − *b* × (ICV − mean ICV). The coefficient *b* represents the slope of regression of a region of interest volume on ICV.

Hippocampal Microstructure

Microstructure of the hippocampus was assessed by mean diffusivity (MD) estimated using diffusion tensor imaging (DTI), in line with previous studies [den Heijer et al., 2012; Kerti et al., 2013]. Therefore, a tensor model was fitted to the motion-corrected DTI data to create individual 3D images of MD. Then, individual T1-weighted images were co-registered to the b0 images using rigid-body transformations. These registrations were used to transform masks of the left and right hippocampus and subfields (derived by FreeSurfer-segmentation on the T1 images) to the MD maps, for extraction of the mean individual hippocampal MD values.

Statistical Analysis

To detect significant differences between pairwise-matched MCI and HC in the above described parameters, we calculated paired *t*-tests or Wilcoxon signed-rank tests, as appropriate. To further adjust for potential confounders such as age, sex and body fat, analyses of covariate (ANCOVA) with “matching-pair” as additional covariate were calculated in men and women separately. For differences in volume and microstructure of the hippocampus between MCI and HC, we adjusted for age, sex, scanner site, and “matching-pair” using ANCOVA. Linear associations between leptin, hippocampus measures, and memory performance were assessed using partial correlations on crude or rank-transformed data, adjusting for age, sex, education, body fat, scanner site, “matching-pair,” and

group (MCI or HC), as appropriate. In addition, simple mediation analyses were calculated to test if hippocampus measures would indirectly mediate potential effects of leptin on memory performance independent of confounders, according to Preacher and Hayes [2004].

Leptin, insulin, cholesterol, and triacylglycerides were log-transformed to reduce their skewed distributions and to improve visualization. Leptin levels were sex-standardized due to the large sex differences in circulating levels [Benedict et al., 2014]. Normal or near-normal distributions were considered if variables displayed unimodal distributions with a skewness of > −1 and < 1. Levels of significance were set at $P \leq 0.05$. Further, a Bonferroni-correction for multiple testing was applied for group differences in serum concentrations considering seven markers of interest ($\alpha = 0.05/7 = 0.0071$). Significance levels of further analyses were not corrected for multiple comparisons due to the exploratory nature of these analyses. Mediation analyses were evaluated using a bootstrap method with 10,000 resamples. Analyses were done using SPSS 22 (PASW, SPSS, IBM).

RESULTS

Cognitive Performance

As expected, MCI patients showed significantly poorer memory performance compared with HC (all $P < 0.01$; see Table II for details). In addition, MCI patients performed significantly poorer on tests of processing speed and executive functions (all $P < 0.019$; see Table II for details).

Fasting Serum Parameters

No significant differences were found for markers of glucose and lipid metabolism (all $P > 0.05$, for details, see Table III). We observed significantly lower serum leptin concentrations in MCI patients compared with HC (Wilcoxon-signed-rank test, standardized $W = 4.6$, $P \leq 0.001$, Bonferroni-corrected, Fig. 1). The difference in the median z-scores was 1.26, which relates to a mean difference of 10 ng/ml leptin between MCI and HC.

TABLE III. Fasting serum parameters of mild cognitive impairment (MCI) patients and healthy controls (HC)

Parameter	MCI (n = 40)	HC (n = 40)	P
Leptin, ng/mL	5.9 ± 8.0	15.9 ± 11.6	<0.001 ^a
Glucose, mg/dL	96.6 ± 10.8	94.6 ± 9.5	0.33 ^b
Insulin, μU/mL	8.5 ± 4.8	8.3 ± 4.0	0.97 ^b
HbA1c, % ^a	5.76 ± 0.3	5.84 ± 0.3	0.20 ^b
Total cholesterol, mg/dL	224.0 ± 35.2	219.1 ± 41.5	0.46 ^b
LDL-to-HDL ratio	2.3 ± 0.8	2.2 ± 0.8	0.63 ^b
Triacylglycerides, mg/dL	109.6 ± 49.7	104.9 ± 40.9	0.78 ^b

Significant differences are indicated by bolding the number. Data are given as mean ± SD

HbA1c, hemoglobin A1c; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

^aWilcoxon signed-rank test.

^bPaired *t*-test.

Lower leptin in MCI compared with HC was also found when looking at women and men separately (paired *t*-tests, females: *t* (19) = -4.0, *P* = 0.001, males: *t* (19) = -4.3, *P* ≤ 0.001). In addition, non-standardized values of leptin correlated with higher body fat (*r* = 0.48, *P* ≤ 0.001) and higher age (*r* = 0.27, *P* = 0.017); however, adjusting for age and body fat did not attenuate the effects of group (ANCOVA, MCI vs. HC, *F*(1, 76) = 36.7, *P* ≤ 0.001). This was also true when additionally adjusting for glucose and lipid parameters (effect of group, *F*(1, 71) ≥ 35.1, *P* ≤ 0.001).

Hippocampus and Memory Performance

MCI patients had smaller volumes and higher MD in the whole hippocampus and within the subfields when compared with HC (see Table IV). These differences reached significance in women for volumes of the CA1, CA2/3 and CA4/DG subfields, after adjustment for age and scanner site (sex-by-group interactions, all *F*(1, 74) > 5.9, all *P* < 0.018). Turning to microstructure, significant differences in MD were found for left, right, and mean hippocampus (all *F*(1, 73) > 7.3, all *P* < 0.048) and for the CA1 subfield (*F*(1, 73) = 4.5, *P* = 0.038) in the whole group after adjustment for age, sex and scanner site.

In addition, memory performance was associated with measures of the hippocampus, after adjusting for the effects of group (MCI or HC), age, sex, scanner site, and education, using partial correlations. Accordingly, larger volumes of the left, right and mean hippocampus correlated significantly with better delayed recall, retention of words, and recognition memory (all *r* ≥ 0.24, all *P* ≤ 0.04, see Table V). These correlations were also found for the CA2/3 and CA4/DG subfields for delayed recall and recognition (all *P* ≤ 0.046). An overall similar pattern was

found for learning ability, yet failed to reach significance (all *P* ≤ 0.11).

For microstructure, the associations were less clear, however better performance correlated significantly with lower MD for recognition memory in the CA2/3 and CA4/DG, and for learning and recall in the CA4/DG subfield (all *P* ≤ 0.038, see Table V for details).

Correlations with Leptin

To further define if differences in leptin correlated with cognitive and structural changes, we first calculated partial correlations adjusting for the effects of age, sex, group (MCI or HC), scanner site, body fat, and education, as appropriate. Accordingly, higher leptin correlated with larger volumes and lower MD within the hippocampus, reaching significance for volume of the right hippocampus (*r* = 0.27, all *P* = 0.022, Table V) and for MD in the left

Figure 1.

Serum leptin concentrations in mild cognitive impairment (MCI, *n* = 40) patients and healthy controls (HC, *n* = 40). Note that MCI patients showed significantly lower concentrations than pairwise matched HC (Wilcoxon-signed-rank test, *P* < 0.001). Leptin was analyzed on the log scale and standardized to sex. Bars depict group's median, gray dots give individual values for males, black triangles give individual values for females. ***, *P* < 0.001.

TABLE IV. Volume and mean diffusivity of the hippocampus in mild cognitive impairment (MCI) patients and healthy controls (HC)

Hippocampus		MCI (<i>n</i> = 40)	HC (<i>n</i> = 40)	<i>P</i> _{t-test}	<i>P</i> _{adjusted}
Volume (mm ³)	LH	3664 ± 815	3903 ± 689	0.12	0.28
	RH	3782 ± 826	4056 ± 602	0.07	0.29
	Mean	3723 ± 807	3980 ± 634	0.086	0.28
	CA1	332 ± 63	340 ± 58	0.48	0.049^a
	CA2/3	921 ± 197	966 ± 98	0.23	0.022^a
	CA4/DG	520 ± 110	547 ± 49	0.18	0.012^a
Mean diffusivity (*10 ⁻³ mm ² /s)	LH	1.18 ± 0.09	1.17 ± 0.10	0.51	0.017
	RH	1.20 ± 0.10	1.17 ± 0.10	0.15	0.048
	Mean	1.19 ± 0.09	1.17 ± 0.09	0.27	0.030
	CA1	1.17 ± 0.16	1.13 ± 0.17	0.35	0.038
	CA2/3	1.23 ± 0.14	1.20 ± 0.14	0.31	0.33
	CA4/DG	1.10 ± 0.13	1.06 ± 0.09	0.076	0.24

Significant differences are indicated by bolding the number. Data are given as mean ± SD.

LH, left hemisphere; RH, right hemisphere; CA, Cornu ammonis; DG, dentate gyrus. *P*-values according to t-tests or adjusted analysis of covariance.

^aIn women.

hippocampus and in the CA1 subfield (all $r = -0.23$, all $P \leq 0.049$, Table V). No significant correlations were found between leptin and memory performance (all $P > 0.05$).

Mediation Analyses

Next, to explore if higher leptin would indirectly contribute to better memory performance through its positive effect on hippocampus volume or microstructure, we calculated simple mediation analyses that were corrected for age, sex, group, scanner site, body fat and education. Accordingly, leptin indirectly influenced learning ($ab = 0.72$, 95% CI = 0.06, 2.09, Fig. 2), delayed recall ($ab = 0.37$, 95% CI = 0.03, 0.97), retention of words ($ab = 0.22$, 95% CI = 0.01, 0.64) and recognition ($ab = 0.50$, 95% CI = 0.03, 1.37) through its effect on right hippocampus volume. In addition, lower MD of the left hippocampus partly mediated the positive effect of leptin on recognition memory ($ab = 0.27$, 95% CI = 0.01, 0.84). The bias-corrected bootstrap 95% confidence intervals for the indirect effects were entirely above zero. No significant mediation effects were found for other measures of the hippocampus.

DISCUSSION

We here showed that prodromal AD is associated with lower serum leptin independent of body fat, markers of glucose and lipid metabolism, sex and age, using a homogenous cohort of 40 MCI patients and 40 pairwise-matched HC. Moreover, lower leptin was associated with reduced volume and microstructural integrity of the hippocampus. While leptin and memory were not significantly correlated, mediation analyses indicated that lower leptin contributed to poorer memory performance through

its negative effect on right hippocampus volume and left hippocampus microstructure.

Leptin and Cognitive Impairment

Our findings of lower leptin associated with MCI are in line with several previous studies. For example, Lieb et al. [2009] reported based on the Framingham prospective cohort study that older adults (*mean age* = 79 years) in the low leptin range had a significantly higher risk to develop AD 12 years later. This was also found in another large sample of older subjects (70–79 years) [Holden et al., 2009]. However, in a third epidemiological study with older Mexican-Americans (*age* > 60 years), higher leptin was protective against cognitive decline only in subjects with a smaller waist circumference [Zeki Al Hazzouri et al., 2012]. Similar results were found by this group in a cohort of older women (*mean age* = 83 years), showing a positive effect of higher leptin only for women with normal weight, as defined by a BMI of less than 25 kg/m² [Zeki Al Hazzouri et al., 2013]. The authors suggested that higher leptin in obese subjects might no longer exert protective effects, as obesity induces leptin resistance [reviewed in Myers et al., 2008], similar to the mechanism of insulin resistance. For example, obesity-induced hyperleptinemia, diabetes and inflammation could compromise leptin transport across the blood brain barrier and also central leptin signaling [Arch, 2005; Banks et al., 2004; Chen et al., 2006], thus leading to leptin resistance [Myers et al., 2008]. A lack of associations between higher leptin and lower AD risk has also been found in the Framingham study in subgroups of very high BMI and waist-to-hip-ratio [Lieb et al., 2009]; however, note that number of AD cases was limited in these groups. In contrast, both studies of Zeki Al Hazzouri et al. [2012, 2013] included a

TABLE V. Correlation of memory performance and leptin with volume and mean diffusivity of the hippocampus

		Memory performance								Correlation with leptin	
		Learning		Recall		Retention of words		Recognition			
		<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>		
Hippocampus											
Volume (mm ³)	LH	0.21	0.069	0.27	0.019	0.24	0.040	0.24	0.034	0.15	0.19
	RH	0.22	0.058	0.31	0.008	0.25	0.032	0.32	0.005	0.26	0.023
	Mean	0.22	0.057	0.30	0.01	0.25	0.032	0.29	0.012	0.21	0.070
	CA1	0.10	0.38	0.18	0.12	0.22	0.060	0.10	0.40	0.09	0.42
	CA2/3	0.19	0.11	0.23	0.046	0.19	0.11	0.28	0.016	0.17	0.14
	CA4/DG	0.19	0.11	0.26	0.027	0.23	0.050	0.28	0.014	0.17	0.15
Mean diffusivity (10 ^{−3} mm ² /s)	LH	−0.01	0.93	−0.01	0.93	0.01	0.94	−0.20	0.089	−0.27	0.019
	RH	−0.04	0.75	0.07	0.52	0.14	0.22	−0.19	0.11	−0.13	0.28
	Mean	−0.03	0.83	0.03	0.77	0.08	0.49	−0.21	0.076	−0.22	0.063
	CA1	−0.06	0.59	0.01	0.91	0.06	0.60	−0.12	0.31	−0.29	0.013
	CA2/3	−0.07	0.53	−0.04	0.76	0.03	0.78	−0.25	0.031	−0.23	0.052
	CA4/DG	−0.24	0.038	−0.25	0.032	−0.16	0.16	−0.30	0.009	−0.04	0.77

Significant differences are indicated by bolding the number. Data give *r*- and *P*-values according to partial correlations adjusted for group, sex, age and scanner site, matching-pair, and for education (memory performance) and body fat (leptin), respectively (*n* = 80). LH, left hemisphere; RH, right hemisphere; CA, Cornu ammonis; DG, dentate gyrus.

large number of overweight and obese individuals. In our study, we did not observe interactions with measures of obesity, yet a possible resistance to leptin might have had only negligible effects, as we excluded diabetes patients a

priori and only 9 subjects had a BMI above 30 kg/m², with a maximum BMI of 36.7 kg/m². In addition, the careful matching between MCI and HC subjects in our sample with regard to age and body fat could have also overcome

Figure 2.

Simple mediation model illustrating that higher leptin contributed to better learning ability through its positive effect on right hippocampus volume. Values of the indirect effect are given as *ab*-path, corrected for age, sex, group, scanner site, matching-pair, body fat and education, with a bias-corrected bootstrap 95% confidence interval (BCCI). Left-hand panel shows

differences in fasting serum leptin concentrations between mild cognitive impairment (MCI) and healthy controls (HC) ($P < 0.001$), right-hand panel shows the correlation between volume of the right hippocampus and verbal learning ability ($n = 80$). [Color figure can be viewed at wileyonlinelibrary.com]

the confounding effects of leptin resistance with higher adipose mass. Notably, two cohorts including MCI patients yielded mixed results, showing in the ADNI cohort that higher leptin was associated with MCI and AD compared with HC [Johnston et al., 2014], but not with progression of MCI to AD [Oania and McEvoy, 2015], yet Kamogawa et al. [2010] did not observe significant differences in leptin between MCI and HC. The latter study might have failed to detect significant changes due to unbalanced distributions of body fat, sex and age between groups, which could have resulted in a smaller statistical sensitivity given that leptin is highly correlated with these conditions.

Structural Correlates and Underlying Mechanisms

In line with previous studies [reviewed, e.g., in Drago et al., 2011], MCI patients exhibited poorer memory performance and marginal reductions in volume and microstructure of the hippocampus compared with controls. Moreover, we found that poorer memory performance was correlated with smaller volumes of the right and left hippocampus, and more specifically, with smaller volume and reduced microstructural integrity of the CA2-4 and DG subfields, similar to earlier findings [Drago et al., 2011; Granziera et al., 2015; Kerti et al., 2013; van Norden et al., 2012; Yakushev et al., 2011]. Interestingly, lower serum leptin correlated with hippocampal measures in our cohort, underscoring the role of leptin for AD-related changes in hippocampal structure and function [reviewed, e.g., in Irving and Harvey, 2014]. Consistently, Lieb et al. [2009] observed a positive correlation between serum leptin and hippocampus volume, and higher leptin was associated with higher regional gray matter volume, including the right hippocampus, in a group of 34 healthy older subjects, using voxel-based morphometry (VBM) [Narita et al., 2009]. However, in a large sample of 517 AD, MCI and control subjects of the ADNI cohort, Rajagopalan et al. [2013] reported that higher serum leptin was associated with lower brain volumes in frontal, temporal, parietal, occipital lobes, brainstem and in the cerebellum, using a tensor-based morphometry approach. Again, this study included nearly twice as much obese and overweight subjects compared with normal or underweight. Accordingly, the authors discussed that higher serum leptin in this cohort was related to leptin resistance, thus displaying a marker of obesity in their specific cohort [Rajagopalan et al., 2013]. This might have masked potential beneficial effects of leptin in subjects without metabolic alterations. Higher serum leptin due to leptin resistance would also help to explain results of patients with T2DM, showing that higher leptin correlated to worse cognitive performance in men [Labad et al., 2012].

Taken together, a careful evaluation of age, sex, body fat and metabolic conditions seems crucial to better

understand the effects of leptin in humans. Interestingly, two small reports examining the effects of leptin replacement therapy observed rapid increases in regional gray matter volume after leptin intervention [London et al., 2011; Matochik et al., 2005], indicating that leptin may induce gray matter modulations along with functional improvements. In line with this hypothesis, adjusted simple mediation analyses indicated that higher leptin in our cohort contributed to better memory performance through its positive effect on right hippocampal volume and left hippocampus microstructure.

Underlying Mechanisms

Leptin has been suggested to exert beneficial effects on hippocampal structure and function via several mechanisms [reviewed in Irving and Harvey, 2014; Paz-Filho et al., 2010]. For example, it has been shown that long-term leptin administration increases neurogenesis in adult mice [Garza et al., 2008, 2012] and that leptin signaling leads to synaptogenesis and dendritic morphogenesis in the hippocampus via mitogen-activated protein kinase and cAMP-response element-binding protein pathways [Dhar et al., 2014; O'Malley et al., 2007]. Animal models for AD also showed that leptin can improve tau pathology [Greco et al., 2008], amyloid-beta clearance [Fewlass et al., 2004], and related deficits in long-term potentiation and memory [Tong et al., 2015]. However note that a recent study reported a negative correlation of leptin with A β , measured in the CSF of female AD patients, which was discussed to contribute to the higher risk of developing AD in women [Diehl-Wiesenecker et al., 2015]. Moreover, recent human post mortem analyses detected higher leptin concentrations in the hippocampus of AD patients, yet with decreased leptin receptor mRNA and de-localized leptin receptor proteins, pointing to dysregulated leptin signaling [Bonda et al., 2014]. In addition, immunohistochemical staining showed that leptin was mainly located in reactive astrocytes but not in neurons of AD patients, whereas in healthy subjects, leptin was present mainly inside neurons [Maioli et al., 2015].

In sum, a lack of leptin in neuronal cells, together with insufficient neurotrophic or neuroprotective signaling of leptin within the hippocampus, might have led to structural and functional decline in patients at high risk for AD, in line with our findings. However, whether leptin administration could help to diminish or reverse the above described detrimental changes in humans remains to be tested in interventional trials.

Limitations

Several limitations should be considered when interpreting our findings. First, due to the cross-sectional nature of our analyses, we are unable to track causal relationships. However, mediation analyses are commonly used tools in

evaluating possible underlying mechanisms [see, e.g., Kerti et al., 2013; Mander et al., 2013; Preacher and Hayes, 2004]. Second, our analyses relied on peripheral measurements of leptin, since central leptin concentrations from cerebrospinal fluid (CSF) or neuropathological samples were not available in our patients. However, the correlation of peripheral leptin levels with CSF leptin levels has been shown to be high [Schwartz et al., 1996]. Note also that leptin was measured at a single time-point only. Thus, we cannot exclude that due to possible differences in the opposite direction at other times of the day, mean 24-hour plasma leptin concentration might have been similar between groups. However, note that leptin was always assessed in the morning (fasting levels) over the entire cohort, and that in general, differences in the diurnal rhythm/amplitude of leptin production have been described to depend primarily on age, sex, obesity, and insulin levels [Saad et al., 1998], which were balanced between MCI and controls in the present study. Third, although self-reported average sleep length at night was found to be similar between groups, group differences in sleep duration not captured by the sleep questionnaire might have biased cognitive performance and daytime leptin levels [e.g., Yoo et al., 2007; Spiegel et al., 2004]. In addition, the use of two different scanner sites might have led to reduced statistical power or a systematic error, thus associations between hippocampus measures, cognition and leptin might have even been underestimated. However, note that analyses were adjusted for scanner site. Strengths of our study lie in the large cohort of MCI patients with exclusion of T2DM, and in the careful matching and adjustment for differences in sex, age, and body fat measured using bioelectrical impedance analysis, as well as glucose and lipid metabolic markers, known confounders of leptin effects on the brain.

CONCLUSIONS

Using a homogenous cohort of 40 non-diabetic MCI patients and 40 pairwise-matched HC, we found significantly lower serum leptin concentrations in this population at high risk for AD, independent of sex, age, body fat, and glucose and lipid metabolism. Based on mediation analyses, our data further suggest that inefficient leptin signaling could partly contribute to decreases in memory performance through negative effects on hippocampal structure. This is in line with postulated pro-cognitive effects of leptin based on experimental evidence showing leptin-induced improvements in hippocampal neuro-, synapto-, and morphogenesis, as well as AD-related pathologies. Our findings should now be substantiated in larger cohorts of well-matched healthy and cognitively impaired subjects, followed by interventional trials on leptin-enhancing approaches.

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