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Insulin Levels are Decreased in the Cerebrospinal Fluid of Women with Prodomal Alzheimer's Disease

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Abstract. Previous studies have failed to reach consensus on insulin levels in cerebrospinal fluid of Alzheimer's disease (AD) patients and on its relation to pathological features. We performed a new analysis in patients at different stages of AD, and investigated the relationship of insulin levels with biochemical disease markers and with cognitive score. We included 99 patients from our Memory Clinic (Karolinska University Hospital, Sweden), including: 27 patients with mild AD, 13 that progressed from mild cognitive impairment (MCI) to AD in two years time, 26 with MCI stable after two years, and 33 with subjective cognitive impairment. Insulin was significantly decreased in the cerebrospinal fluid of both women and men with mild AD. Insulin deficits were seen in women belonging to both MCI groups, suggesting that this occurs earlier than in men. Insulin was positively associated with amyloid-β 1-42 (Aβ_{1-42}) levels and cognitive score. Furthermore, total-tau/(Aβ_{1-42}*insulin) ratio showed strikingly better sensitivity and specificity than the total-tau/Aβ_{1-42} ratio for early AD diagnosis in women.

Keywords: Alzheimer's disease, cerebrospinal fluid, insulin, mild cognitive impairment, women

INTRODUCTION

The functions of insulin governing peripheral glucose homeostasis and its involvement in diabetes mellitus are well known. However, less is known about the roles of insulin beyond the periphery, in the central nervous system (CNS). Insulin receptors are found throughout the CNS, although it is unclear whether brain insulin comes only from the blood through the blood-brain barrier (BBB) [1] or is also synthesized by CNS cells [2,3]. Insulin participates in a number of brain functions, such as regulation of food intake and body weight [4], and in cognition by modulating synaptic plasticity and long-term potentiation [5,6]. Diabetes mellitus type 2, which is mainly associated with insulin resistance, is a risk factor for dementia and AD [7].

Evidence of insulin resistance in AD is suggested by reports of reduced glucose utilization and deficient energy metabolism in early stages of the disease [8,9]. Expression of insulin, insulin-like growth factor (IGF)-I and -II, as well as their receptors, was found to be increasingly down-regulated in postmortem brain tissue of AD patients, in correspondence with the severity of the disease [2,3]. *In vitro* and *in vivo* animal studies fur-

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ther support the association of insulin with the classical pathogenic hallmarks of AD, namely amyloid protein precursor (AβPP)/amyloid-β(Aβ) and tau [10–12].

The possible association of brain insulin levels with AD pathogenesis needs to be assessed at all stages during the course of the disease. Cerebrospinal fluid (CSF) measurements can be used to investigate all stages, even the earliest ones. Three studies have, so far, investigated CSF-insulin levels in AD. Craft et al. reported decreased CSF-insulin in advanced AD [13]. In contrast, the two other studies showed increased levels [14] or no changes [15]. None of these studies found correlations found between insulin levels and pathological features or the cognitive status of AD patients.

The present study aims to further clarify the meaning of CSF-insulin levels in AD. We have investigated CSF-insulin levels in memory clinic patients with mild-AD, subjective cognitive impairment (SCI), and in the state considered relatively high-risk for AD [16], mild cognitive impairment (MCI). Moreover, we have divided the MCI group into those who progressed to AD in two years time, and those who did not in this time framework. The questions addressed in this paper include: a) Are CSF-insulin levels abnormal in AD or MCI?; b) Is gender or apolipoprotein E (ApoE) genotype differentially affecting CSF-insulin in AD or MCI?; c) Is insulin associated to some AD hallmark?; and d) Does insulin has prognostic value for early diagnosis?

METHODS

Study population

The patients included in the study ($n = 99$) were from the Memory Clinic at the Karolinska University Hospital in Huddinge, Sweden: 33 had subjective cognitive impairment (SCI), 39 MCI, and 27 mild-AD. In the MCI group, 13 had progression-to-AD (PMCI) within two years time, and 26 remaining stable (SMCI). These patients were all living independently in the community. They were evaluated according to a standard comprehensive assessment protocol including clinical examination, brain imaging, electroencephalography, analyses of blood (serum albumin, glucose) and CSF (including albumin, total tau (T-tau), phospho-tau (P-tau), and Aβ_{1-42}) and a detailed neuropsychological evaluation. Dementia and AD were diagnosed according to DSM-IV and NINCDS-ADRDA criteria. MCI patients were (1) not demented, (2) had (self and/or an informant) reported cognitive decline and impairment on objective cognitive tasks, and (3) had preserved basic ADL/minimal impairment in complex instrumental functions. SCI patients had cognitive complaints without impairment on objective cognitive tasks. Women under hormonal replacement therapy, as well as patients with psychiatric disorders (i.e., depression, alcohol abuse) or other conditions (i.e., diabetes, brain tumors, normal pressure hydrocephalus) were not included. The study was conducted under the guidelines of the Declaration of Helsinki and approved by the ethics committee of the Karolinska Institutet.

CSF measurements

CSF was collected for diagnostic purpose by lumbar puncture as previously described [17,18]. CSF samples were obtained by lumbar puncture performed in the sitting position. CSF extraction is routinely performed at the Karolinska University Hospital Memory clinic in Huddinge (Sweden) as part of the medical examination. The extractions were performed in the mornings in fasting patients. CSF samples were obtained from L3/L4 or L4/L5 interspaces after local anesthetic infiltration in the skin. After disposal of the first ml. the following 10 ml were collected in polypropylene tubes. No sample contained more than 500 erythrocytes/μl CSF samples was used. Samples were gently mixed to avoid gradient effects and centrifuged at 2000$\times$g for 10 min to eliminate cells and insoluble material. Supernatants were aliquoted, immediately frozen and stored at -80°C pending biochemical analyses. Tau was determined using a sandwich enzyme-linked immunoabsorbent assay (ELISA) [19]. P-tau (P-Thr181) was determined using a sandwich ELISA, with monoclonal antibody HT7 (recognizing all forms of tau) used as capturing antibody, and AT270 (specific to P(Thr181)-tau) used as a detection antibody [20]. A$\beta_{1-42}$was determined using a sandwich ELISA as previously described [18]. All kits were purchased from Innogenetics NV, Ghent, Belgium. Insulin levels were measured by a highly sensitive radioenzymatic assay, using Ultra Sensitive Human Insulin RIA kit (Linco Research. HI-11K).

Data analysis

Normal distribution of data was checked by Shapiro-Wilks. Analytics are presented as mean values $\pm$ standard error mean (SEM). Linear regression was used to analyze the association of CSF-insulin levels with age, BMI and plasma glucose levels. Insulin levels were

Table 1
Demographic and clinical characteristics of participants

	SCI	SMCI	MCI-AD	Mild-AD
Demographics				
n	33	26	13	27
Gender (male/female)	14/19	17/9	4/9	8/19
Age (years)	57.5 (1.1)	61.6 (2.0)	63.1 (2.3)	68.6 (1.8)^{a,b}
Education (years)	14 (0.6)	12.4 (0.7)	14 (1.1)	9.7 (0.6)^{a,b,c}
ApoE4 carriers (%)	39	50	84	70
MMSE (points)	29.1 (0.2)	28.3 (0.3)	27.5 (0.3)^{a}	23.6 (0.7)$^{a,b',c}$
Clinical data				
Body mass index (kg/m^2)	25.1 (0.7)	26.3 (1.1)	22.2 (0.8)^{b}	22.9 (0.9)^{b}
Fasting plasma glucose	5.4 (0.9)	5.5 (1.1)	5.1 (0.8)	5.5 (0.9)
Alb_{CSF}/Alb_{serum}	5.9 (0.4)	6.6 (0.6)	5.5 (0.8)	6.8 (0.5)
CSF Aβ_{1-42}(ng/L)	844.9 (29.0)	677.9 (45.2)^{a}	420.2 (28.8)$^{a,b'}$	468.9 (26.9)$^{a,b',c}$
CSF T-tau (ng/L)	273.3 (20.2)	401.9 (110.8)	456.3 (64.7)	667.9 70.4)^{a,b}
CSF P-tau (ng/L)	50.8 (3.4)	55.5 (7.8)	70.6 (12.5)	94.2 (8.2)^{a,b}

Numeric values are presented as number of patients, percentage or mean (SEM).
SCI: subjective cognitive impairment; SMCI: stable mild-cognitive impairment; MCI-AD: mild cognitive impairment with AD progression; AD: Alzheimer's disease; MMSE: Mini-Mental State Examination; CSF: cerebrospinal fluid; Alb: albumin.
$^{a}p < 0.001$ vs. SCI; $^{b}p < 0.05$ vs. SMCI; $^{b'}p < 0.001$ vs. SMCI; $^{c}p < 0.001$ vs. PMCI.

analyzed using ANOVA after removing the variance of the covariates gender and age (ANCOVA). Pairwise comparisons were performed by HSD Tukey's post-hoc test. Associations between Aβ_{1-42}, P-tau, severity of dementia (MMSE score), and insulin levels were assessed by partial correlation analysis after correcting for the grouping effect. Cut-off for sensitivity and specificity and area under the curve (AUC) were derived from receiver operating characteristic (ROC) curves. Significance was defined as $p \leqslant 0.05$. Analyses were performed with SPSS 12.0 (SPPS, Chicago, USA) and MedCalc 10.0.2 (MedCalc Software, Belgium).

RESULTS

General characteristics of the population

Demographic and clinical characteristics of study participants are shown in Table 1. AD patients were significantly older than SMCI and SCI ($F_{(3,95)} = 8.46$; $p < 0.05$ and $p < 0.001$, respectively). MMSE score was lowest in AD patients ($F_{(3.95)} = 36.06, p < 0.001$). However, patients with PMCI had also a lower score when compared to SCI ($p < 0.001$).

AD patients presented lower CSF levels of Aβ_{1-42}, and higher T-tau and P-tau when compared to either SCI ($F_{(3,82)} = 18.14$, $p < 0.001$; $F_{(3,85)} = 5.93$, $p < 0.001$; $F_{(3,73)} = 8.72$, $p < 0.001$, respectively) or SMCI patients ($p < 0.05$) after correcting for age. CSF-Aβ_{1-42} levels of PMCI group were lower than SMCI and SCI ($p < 0.05$ and $p < 0.001$, respectively).

Insulin is decreased in CSF of AD patients

Linear regression analysis did not show any significant interaction between CSF levels of insulin and age (R = 0.15, $n = 80$, $p = 0.16$), BMI (R = 0.11, $n = 43$, $p = 0.48$) or plasma glucose levels (R = 0.11, $n = 79$, $P = 0.33$). A significant reduction (of approximately 22%) of CSF-insulin was found in patients with mild-AD compared to SCI (0.414 $\pm$ 0.036 versus 0.566 $\pm$ 0.028 μU/mL, one-way ANOVA followed by HSD Tukey's test, $F_{(3,72)} = 3.22$, $p < 0.05$). The PMCI group tended to have decreased insulin levels, although this was not statistically significant (0.480 $\pm$ 0.054 μU/mL; $p = 0.19$). Neither were significant differences ($p = 0.98$) found between patients with SMCI (0.548 $\pm$ 0.046 μU/Ml) and SCI (Fig. 1A).

Figure 1B shows the gender-stratified distribution of CSF-insulin levels along the 4 diagnostic groups. Women presented lower levels of CSF-insulin than did men (0.487 $\pm$ 0.026 vs. 0.578 $\pm$ 0.036 μU/mL, $F_{(1,79)} = 4.56$, $p < 0.05$); and a significant interaction between grouping and gender was found ($F_{(3,79)} = 2.65$, $p < 0.05$, two-way ANOVA). No difference was found between men (0.433 $\pm$ 0.034) and women (0.436 $\pm$ 0.052) in the mild-AD group ($p = 1.00$, HSD Tukey's test). However, significantly lower CSF-insulin levels were found in women from the SMCI group (0.615 $\pm$ 0.054 for men vs. 0.407 $\pm$ 0.057 μU/mL for women; $p < 0.01$, HSD Tukey's test). Women with PMCI showed also a trend of reduction, although non-significant with the number of individuals in the study,

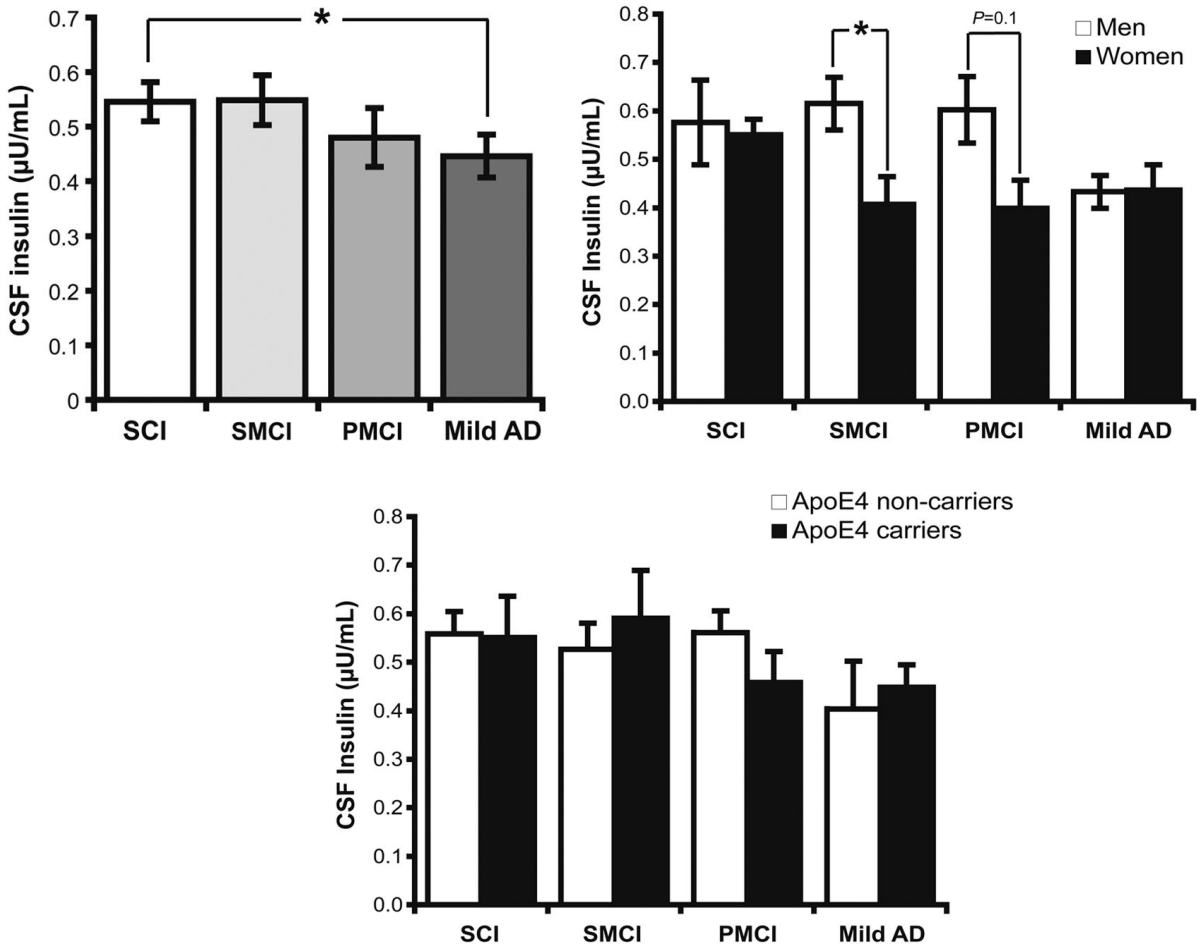

Fig. 1. CSF levels of insulin are decreased in mild-AD. A) Fasting CSF-insulin (μU/mL) with standard errors for the overall subjective cognitive impairment (SCI), stable MCI (SMCI), MCI with AD progression (PMCI) and mild-AD. Mild-AD subjects showed significant lower levels of CSF-insulin than SCI ($p < 0.05$). B) Fasting CSF-insulin (μU/mL) with standard errors for male (white columns) and female (black columns) SCI, SMCI, PMCI and mild-AD groups. SMCI women showed reduced levels of CSF-insulin when compared to men of the same group ($p < 0.05$), and women from PMCI group presented the same trend of reduction by nearly reaching significance ($p = 0.1$).

when compared to men with PMCI (0.602 $\pm$ 0.069 versus 0.399 $\pm$ 0.058 μU/mL, $p = 0.08$, HSD Tukey's test).

Levels of CSF-insulin between apoE ε4-carriers and non-carriers did not differ (0.513 $\pm$ 0.031 for ε4-carriers vs. 0.547 $\pm$ 0.032 for ε4 non-carriers, Student's $t_{(69)} = 0.932$, $p = 0.36$), and no interaction between grouping and ApoE genotype was found ($F_{(3,78)} = 0.27$, $p = 0.85$, two-way ANOVA) (Fig. 1C).

Insulin was also significantly and positively correlated to $\text{Albumin}_{CSF}/\text{Albumin}_{serum}$ ratio (Spearman's rho $= 0.231$, $p < 0.05$). Furthermore, this ratio was lower in women than in men (5.26 $\pm$ 1.98 vs. 7.64 $\pm$ 2.81; $F_{(1,98)} = 22.01$, $p < 0.01$, two-way ANOVA). These differences were attributed to a significantly lower ratio in women both in SCI (4.95 $\pm$ 0.37 vs. 7.09 $\pm$ 0.73; $p < 0.01$, HSD Tukey's test) and SMCI groups (4.48 $\pm$ 0.59 vs. 7.81 $\pm$ 0.73; $p < 0.05$, HSD Tukey's test). Women from PMCI and AD showed a weaker trend toward decrease when compared to men from the same groups, although this was non-significant (4.82 $\pm$ 0.74 vs. 7.18 $\pm$ 1.67, $p = 0.270$; 6.85 $\pm$ 0.48 vs. 8.48 $\pm$ 0.91, $p = 0.122$; respectively).

CSF-insulin levels correlate with CSF-Aβ_{1-42} among women in the non-AD groups.

No correlation between overall CSF levels of insulin and T-tau ($r = -0.06$, $n = 75$, $p = 0.31$), P-tau ($r = -0.03$, $n = 65$, $p = 0.41$), Aβ_{1-42} ($r = 0.10$, $n = 70$,

$p = 0.21$) or MMSE ($r = 0.21$, $n = 76$, $p = 0.09$) was found after correcting for grouping effect. However, CSF-insulin levels among the women belonging to SCI and SMCI groups (patients without AD or with no conversion in 2 years time) were positively associated to CSF-Aβ_{1-42} ($r = 0.44$, $n = 19$, $p < 0.05$; corrected for grouping effect). This correlation was absent in women from the PMCI and AD groups ($r = -0.15$, $n = 20$, $p = 0.26$).

Insulin improves the predictive accuracy of CSF biomarkers tau and Aβ_{1-42} in women

Numerous studies have explored the use of the CSF concentration of classical AD hallmarks as biomarkers for an early diagnosis [21]. The ratio between T-tau and Aβ_{1-42} levels has been proposed as the most sensitive for diagnostic proposes [21]. In view of our results, we next investigated if CSF-insulin could be an additional AD biomarker in women. Both sensitivity and specificity of CSF T-tau/Aβ_{1-42} ratio, when distinguishing PMCI and mild-AD from SCI and SMCI, were improved in women after adding insulin to the equation (T-tau/(Aβ_{1-42}*insulin) ratio), (see ROC analysis in Fig. 2). The best cutoff level for T-tau/Aβ_{1-42} ratio (0.700) resulted in a sensitivity of 87.5% (95% CI, 67.6 to 97.2%) and a specificity of 92.3% (95% CI, 74.8 to 98.8%) with an area under the curve (AUC) of 0.918 $\pm$ 0.041, while the best cutoff level for T-tau/(Aβ_{1-42}*insulin) ratio (1.182) resulted in a sensitivity of 100.0% (95% CI, 82.2 to 100%) and a specificity of 95.0% (95% CI, 75.1 to 99.2%) with an AUC of 0.995 $\pm$ 0.011.

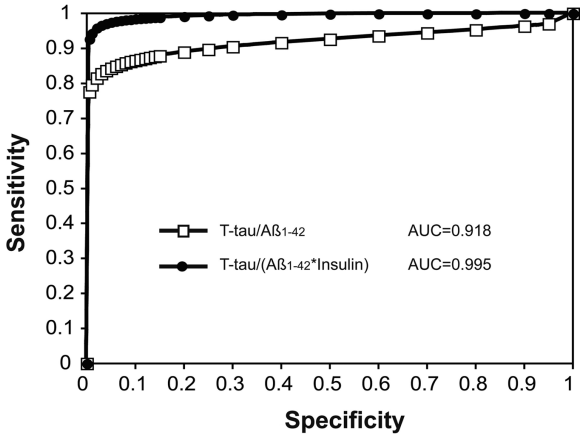

Fig. 2. Insulin improves sensitivity and specificity of classical CSF-AD biomarkers in women. The plot shows a ROC curve analysis for women from AD groups (PMCI and mild-AD) versus non-AD groups (SCI and SMCI groups). The optimal cut-off value for T-tau/Aβ_{1-42} ratio (open squares) was 0.700, resulting in a sensitivity of 87.5%, a specificity of 92.3% and an AUC of 0.918. The optimal cut-off value for T-tau/Aβ_{1-42}*insulin ratio (filled circles) was 1.182, resulting in a sensitivity of 100%, a specificity of 95% and an AUC of 0.995.

DISCUSSION

This study reports lower CSF-insulin levels in patients with mild-AD compared to SCI. The decrease in CSF-insulin was seen earlier during the disease course in women, and was independent of the ApoE genotype. In addition, we found that a combination of insulin, Aβ_{1-42} and tau measurements in CSF was of great sensitivity and specificity for distinguishing women that have mild-AD (MMSE: 23.6) or that will develop AD within two years time (PMCI), from other groups (SCI and SMCI).

Central insulin signaling has become a focus of attention in studying AD pathophysiology. Experimental data has revealed the importance of brain insulin in controlling tau phosphorylation, protecting against Aβ accumulation and toxicity and in promoting synaptic plasticity, neuronal growth and survival [22]. Epidemiological studies have reported an association of insulin resistance and its subsequent compensatory hyperinsulinemia with cognitive decline in elderly subjects [23, 24], and with a higher risk of developing AD [25,26]. However, most studies have focused on peripheral insulin; studies on central insulin showing decreased expression of insulin and insulin receptor [2,3] have been carried out in postmortem tissue. The present study investigates central insulin in memory clinic patients by measuring CSF levels at different stages of AD.

We found decreased levels of CSF-insulin in patients with mild-AD. Our results were significant even after controlling for age and gender. These findings are in agreement with previous studies reporting lower CSF-insulin in more advanced AD [13,27]. Two other studies found either increased levels [14] or no changes [15]. One possible explanation for these discrepancies could be BBB integrity, which was not considered in previous reports [14,15]. Disruption of BBB occurs in the later stages of AD [28], and a subsequent leaking of insulin from blood to brain could explain the finding of higher insulin levels in CSF. Interestingly, increased plasma levels of insulin have been reported in late AD [13,22,29]. The apparent discrepancy between peripheral and central insulin in AD might be due to down-regulation of insulin transport into the brain in conditions of insulin resistance [30]. To the contrary,

others have proposed that this discrepancy might be due to an altered insulin production in the brain [2,3]. The local production of insulin in the brain, as well as the factors that regulate the transport of peripheral insulin into the brain, are largely unknown. The interplay between the peripheral and central insulin productions and homeostasis needs to be further investigated, since it is of vital importance to understand the role of insulin in neurodegenerative disorders like AD.

Previous reports have shown that non-homozygotes for the ε4 allele had higher plasma insulin than ε4 carriers [13,27]. In the present study, no significant differences in CSF-insulin were found between ApoE ε4 carriers and non-carriers, although it is possible that the number of samples in our study would limit the detection of differences in ApoE subgroups.

We have not found an association between CSF-insulin and cognitive status. Only one previous study reporting changes in CSF-insulin showed its relationship to MMSE score in later stages of the disease [13]. Also peripheral insulin has been found to be positively associated with cognition [23], and with plasma Aβ_{1-42} in amnesic MCI and healthy individuals [31,32]. It has been shown that raising brain insulin levels by intranasal insulin administration facilitates verbal memory, attention and functional status in patients with early AD and in memory-impaired older adults [33]. Raising brain insulin levels also improved Aβ_{1-42} clearance to CSF in older adults [34].

The mechanisms by which insulin participates in Aβ clearance are not fully understood. Tamaki et al. reported that insulin induces expression of low-density lipoprotein receptor-related protein (LRP-1) [35]. LRP-1 is a key molecule in Aβ clearance from the brain through the BBB [36], and probably through CSF. Members of the LRP family have been found in the choroid plexus [37].

Insulin was also shown *in vitro* to reduce the intracellular accumulation of Aβ by accelerating AβPP/Aβ trafficking from the trans-Golgi network to the plasma membrane [12]. An insulin-mediated enhancement of Aβ trafficking to extracellular compartments may also contribute to a more effective clearance to the CSF.

In addition, a role for insulin in the regulation of Aβ levels can be envisaged. Deficient insulin signaling was correlated with reduced insulin degrading enzyme (IDE) in AD brains and in Tg2576 transgenic animals [38]. IDE is not only a proteolytic degrading enzyme for insulin but also for Aβ and a number of other peptides [39].

We have found a positive association between CSF-insulin and CSF-Aβ_{1-42} in women belonging to SCI and SMCI groups. Why levels of CSF-insulin are associated with levels of CSF-Aβ_{1-42}, in these groups but not in pre-AD or AD cases remains to be determined. A possibility could be that brain insulin might favor Aβ degradation/clearance to the CSF in non-pathological conditions, while levels of both insulin and Aβ are balanced. In AD, however, lower brain insulin levels coexist with Aβ overproduction. This imbalance could result in deficient Aβ degradation/clearance and thus in increased neuronal exposure to toxic Aβ species. If this is the case, an induction of insulin in the brain could be beneficial for AD patients.

We found that the decrease of CSF-insulin levels occurred earlier in women than in men. In fact, women belonging to both MCI groups (SMCI and PMCI) showed lower CSF-insulin levels than did men. This gender difference was not seen in mild-AD cases. There is little data on gender differences in insulin levels/function in AD, and no reports regarding AD progression. Female AD subjects have been shown to have lower plasma insulin levels compared to males [27]. An interaction between some gonadal hormonal system (that could be affected in post-menopausal women with AD) and brain insulin synthesis or degradation, could also be hypothesized. However, insulin, like other peptides or regulatory proteins, is known to cross the BBB by a saturable transport mechanism [1]. Thus, the correlation between CSF and serum levels of insulin is non-linear; and the rate of increase in CSF levels becomes proportionately smaller at higher serum levels [40]. Indeed, the transport rate of insulin has been shown to be altered in different conditions with abundant peripheral insulin, such as obesity, diabetes mellitus or insulin resistance [1]. A similar disruption in insulin transport through the BBB has been previously proposed in AD [13], where the levels of insulin in plasma are increased [13,22]. Thus, a decreased transport of insulin from plasma to brain, due to saturation of the insulin BBB transporter, could contribute to the lower CSF-insulin levels in AD seen in our study.

Possible gender differences in BBB integrity could be also of importance. BBB dysfunction is indeed a feature of AD [41], and gender differences are important in BBB permeability under pathological conditions [42] but not under normal conditions [43]. In line with this hypothesis, BBB integrity has been shown to be primarily affected in male AD patients [44]. In the present study, the ratio between CSF and plasma levels of albumin was used as an indicator of BBB integrity [28]. We show that albumin CSF-to-plasma ratio positively correlated to CSF-insulin, suggesting the possibili-

ty that increased uptake of albumin from periphery to brain could be associated with higher infiltration of insulin from plasma. Moreover, albumin CSF-to-plasma ratio was reduced in women compared to men in both MCI groups, indicating a gender-dependent disruption of BBB integrity in early phases of AD. Thus, it is tempting to speculate that men with MCI would show higher CSF-insulin levels due to a higher uptake of peripheral insulin, resulting from the degraded performance of the BBB. However, in disagreement with this hypothesis, we also found that later in the disease (mild-AD) both genders had similar lowered CSF-insulin levels and similarly disrupted BBB markers, compared to controls.

In summary, why women have lower levels of CSF-insulin at early stages of the disease is puzzling, and the possibilities of hormonal dependent mechanisms affecting insulin synthesis, transport or degradation, as well as the contribution of gender differences in BBB integrity should be further investigated.

The significant decrease in CSF-insulin levels seen in women at early stages of AD led us to investigate the relevant potential of using this parameter for early diagnosis. AD biomarkers in CSF are being intensively investigated [21]. At present, elevated CSF total, or phosphorylated tau proteins and low CSF-Aβ_{1-42} are the only biomarkers with enough sensitivity and specificity to serve as useful diagnostic biomarkers for identifying AD in the early stages [17,45]. In agreement with the largest study published to date [45], we established the ratio total tau/Aβ_{1-42} achieved in women with a sensitivity of 87% and a specificity of 92% when differentiating PMCI and mild-AD from SCI and SMCI. Interestingly, when insulin was combined with the total tau/Aβ_{1-42} ratio, the sensitivity increased to 100% and the specificity accuracy to 95%. The enhanced prediction accuracy might be attributed to the lack of correlation between CSF-insulin and CSF-Aβ_{1-42}, or tau. Statistically, when two markers are not correlated, the increase in prediction accuracy is incremental. The appearance of such striking improvement in AD diagnosis in women from the PMCI group suggests that, just by adding simple, well implemented and inexpensive insulin measurements to the classical AD CSF biomarkers, one could diagnose AD in females, at least, two years earlier. Confirmatory studies using larger samples are essential to establish the clinical significance of this finding.

In summary, the present study presents evidence that insulin levels are reduced in AD brains. This reduction is first seen in women at early stages of the disease. Overall, our findings support the role of brain insulin dysfunction as an early event in AD pathophysiology, and suggest that by CSF-insulin could improve early diagnosis of AD in females.

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Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=507).

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