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Low Plasma Leptin in Cognitively Impaired ADNI Subjects-Gender Differences and Diagnostic and Therapeutic Potential

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Abstract: Analysis of data derived from the Alzheimer's Disease Neuroimaging Initiative (ADNI) program showed plasma leptin levels in individuals with Mild Cognitive Impairment (MCI) or Alzheimer's disease (AD) to be lower than those of subjects with normal cognition (NC). Approximately 70% of both men and women with MCI have plasma leptin levels lower than the median values of NC. Additionally, half of these subjects carry at least one apolipoprotein-E4 (*APOE*-ε4) allele. A subgroup of participants also had cerebrospinal fluid (CSF) leptin measured. Plasma leptin typically reflected the levels of leptin in CSF in all groups (Control/MCI/AD) in both genders. The data suggest that plasma leptin deficiency provides an indication of potential CNS leptin deficiency, further supporting the exploration of plasma leptin as a diagnostic marker for MCI or AD. The important question is whether leptin deficiency plays a role in the causation of AD and/or its progression. If this is the case, individuals with early AD or MCI with low plasma leptin may benefit from leptin replacement therapy. Thus, these data indicate that trials of leptin in low leptinMCI/early-stage AD patients should be conducted to test the hypothesis.

Keywords: Leptin, Alzheimer's disease, Alzheimer's disease neuroimaging initiative, mild cognitive impairment, apolipoprotein-E, leptin replacement.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative brain disorder pathologically characterized by: a) neuritic plaques, which contain deposits of β-amyloid protein (Aβ), formed either by inadequate clearance of Aβ or the aberrant processing of amyloid precursor protein (APP), b) pathological neurites, with paired helical filaments formed by hyperphosphorylated tau protein, and c) neurofibrillary tangles, resulting from accumulation of paired helical filaments in neuronal cell bodies. Our previous research has shown that leptindirectly regulates levels of Aβ both *in vitro* and *in vivo* [1-3]. In cell culture studies, leptin reduces extracellular Aβ secretion and increases apolipoprotein E (*APOE*) dependent Aβ uptake through a mechanism that involves regulation of lipids in neuronal membranes and lipid rafts [3]. In addition, leptin reduces the accumulation of phosphorylated tau in cells in culture as well as in brains of transgenic mice [4]. Leptin modulates these pathological hallmarks of AD through common upstream targets, including AMP-activated protein kinase (AMPK) [2, 19] a cellular energy sensor. AMPK is activated by leptin and can inhibit tau phosphorylation through AMPK –dependent deactivation of glycogen synthase kinase-3beta (GSK3β) [1].

Leptin is an adipocyte-derived hormone thought to be involved in appetite and energy homeostasis [5]. Additionally, in animals, leptin affects cognition [6], is involved in reward pathways [7], and protects hippocampal neurons and synapses from Aβ toxicity [8]. Indeed, genetically leptin deficient mice (ob/ob) have lower brain weights and are cognitively impaired, bearing disrupted synaptic and glial proteins [9]. Administration of leptin to these mice facilitates memory and learning and enhances synaptic plasticity, in addition to weight loss [10]. In humans, leptin administration resulted in improvement in neurocognition in a five year old congenitally leptin deficient patient [11]. A correlation has been found between low leptin levels and poor learning and memory performance in HIV-infected men [12] as well as indi-

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[#]*Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf*

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viduals with mood and eating disorders [13, 14]. Epidemiological studies have clearly associated low leptin levels in elderly with increased risk of AD [15-18].

These observations are consistent with the presence of a high concentration of functional leptin receptors in the hippocampus [21] and suggest that leptin may be a viable therapeutic for AD and Mild Cognitively Impaired (MCI) [22, 23]. In combination, the data suggest an important role for leptin signaling in the pathobiology leading to cognitive impairment in AD [22, 23].

To confirm previous observations, and further investigate the specificity of leptin deficiency in individuals with MCI and AD compared to subjects with normal cognition (NC), we analyzed biomarker data available from participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI) program [24, 25]. The data derive from participants in a multiyear study, subjected to a battery of analyses including neuro-psychological testing, plasma testing, cerebrospinal fluid (CSF) analysis and brain imaging (not analyzed here). We focused on a number of metabolic peptides as well as body mass index (BMI), gender and *APOE* genotype in subjects at baseline with plasma leptin data. Herein we show gender differences in leptin levels of NC participants as well as in those of MCI and AD cases, with approximately two-fold higher levels of leptin in women compared to men. More importantly we show that approximately 70% of MCI subjects have leptin levels below the NC median value. In addition, we investigated whether leptin deficiency correlates with other potential AD biomarkers (CSF Aβ, CSF p-tau/tau) and any *APOE* genotype [26] and discuss the potential implications regarding patient selection for interventional or preventative trials as well as the prospective of leptin as a treatment for AD.

2. METHODS

2.1. Participants

All participant data were obtained from the ADNI -1 database. This is a public-private cooperative partnership involving the National Institute on Aging (NIA), academic medical centers and trial sites and pharmaceutical companies with the goal to provide insights into the progression of AD pathology to help lead to effective treatments or preventative interventions (www.loni.ucla.edu/ADNI). A total of 819 subjects were enrolled at baseline, cognitively normal (NC) elderly persons (229), those with MCI (398) and a smaller group with mild AD (192). The clinical characterization of the participants, and the overall study design for three years of the project duration have been described previously [27]. Subject selection criteria, demographic data, baseline assessments, concomitant medications along with assessment sites are available within the ADNI website. In addition, standard protocols are available along with raw data and detection limits for all assay procedures utilized.

2.2. Plasma and CSF Leptin Levels

Blood samples were collected from subjects following an overnight fast, and the corresponding plasma was frozen within 120 minutes and shipped to ADNI for analysis (Data-primer Biomarkers Consortium, ADNI website).Plasma and CSF leptin levels were determined as previously described by the ADNI Biomarkers Consortium [28] using Luminex immunoassay technology. Plasma leptin levels were determined as part of a panel of 190 analytes and CSF leptin levels were determined as part of a panel of 159 analytes as initially described by Hu et al [24]. This technology uses a flow-based laser to detect fluorescent polystyrene microspheres with unique color codes for each analyte and derived data is still considered to be exploratory. For plasma leptin, the least detectable dose was 0.12ng/ml with reference standards diluted in buffer not plasma. The CSF leptin samples were run against standards in reference buffer (not CSF), with a least detectable dose of 0.047 pg/ml and a 22.2% intra-assay variation (Data-primer Biomarkers Consortium, ADNI website).

2.3. Data Analysis

All data for the present study were derived from measurements at baseline and represent a cross-sectional analysis. A master database was created into which data of interest were imported and organized by diagnosis (NC, MCI or AD) and subject ID number for the original 819 subjects. Only subjects with available baseline plasma leptin measurements (566) were analyzed further. BMI values and APOE genotype were typically available for all ADNI participants. Of these 566 subjects,112 had undergone lumbar puncture, providing data points for CSF β-amyloid and tau levels at baseline. Of this group, the majority had CSF leptin determinations (n=91) and these were used for correlation studies of markers. Statistical analysis was performed in SPSS 17.0 (Chicago IL) along with linear modeling.

3. RESULTS

3.1. Basic Demographic Features of ADNI-1 Subjects with Measured Plasma Leptin Levels

Characteristics of the participants in this analysis involving a subgroup of ADNI-1 subjects with available plasma leptin data are shown in (Table **1**). This subgroup at baseline is comprised of 58 NC, 396 MCI and 112 AD subjects with 24, 71 and 16 CSF specimens available for each group respectively. The NC and MCI groups had significantly higher MMSE scores compared to those of the AD group. The mean body mass index (BMI) was lower in MCI and AD cohorts, significantly so for the latter compared to NC (Table **1**), as reported previously [29]. Baseline plasma leptin values were significantly lower in the MCI group, compared to NC and while lower in the AD subjects, values were not significantly different from MCI subjects (Table **1**), in agreement with previous studies [16, 17, 30]. When analyzed by gender (Fig. **1**), plasma leptin values in men were approximately half those of women regardless of group (NC, MCI or AD). MCI men had significantly lower plasma leptin than NC men, but this was not the case for MCI women compared to NC women unless only women of BMI <27 were included for the comparison.

In general, as has been previously shown, plasma leptin values positively correlate to BMI [31], in both genders and in all groups (Fig. **2**), with women exhibiting higher leptin values per BMI unit (Table **2**). Mixed linear modeling of leptin vs BMI is shown in (Fig. **3**), with data and statistics

Table 1. Basic Demographic Features of ADNI-1 Subjects with Measured Plasma Leptin Levels.

	Normal Cognition (n=58)	MCI (n=396)	AD (n=112)	P
Age, yr (SD)	75.2 (5.8)	74.9 (7.5)	75.0 (8.0)	0.948
Male (%)	30 (52%)	256 (65%)	65 (58%)	0.104
MMSE	28.9 (1.1)	27.0 (1.8)	23.6 (1.9)	**<0.001**
BMI	27.0 (4.1)	26.1 (4.0)	25.6 (3.8)	**0.023 (NC vs. AD)**
APOE4	5 (9%)	211 (53%)	76 (68%)	**<0.001**
Plasma leptin	18.3 (15.8)	12.6 (13.1)	14.5 (15.4)	**0.011 (NC vs. MCI)**
Plasma leptin/BMI ratio	0.663 (0.551)	0.460 (0.431)	0.544 (0.547)	**0.005**
CSF	n=24	n=71	n=16	
Aβ42	257.9 (25.1)	161.3 (57.1)	130.6 (21.6)	<0.001
t-Tau	63.7 (22.6)	96.5 (49.2)	146.9 (77.8)	<0.001
p-Tau_{181}	21.0 (7.6)	33.3 (1.7)	43.5 (16.0)	<0.001
t-Tau/Aβ42	0.248 (0.090)	0.708 (0.479)	1.120 (0.620)	<0.001

In the NC faction, 52% were men, in the MCI faction 65% were men and in the AD faction 58% were men. The MCI group had mean MMSE score of 27±1.8 and the AD group had 23.6±1.9 ($p<0.001$), compared to 28.9±1.1 for the NC group. The average body mass index (BMI) was lower in the MCI and AD factions and significantly for the AD group compared to NC ($p=0.023$). The plasma leptin was 18.3 ±15.8 ng/ml in NC, 12.6±13.1 ng/ml in MCI and 14.5±15.4 ng/ml in the AD group and significantly lower in MCI group, compared to NC ($p=0.011$). For the AD subjects, mean plasma leptin values were also lower when compared to NC, but were not significantly different to MCI subjects. When plasma leptin/BMI ratio values in all men and women for each group are combined, there is a significant difference between NC and MCI or AD ($p=0.005$). In addition, Aβ42, t-Tau, p-$Tau_{181,}$ and t-Tau/Aβ42 and APOE4 significantly correlate with disease progression ($p=0.001$ compared to NC)

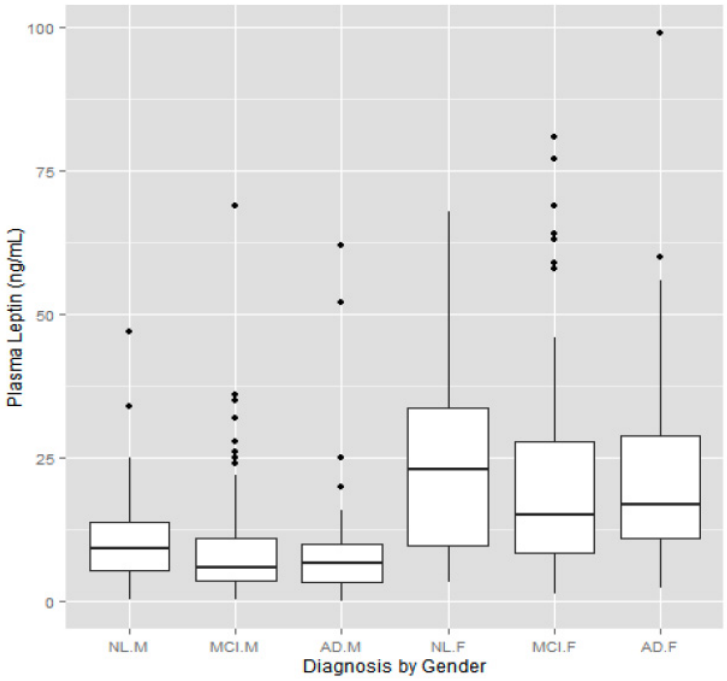

Fig. (1). Low Plasma Leptin Levels are Associated with MCI and AD in Men and Women. Traditional box and whisker plot of plasma Leptin. Plasma aliquots were interrogated in 2009 by Rules –Based Medicine (RBM, Auston TX) for levels of 190 analytes in 566 participants, including leptin, using the multiplex Human Discovery MAP ^{TM} panel and a Luminex 100 Platform (see Table **1** for characteristics). Dynamic range for each plasma analyte including leptin, is provided at the ADNI Website. A total of 352 subjects also had CSF AD biomarker levels provided by the ADNI Biomarker Core. NL-M: Normal men; MCI-M: MCI Men; AD-M: Men with AD; NL-F: Normal women; MCI-F: MCI Women; AD-F: Women with AD. Boxes represent the interquartile range (1^{st} to 3^{rd} quartiles) with the median line bolded. Whiskers are extended to 1.5 times the interquartile range and outliers are indicated with dots.When analyzed by gender, plasma leptin values in men were approximately half those of women regardless of subject faction (NC, MCI or AD) ($p =0.001$). MCI men had significantly lower ($p=0.02$) plasma leptin (8.31±7.51 ng/mL) than NC men (11.76±9.86 ng/mL). This was not the case for MCI women compared to NC women (20.40±16.91 vs 25.22±17.9 ng/ml, $p=0.18$) unless only women of BMI <27 were included for the comparison (13.72±9.88 vs 20.31±17.71 ng/ml, $p=0.02$, not shown).

presented in (Table **2**). Overall, leptin levels change more per BMI unit in NC men, than in men with MCI or AD (Fig. **3**, Panel **A**). Such a correlation was not observed for women (Fig. **3**, Panel **B**), where the change in leptin level per BMI unit was the same in all groups unless women of BMI <27 were used for the analysis (Fig. **3**, Panel **C**). Although these are cross-sectional observations, this could imply that plasma leptin levels drop faster than the rate of BMI drop in the continuum from NC to MCI or AD. When plasma leptin/BMI ratio values in all men and women for each group are combined, (Table **1**) there is a significant difference between NC and MCI or AD.

Table **1** also includes an analysis of Aβ42, t-Tau, p-$Tau_{181,}$ and t-Tau/Aβ42, measured in CSF for all groups. These values were significantly different in MCI and AD compared to NC. In addition, there was an increased prevalence of APOE4 from NC to MCI to AD (Table **1**).

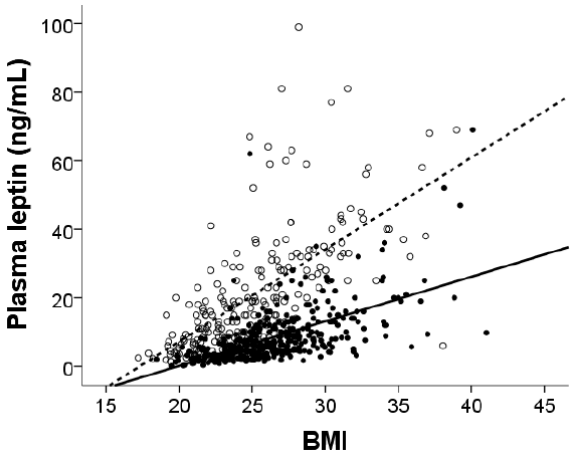

Fig. (2). Relationship Between Plasma Leptin and BMI According to Gender. Plasma leptin levels correlate with BMI in men (solid circle, R=0.588) and women (empty circle, R 0.645), but women have a greater increase in leptin levels per unit of BMI change than men.

3.2. Plasma Leptin Versus CSF Leptin

A subgroup of ADNI-1 participants with baseline plasma leptin determinations had also provided CSF samples following lumbar puncture at baseline for routine analysis of disease biomarkers (Aβ42, p-tau-181 and tau) as well as multiplex analysis of analytes of interest. Thus, some data concerning CSF leptin was available. The concentration of leptin in the CSF correlated with plasma leptin for all men and women, albeit two orders of magnitude lower (Fig. **4**). The ratio of plasma leptin (PL) over CSF leptin (CL; PL/CL) was not significantly different across the groups (Fig. **4**). This ratio increases significantly in obese subjects who are characterized by hyperleptinemia (high plasma leptin values) [31] a phenomenon that contributes to leptin resistance. It is believed that this is due to the saturability of the leptin transporter at the blood-brain-barrier (BBB). It appears that the CSF leptin concentrations may gradually reach a plateau at high plasma leptin concentrations herein as well (Fig. **4**, Panel **A** and **B**). There was a significant decrease of the PL/CL ratio in AD men compared to NC (not shown) perhaps reflecting the higher percentage of AD men with low plasma leptin, and corresponding to the beginning of the logarithmic curve that would best describe the plasma / CSF leptin association, based on an active BBB transporter (Fig. **4**).

3.3. Other CSF Markers Versus Plasma Leptin

Data for Aβ42, p-tau-181 and tau in NC, MCI, MCI with leptin at the lowest quartile (MCI(q1)) and AD are shown in (Fig. **5**). Despite a trend in increasing abnormalities from NC to MCI to MCI(q1) to AD for those CSF markers, there were no significant differences between the non NC groups, but each group was significantly different compared to NC (Fig. **5**). The trend was more apparent for p-Tau181 in both, men and women (B, E), Aβ1-42 for men (A) and tau for women (F).

3.4. Normal Leptin Quartiles and Changed Distribution in MCI Subjects

The leptin quartile bins in ADNI-1 NC men (N=30) and women (N=28), despite the relatively low sample numbers, were in agreement with those described previously by Lieb *et al* (2009) [17]. When MCI men and women were categorized into these bins and % of total MCI (for each gender) was calculated, it was found that 45.3% of men and 32.1% of women with MCI had leptin levels falling within the first bin of normal subjects. Further, 71.1% of men with MCI and

Table 2. Main Effects on Plasma Leptin Levels From Gender-Specific Mixed Linear Modeling. Values Shown are F Statistics with p values in parentheses. For Parameter Estimates, see Table 3.

	Men	Women
Age	**4.36 (0.038)**	0.102 (0.750)
BMI	**124.9 (<0.001)**	**96.93 (<0.001)**
Diagnosis	2.76 (0.064)	0.019 (0.981)
Diagnosis x BMI	**3.21 (0.041)**	0.022 (0.979)
Presence of APOE4	0 (0.993)	2.377 (0.125)

Mixed linear regression analysis of the effects of BMI and diagnosis on plasma leptin levels of the ADNI cohort. Values shown are F statistics and p values are in parenthesis. In men, leptin changes were significantly associated with BMI (p=0.001) and plasma leptin change more per unit increase of BMI in control subjects than in those with MCI/AD (p=0.041). Plasma leptin levels were also associated with BMI in women (p=0.001). Presence of the APOE4 allele did not influence plasma leptin levels in either gender.

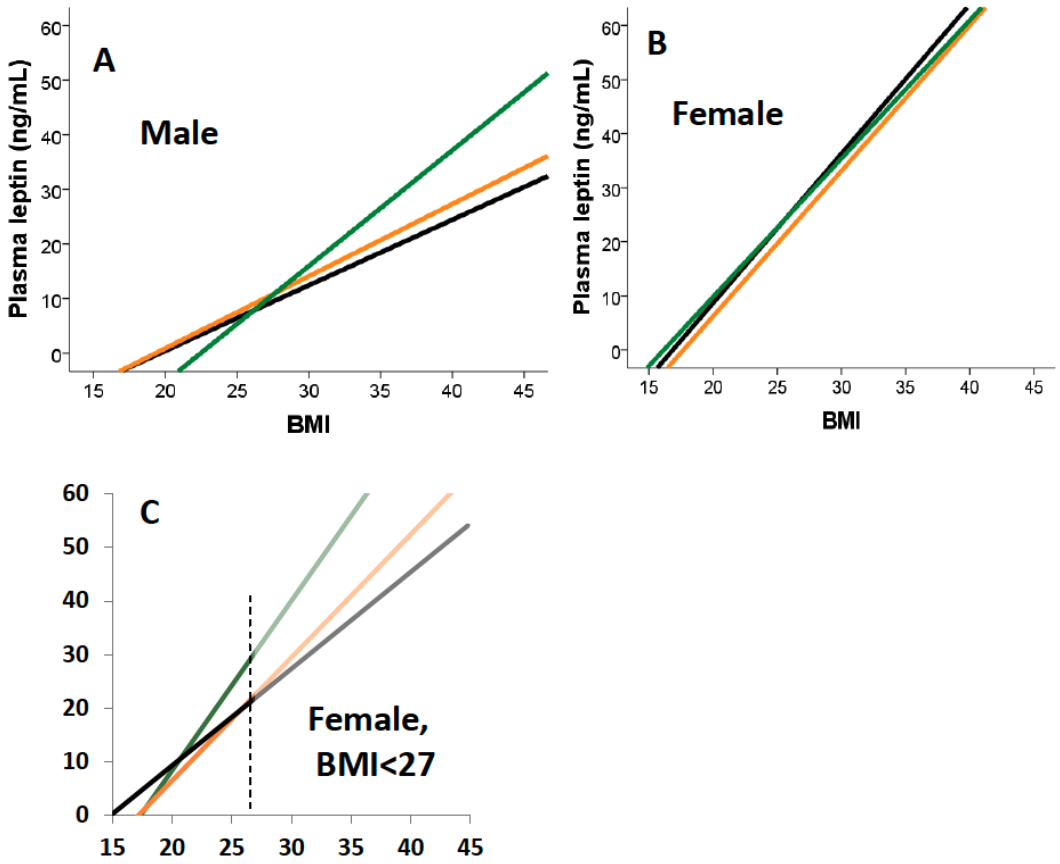

Fig. (3). Linear Correlations Between Plasma Leptin Levels and BMI According to Diagnosis in Men, Women and Women with BMI<27. In the mixed linear models, plasma leptin level was the dependent variable, with age, presence of APOE4 allele, BMI, diagnostic category, and the interaction between diagnostic category and BMI as fixed variables, and BMI as a random variable. Men (Panel A) with normal cognition experience greater change in leptin levels per BMI unit change than men with MCI or AD (p=0.041). This was not true in women (Panel B), except for those with BMI<27 (Panel C). Green line: NC; orange line: MCI; black line: AD.

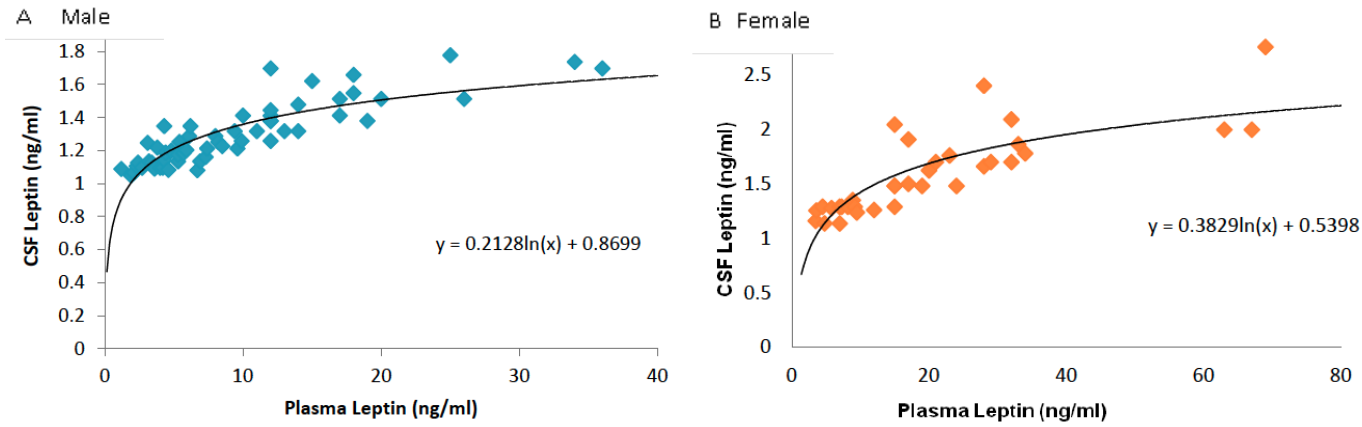

Fig. (4). Relationship Between CSF Leptin and Plasma Leptin for Men and Women. Scatchard plots showing CSF leptin versus plasma leptin levels for all men (**A**) and all women (**B**). The mean plasma leptin: CSF leptin ratios for men were 101.42±31.94, 86.22±42.87 and 74.97±36.68 for NC, MCI and AD respectively. These values were 112.13±46.75, 93.28±42.60 and 98.84±56.84 for the corresponding groups of women. The curve of all data points is logarithmic, depicting saturability of CSF leptin levels at high plasma levels. Generally, the ratio of plasma versus CSF leptin was similar across groups and genders. However, a significant lower ratio was found in men, but not women, with AD (74.97±36.68) compared to NC (101.42±31.94) (p=0.008), indicating that at least transport of leptin into the brain is not affected by the disease. In obesity, this ratio becomes larger compared to controls, possibly due to transporter saturability.

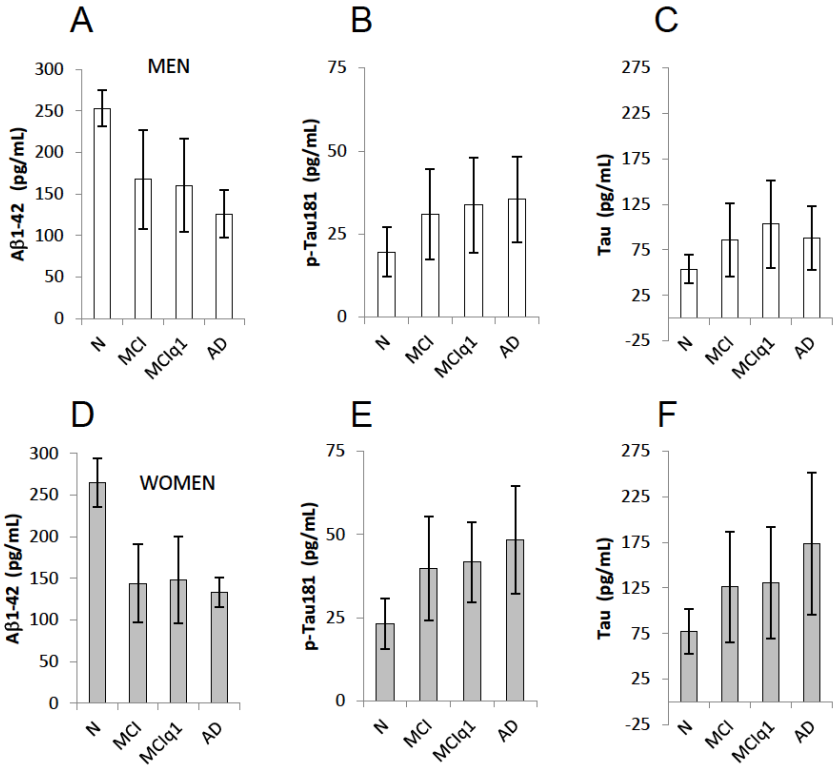

Fig. (5). CSF Biomarker Profile for NC, MCI, Lowest Leptin Quartile MCI(q1) and AD. CSF biomarkers Aβ1-42, p-Tau181 and Tau are plotted for men (**A**-**C**) and women (**D**-**F**) for the ADNI-1 participants (Normal (N), MCI, AD) with plasma leptin determinations. The lowest –Leptin quartile within the MCI was also plotted separately (MCI(q1). Data represent the mean±SD. There were no significant differences between the non NC groups despite a trend in increasing abnormalities from NC to MCI to MCI(q1) to AD. However, each group was significantly different compared to NC ($p<0.006$). The trend was more apparent for p-Tau181 in both, men and women (B, E), Aβ1-42 for men (A) and tau for women (F).

Table 3. Parameter Estimates of Factors Which Influence Plasma Leptin Levels According to Gender.

	Men	**Women**
Intercept	**-34.28 (p<0.001)**	**-48.08 (p=0.009)**
Age	**0.10 (p=0.038)**	0.04 (p=0.751)
BMI	**1.36 (p<0.001)**	**2.59 (p<0.001)**
Diagnosis (compared to control) MCI AD	 **23.69 (0=0.019)** 21.61 (p=0.057)	 -3.25 (p=0.848) -2.12 (p=0.916)
Diagnosis x BMI MCI x BMI AD x BMI	 **-0.91 (0.012)** -0.78 (0.056)	 0.08 (p=0.901) 0.16 (p=0.837)
APOE4	-0.01 (0.993)	-3.04 (0.125)

Parameter estimates of the factors influencing plasma leptin for each gender as estimated from mixed linear modeling. The table shows leptin change for each parameter, with p values in parenthesis. Most significantly, for the men, leptin changes more in the MCI group compared to controls.

70% of women had circulating plasma leptin levels below the median values of NC subjects (Fig. **6**).

3.5. Relationship Between Leptin and APOE Genotype

In (Table **1**) we show and confirm the increased prevalence of *APOE*ε4 allele in MCI and AD subjects compared to NC [32]. In (Table **4**) we examine the frequencies of all *APOE* genotypes in NC, MCI and AD and further analyzed whether there was any enrichment in the low leptin subgroups. We report increased frequencies of *ε3/ε4* and *ε4/ε4* and decreased frequencies of *ε2/ε3* and *ε3/ε3* alleles from NC to MCI/AD. There was no significant association of any particular *APOE* genotype with any of the leptin subgroups.

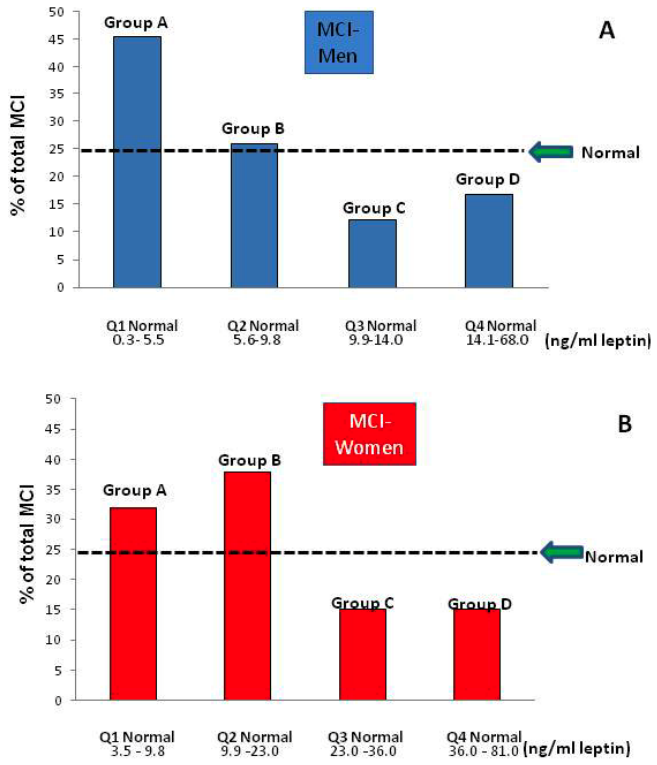

Fig. (6). Approximately 70% of MCI Subjects Have Plasma Leptin Values Lower Than the Median Leptin Value of Normal Elderly. The range of plasma leptin values found in the quartiles in normal men and women from ADNI (Panel A) were used to divide the MCI men and women into four groups (Panel B, groups A-D), corresponding to Q1-Q4 of normal quartiles respectively (Panel A). In Panel B, Groups A and B combined represent the percent of MCI subjectswith leptin values lower than the median values of leptin in normals (9.8ng/ml for men and 23ng/ml for women). Men: white boxes; Women: black boxes.

DISCUSSION

We report that leptin levels in the plasma of ADNI-1 subjects are approximately two fold higher in women than in men in all groups, in agreement with previous reports [33, 34]. However, 70% of the MCI subjects, regardless of gender, had plasma leptin levels lower than the median values of NC, suggesting that plasma leptin level may be a useful biomarker for predicting risk for dementia. Incidently, the NC group in this study was very similar to that reported for a larger group (n=785) of elderly [17] with regards to their plasma leptin levels.

ADNI-1 MCI men exhibited significantly lower leptin levels compared to NC men and these levels were independent of BMI. However, leptin levels ofMCI women, while lower than NC women, were only significant when subjects were selected based on low BMI (BMI<27, p=0.02: BMI<25, p=0.01), in agreement with a recent study [30]. Our cross-sectional observations imply that plasma leptin levels in MCI men and possibly non-obese women are different to those predicted by BMI values in NC, thus leptin as a marker could strengthen the predictive power of low BMI which has been consistently linked to disease onset [29, 35, 36], and associated with biological markers of core brain pathology of AD [29]. The present data suggest but do not prove that for both genders, the decline in circulating leptin itself may have a role in the cascade of AD causation. This could be the result of a deregulation of energy homeostasis due to a disruption of an important feedback between hippocampus and hypothalamus in an early phase of disease continuum. However, proper trials in the AD and MCI populations are needed to address this and certainly longitudinal data with information on conversion rates of MCI to AD could be particularly powerful.

Analysis of AD-related biomarkers ($A\beta_{1-42}$, p-Tau181, Tau) in CSF samples of normal subjects, MCI and AD groups confirmed previous findings that decreased $A\beta_{42}$ and increased Tau are associated with MCI and AD [37-40]. However, longitudinal data will be required to determine whether low leptin levels are involved in the causal stream related to the decline of CSF $A\beta_{1-42}$ [40] or increase in CSF tau. Interestingly, a non-statistical trend of a worsening CSF biomarker profile (higher p-tau181, higher tau and lower Aβ1-42) was observed from NC subjects to MCI subjects to MCI(q1) to AD in the available cross-sectional data. Plasma leptin may represent a useful biomarker for the early detection of AD or the identification of those MCI who are at risk to convert to AD [32, 37, 38, 40], adding to both the specificity and sensitivity of established CSF biomarkers (Aβ42, tau, p-tau). Elderly individuals with MCI could be screened for a multianalyte profile [25] and those with low leptin levels could be monitored with a practical screening test for memory difficulties to define the earliest indications of the incipient development of AD related MCI and dementia [37].

ADNI was established to provide insights into the progression of AD over time, identify useful biomarkers [24, 25], and facilitate drug development programs [27, 41]. A critical application of the leptin data is to consider relevant direction for the development of successful therapeutic strategies. In particular, the analysis presented here, consistent with several prior studies of leptin and development of dementia, suggests that an important consideration is whether institution of leptin-replacement therapy would decrease the probability of progressive cognitive decline and the development of MCI and AD dementia. In conjunction with the preclinical findings from *in vitro* and *in vivo* studies using models of the disease [2-4], these findings support the possibility that restoring leptin levels to physiological levels in MCIs at high risk for converting to AD dementia is a reasonable basis for therapy. This substantial body of data strongly supports a clinical trial of leptin replacement in MCI and AD subjects with low leptin levels.

Within the MCI group there was no clear association of leptin levels and frequency of any specific *APOE* allele. These results suggest that *APOE* isoforms and plasma leptin-levels may represent independent risk factors for MCIs or AD subjects [32, 38]. On the other hand, and as we have shown in *in vitro* studies [3] leptin's beneficial activity on *APOE*-dependent Aβ-uptake may be sensitive to the *APOE* genotype. One explanation could be that a leptin–*APOE*-Aβ interplay may be important at earlier stages of the disease. With the decrease of weight and fat load common in the elderly, leading to lower leptin levels, there would then be pathologically inadequate leptin stimulation of the brain,

Table 4. APOE Genotype Frequencies in NC, MCI and AD Men and Women. Frequencies in Low Leptin Subgroups, with Plasma Leptin Values Within the Range of Q1 of NC (0-5.5ng/ml for Men, 0-9.8ng/ml for Women) are also Presented.

Group	Apo E genotype *number of carriers , n & (frequency)*						Total
	E2/E2	E2/E3	E2/E4	E3/E3	E3/E4	E4/E4	
NC men	0	7 (0.250)	0	18 (0.683)	3 (0.107)	0	28
NC men <5.5 ng/ml leptin	0	3 (0.375)	0	4 (0.500)	1 (0.125)	0	8
NC women	0	10 (0.357)	0	17 (0.607)	1 (0.036)	0	28
NC women <9.8 ng/ml leptin	0	3 (0.429)	0	4 (0.571)	0	0	7
MCI men	0	9 (0.035)	8 (0.031)	114 (0.445)	98 (0.383)	27 (0.105)	256
MCI men <5.5 ng/ml leptin	0	5 (0.040)	6 (0.048)	55 (0.437)	49 (0.389)	11 (0.087)	126
MCI women	0	8 (0.058)	3 (0.021)	54 (0.388)	54 (0.388)	20 (0.149)	139
MCI women <9.8 ng/ml leptin	0	2 (0.044)	0	17 (0.378)	18 (0.400)	8 (0.178)	45
AD men	0	1 (0.015)	0	19 (0.292)	31 (0.477)	14 (0.215)	65
AD men <5.5 ng/ml leptin	0	1 (0.022)	0	10 (0.345)	12 (0.414)	6 (0.207)	29
AD women	0	0	2 (0.046)	16 (0.340)	20 (0.426)	9 (0.191)	47
AD women <9.8 ng/ml leptin	0	0	1 (0.091)	4 (0.364)	5 (0.455)	1 (0.091)	11

leading to the predisposition to AD. In parallel, middle age obesity and high leptin levels could lead to leptin resistance in the brain, which predisposes to the development of AD dementia.

Because 50% of subjects with low leptin levels also inherited an *APOE*ε4 allele (Table **4**, relative to 22% of a normal population and 60% of an AD dementia population, [32]) the substantial proportion of the MCI population with low leptin levels may have a greatly increased risk for proximate development of AD dementia or *APOE4* is the driver and leptin a silent feature following the *APOE4* population. Further analysis of the ADNI cohort (five years and now longer with ADNI-2) may provide additional useful information for understanding the implications of leptin levels in progression of cognitive impairment and to AD dementia. Importantly, the current analysis has confirmed that low leptin in plasma and CSF is commonly found in MCIs and AD subjects. Furthermore, the feasibility of a leptin replacement trial focusing on an enriched and relatively homogeneous (genetically and phenotypically) MCI group, representing early stage AD, is suggested. This target group can be selected following a prescreening procedure to identify those MCI men and women with low plasma leptin and who are carriers of an *APOE*ε4 allele, in addition having high CSF pTau-181 and low CSF $A\beta_{42}$. As 35% of the MCI population could potentially fulfill those criteria such a strategy is feasible and could potentially improve variability in clinical trials where heterogeneity of the MCI group confounds results, costs and increases and the subject numbers needed for statistical analysis.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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