Plasma Levels of Adipokines in Patients With Alzheimer's Disease – Where Is the "Breaking Point" in Alzheimer's Disease Pathogenesis?

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Summary

Peripheral insulin resistance is associated with decreasing adiponectin and increasing leptin plasma levels, and also with cognitive decline. The effects of adipokines on brain function have been published from both animal and human studies. In particular, the influence of leptin and adiponectin on the development of Alzheimer's disease (AD) has been extensively investigated. However, the association between adipsin and AD is as yet unknown. In 37 patients with AD and 65 controls that followed the same study protocol, we tested whether adiponectin, leptin, and adipsin could be used as biomarkers in the early stages of AD. In contrast with conclusions of cognition studies in insulin resistant states, our study found a correlation of impaired neuropsychological performance with increasing adiponectin and decreasing leptin in AD patients. Nevertheless, no significant differences between patients and controls were found. AD women had significantly increased adipsin compared to controls, and there was a positive correlation of adipsin with age and disease duration. Although adipokines do not appear to be suitable biomarkers for early AD diagnosis, they certainly play a role in the pathogenesis of AD. Further studies will be needed to explain the cause of the adipokine "breaking point" that leads to the pathogenesis of overt AD.

Key words

Alzheimer's disease • Adiponectin • Leptin • Adipsin • Blood-based biomarker

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Introduction

Alzheimer's disease (AD) and other disorders causing dementia are one of the fundamental challenges for the health care and social systems due to their high prevalence in populations and serious impacts on the health and quality of life of affected people. Because the highest prevalence of these disorders is among the eldest age groups, demographic changes are leading to their continued rise. Most cases of AD represent a sporadic form of the disease, with both genetic susceptibility and environmental backgrounds. AD is characterized by extracellular amyloid beta plaques and intraneuronal deposits of neurofibrillary tangles constituted by hyperphosphorylated tau proteins. Although several pathobiochemical and neurochemical changes that lead to the morphologic brain alterations typical for AD have been revealed, causal mechanisms have not yet been clarified. However, it is well documented that metabolic and endocrine impairments play an important role in the AD pathogenesis (Cai *et al.* 2012).

Adipokines, a large class of molecules derived from adipose tissue (including hormones, cytokines, growth factors), play a key role in many metabolic

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functions. These molecules can cross through the blood-brain barrier; in the brain they are active mainly in the hypothalamus, where they have an important role in the control of energy metabolism. This control can be a link between AD and metabolic diseases such as obesity, dyslipidemia, hypertension, impaired glucose metabolism or metabolic syndrome. There is growing evidence that obesity and type 2 diabetes mellitus (T2DM) are associated with cognitive decline, and adipokines probably play a crucial role in this pathological process (Forny-Germano *et al.* 2018). Indeed, dysregulated leptin and adiponectin, the two most abundant and most studied adipokines, directly influence cognitive decline and memory loss, beta amyloid production, aggregation and deposition, tau hyperphosphorylation, neurodegeneration, impaired synaptic plasticity and synapse loss. Adiponectin influence microglia-mediated neuroinflammation and neuronal insulin resistance. The possible mechanisms and the role of adipokines as key mediators of the communication between the periphery and the CNS in health and disease are excellently reviewed by (Forny-Germano *et al.* 2018).

Although peripheral blood adipokines have been considered as suitable biomarkers for AD, current research has provided rather unclear results. In this study we focused on adiponectin, leptin and adipsin, examining whether peripheral levels of these adipokines could be used as biomarkers in the early stages of AD and whether they correlate with cognitive decline.

Adiponectin

Adiponectin, one of the most abundant adipokines, is a hormone secreted by adipose tissue and mainly distributed in the skeletal muscle, liver and pancreatic islet tissue. After binding to the receptors, adiponectin may improve glucose utilization and stimulates fatty acid oxidation. Adiponectin has anti-inflammatory properties, and acts against atherosclerosis and insulin resistance (Kadowaki *et al.* 2006). Decreased insulin sensitivity in lean women with gestational diabetes and polycystic ovary syndrome was shown to be associated with lower adiponectin levels compared to lean controls (Vejrazkova *et al.* 2017). Higher levels of adiponectin have been shown to lower the risk for T2DM (Li *et al.* 2009) or risk for metabolic syndrome (Novotny *et al.* 2014). The adiponectin receptor gene is extensively expressed throughout the body, including the brain (Yamauchi *et al.* 2003). Despite the fact that increased adiponectin levels have been shown to have a beneficial effect on insulin signaling and the management of T2DM, higher levels of adiponectin have also been associated with an increased risk of mortality in patients with cardiovascular disease (Wu *et al.* 2014). Moreover, results of the Framingham Heart Study indicated that in women, elevated adiponectin levels were associated with an increased risk of dementia and AD. The fact that there was a threshold effect in women above which adiponectin levels become a risk factor for dementia suggests that the absence of an adiponectin effect in men could be due to their having adiponectin levels less than a threshold value for increasing the risk of dementia (Van Himbergen *et al.* 2012). Changes in the serum levels of adiponectin and insulin in AD are positively correlated with the severity of dementia (Khemka *et al.* 2014). However, there are studies that have not found significant differences in blood adiponectin levels between AD patients and healthy controls (Warren *et al.* 2012).

Leptin

The most studied adipokine in relation to AD is leptin (see recent reviews Magalhaes *et al.* 2015, McGregor *et al.* 2018). Leptin is secreted primarily, but not exclusively, by adipose tissue, which has an important role in energy storage and availability in the body. Leptin controls the body weight and suppress appetite, and its effect is mediated by the long form of the leptin receptor in the hypothalamus. Leptin directly activates the energy sensors AMP-kinase and sirtuins, and thus decreases tau protein phosphorylation and amyloid beta storing, processes that occur in neurons during metabolic imbalances (Greco *et al.* 2011). Although the major interest in leptin is related to its role in the regulation of energy balance, interest on its effects on brain cognition and neuroprotection is increasing. Leptin might promote the activation of cognitive processes that may retard or even partially reverse selected aspects of AD or ageing memory loss (Folch *et al.* 2012). Besides the regulation of energy homeostasis and neuroendocrine functions in the hypothalamus such as feeding, thermogenesis, and neuroendocrine status, leptin also modulates higher nervous functions, such as behavioral performance related to learning and memory, and hippocampal synaptic plasticity (Oomura *et al.* 2006).

However, studies on the importance of leptin in AD patients have not shown conclusive results. Some studies have shown a protective effect of higher leptin levels on cognitive functions (Holden *et al.* 2009,

Khemka *et al.* 2014), while others have not confirmed this link (Warren *et al.* 2012). In more recent studies, the opinion prevails that peripheral leptin levels are not related to the development of AD (Teunissen *et al.* 2015) and that leptin is therefore not a suitable peripheral biomarker for AD (Oania *et al.* 2015).

Adipsin

Adipsin, also known as complement factor D, is a serine protease synthesized by adipocytes and released into the bloodstream. Adipsin activates the alternative complement pathway, and its main role in the immune system is the suppression of infectious agents. Adipsin has a high level of expression in fat, highlighting the importance of adipose tissue in immunity. Human studies show altered levels of adipsin in insulin resistance (Wang *et al.* 2019), polycystic ovary syndrome (Gursoy Calan *et al.* 2016), metabolic syndrome (Palla *et al.* 2020, Chedraui *et al.* 2014), and cardiovascular diseases (Ho *et al.* 2018). Interestingly, adipsin is selectively decreased in T2DM patients with β cell failure (Lo *et al.* 2014). Elevated adipsin levels have been associated with mild cognitive impairment in patients with T2DM (Guo *et al.* 2019). Adipsin can cross the blood-brain barrier and is present in the human cerebrospinal fluid under pathophysiological conditions. Cerebrospinal fluid adipsin concentrations show a significant correlation with markers of inflammation in the cerebrospinal fluid (Schmid *et al.* 2016).

Because of its ability to cross the blood-brain barrier and the fact that adipsin plays a role in immune processes, adipsin might be a potential predictive factor of neurodegenerative diseases, including AD. However, since adipsin has not been associated or investigated in relation to AD, it is not yet clear exactly how adipsin might act in AD pathogenesis.

Methods

A total of 37 patients (23 women and 14 men) with sporadic AD fulfilling the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable AD, participated in the study. AD patients were recruited to participate by the Department of Neurology, 3rd Faculty of Medicine, Charles University and Thomayer Hospital in Prague, Czech Republic. The control group consisted of 65 persons over the age of sixty years, of whom 42 were women and 23 men. T2DM was an exclusion criterion for both groups. The presence of metabolic syndrome was based on NCEP ATP III criteria (2002). Details regarding the clinical characterization of the tested groups are listed in Table 1.

The diagnosis of AD was confirmed by neuropsychological tests, cerebrospinal fluid analysis (amyloid beta, total tau and phosphorylated tau protein levels), and an MRI of the brain. On MRI scans, the Medial Temporal lobe Atrophy scale (MTA, Scheltens) was used to assess cortical atrophy, and the Fazekas scale was used to assess the effect of ischemic white matter changes on the cognitive impairment in AD patients. Neuropsychological assessments were given as follows. In both AD patients and controls: the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS – population average range 110-119 adjusted to education level with higher values indicating better performance). In AD patients only: the Mini Mental State Exam (MMSE – the maximum score is 30; values below 24 can indicate cognitive impairment) and the Frontal Assessment Battery (FAB – the maximum score is 18, with higher values meaning better performance). And in controls only: the Montreal Cognitive Assessment (MoCA – the maximum score is 30; higher values mean better performance) and the Geriatric Depression Scale (GDS – the maximum score is 15; a score higher than 5 is suggestive of depression). Controls with normal cognitive performance and the absence of hippocampal atrophy and ischemic white matter lesions on MRI underwent the same test protocol as patients with AD, except for cerebrospinal fluid analysis. The majority of the 37 AD patients were examined at the time of AD diagnosis (28 patients), 6 AD patients were examined after having the disease of less than one year (from 2 to 17 months), and only 3 patients were examined after having the disease for more than one year (from 28 to 56 months).

Biochemical characteristics

For the evaluation of biochemical parameters, blood samples were taken in a fasting state in the morning. Blood samples for multiplex evaluations of biomarkers were treated with DPPIV Protease Inhibitor Coctail (Sigma-Aldrich, Saint Louis, MO, USA), centrifuged and stored at -80 °C until analyzed. Adiponectin and adipsin levels were measured using Bio-Plex ProHuman Diabetes Adipsin and Adiponectin Assays (Bio-Rad, Hercules, CA, USA) and leptin levels

were measured using a Bio-Plex ProHuman Diabetes 10-Plex Assay (Bio-Rad, Hercules, CA, USA). Assays were done according to the manufacturer's instructions, and the positions of patient plasma samples and controls were randomly placed in the test plates of each kit. Lipid profile assessments included total and high-density lipoprotein (HDL) cholesterol levels, and triacylglycerol concentrations by an enzymatic colorimetric test (Cobas 6000, Roche Diagnostics, Mannheim, Germany). LDL-cholesterol was calculated by the Friedewald-Levy-Fredrickson formula (Roberts 1988). To assess peripheral insulin sensitivity, the homeostasis model of insulin resistance HOMA-R was calculated, and for insulin secretion the homeostasis model of beta cell function HOMA-F was calculated (Matthews *et al.* 1985). For these calculations, blood glucose levels were measured by an enzymatic reference method with hexokinase, insulin and C-peptide by ECLIA (Cobas 6000, Roche Diagnostics, Mannheim, Germany). Glycated hemoglobin (HbA1c) was assayed by a turbidimetric inhibition method (Cobas 6000, Roche Diagnostics, Mannheim, Germany).

Anthropometric characteristics

Body weight, height, and waist- and hip-circumferences were measured in order to calculate the body mass index (BMI), body adiposity index (BAI) and waist to hip ratio (WHR). BAI, a surrogate measure of body fat, was calculated as described elsewhere (Freedman *et al.* 2012).

Table 1. Clinical, anthropometric and biochemical characteristics of AD patients and controls.

	AD patients	**Controls**	**P value**	**P value[a]**	**P value[b]**
Number (men/women)	37 (14/23)	65 (23/42)			
Age [years]	72.9 (68.3-76.6)	64.9 (63.8-67.8)	**<0.001**		
Presence of metabolic diseases					
Impaired glucose tolerance	6 (16 %)	25 (38 %)	**0.034***		
Hypertension	24 (65 %)	26 (40 %)	**0.027***		
Dyslipidemia	13 (35 %)	29 (45 %)	0.467*		
Metabolic syndrome	14 (38 %)	34 (52 %)	0.229*		
Anthropometry					
BMI women [kg/m^2]	25.3 (21.8-27.9)	26.5 (24.7-28.8)	0.108	0.137	
BMI men [kg/m^2]	26.3 (23.2-27.4)	28.7 (26.3-29.7)	**0.045**	0.148	
WHR women	0.82 (0.78-0.85)	0.84 (0.80-0.85)	0.612	0.430	
WHR men	0.92 (0.88-0.97)	0.93 (0.91-0.99)	0.234	0.143	
BAI women [%]	33.4 (28.7-34.9)	32.3 (29.8-33.9)	0.923	0.328	
BAI men [%]	25.4 (23.8-29.0)	25.3 (24.7-27.7)	0.900	0.969	
Biochemistry					
Glucose [mmol/l]	5.1 (4.9-5.2)	5.4 (5.2-5.6)	**0.004**	**0.003**	**0.017**
C-peptide [nmol/l]	0.79 (0.73-0.94)	0.78 (0.68-0.87)	0.227	0.238	**0.040**
Insulin [mIU/l]	9.3 (7.9-11.2)	8.0 (6.3-10.1)	0.095	0.256	**0.023**
HbA1c [mmol/mol]	38 (37-39)	36.1 (34.5-37.9)	**0.043**	0.356	0.174
HOMA-R	2 (1.72-2.57)	2.11 (1.49-2.40)	0.365	0.710	0.110
HOMA-F	135 (114-172)	95 (84-112)	**<0.001**	0.146	0.150
Triacylglycerols [mmol/l]	1.2 (1.0-1.3)	1.12 (0.95-1.21)	0.360	0.275	0.138
Total cholesterol [mmol/l]	5 (4.5-5.6)	5.13 (4.92-5.25)	0.723	0.643	0.730
HDL-cholesterol women [mmol/l]	1.55 (1.3-2.0)	1.64 (1.46-1.81)	0.682	0.409	0.243
HDL-cholesterol men [mmol/l]	1.25 (1.0-1.5)	1.35 (1.23-1.65)	0.173	0.149	0.092
LDL-cholesterol [mmol/l]	2.92 (2.41-3.45)	2.92 (2.75-3.18)	0.898	0.415	0.450

Data are shown as median (95 % confidence interval), P value by Kruscal-Wallis test, P value[a] adjusted for age, P value[b] adjusted for age and BMI, * Chi-square test.

Table 2. Adipokines in AD patients and controls.

	AD patients	Controls	P value	P value[a]	P value[b]
Men	14	23			
Adiponectin [ng/ml]	8892 (2880-13404)	8411 (6480-13776)	0.500	0.494	0.269
Leptin [ng/ml]	1.44 (0.55-2.64)	2.53 (1.22-3.50)	0.243	0.526	0.579
Adipsin [ng/ml]	753 (630-1518)	762 (561-909)	0.229	0.449	0.149
Women	23	42			
Adiponectin [ng/ml]	20245 (11670-24718)	12515 (9881-14341)	**0.045**	0.300	0.390
Leptin [ng/ml]	4.37 (2.61-8.43)	6.66 (4.82-8.90)	0.225	0.874	0.117
Adipsin [ng/ml]	823 (589-1252)	646 (556-701)	**0.038**	0.125	**0.031**

Data are shown as median (95 % confidence interval), P value by Kruscal-Wallis test, P value[a] adjusted for age, P value[b] adjusted for age and BMI.

Statistical data analysis

A non-parametric Kruskal-Wallis One-way Analysis of Variance, Spearman Rank Correlation and the Chi-square test (NCSS 2019, LLC, Kaysville, UT, USA) were used for comparing anthropometric, biochemical and clinical parameters between the groups.

The study protocol was in accordance with the institutional Ethics Committee and national laws, and all subjects gave their written informed consent to participate in the study.

Results

Results from neuropsychological assessments in AD patients were: RBANS - median 64.5 (54-73; 95 % confidence interval), MMSE - median 21.5 (18-26; 95 % confidence interval), and FAB - median 12 (11-15; 95 % confidence interval). Neuropsychological assessments in controls gave results of: RBANS - median 107 (102-114; 95 % confidence interval), MoCA - median 29 (28-30; 95 % confidence interval), and GDS – 91 % controls ≤ score 5.

Clinical, anthropometric and biochemical characteristics of the AD patients and controls are presented in Table 1. The AD patients were significantly older than controls. Metabolic parameters were adjusted to age as well as to age and BMI. AD patients did not differ from the controls in the lipid spectra, and there was also an equal proportion of people treated with dyslipidemia in AD patients and controls. Under conditions of the same value of insulin resistance (HOMA-R), the AD patients had lower levels of fasting blood glucose and higher insulinemia. AD patients and controls had a similar percentage of subjects with metabolic syndrome, but controls had a higher proportion of impaired fasting glucose. There were significantly more patients treated with antihypertensive drugs in the AD group.

In both men and women, plasma levels of adiponectin and leptin did not significantly differ between AD patients and controls, even after age- and BMI-adjustments. Adipsin levels in men also did not differ in AD patients and controls, but in women, after adjustment for age and BMI, adipsin levels were higher in AD patients compared to controls (Table 2). Adiponectin and leptin levels were higher in women compared to men, both in AD patients and in controls. However, we found no differences between men and women depending on AD diagnosis, i.e. the interactions between gender and AD diagnosis for individual adipokines (data not shown). Significant Spearman correlations of adiponectin, leptin and adipsin with other screened parameters in AD patients and controls are presented in Tables 3-5. In AD patients, there was a positive correlation of adipsin levels with age and the duration of the disease. Neither adiponectin nor leptin correlated with age and the duration of AD in patients or with age in controls. Neuropsychological assessments significantly correlated with adiponectin and leptin in AD patients only. While adiponectin in AD patients correlated negatively with MMSE, leptin correlated positively with RBANS and FAB. The MTA score and Fazekas scales did not correlate with adipokines in our study.

Discussion

The main findings of our cross-sectional study were as follows:

Table 3. Significant Spearman rank correlations of adiponectin levels in AD patients and controls.

Adiponectin in AD patients	r_s	P value
MMSE	-0.3964	0.020
WHR	-0.3560	0.036
HDL-cholesterol	0.5983	<0.001
Triacylglycerols	-0.4903	0.003
Glucose	-0.3594	0.034
Insulin	-0.4209	0.015
C-peptide	-0.4913	0.004
HOMA-R	-0.4629	0.007
Adiponectin in controls		
WHR	-0.2914	0.019
HDL-cholesterol	0.3751	0.002
Triacylglycerols	-0.3897	0.001
Insulin	-0.3321	0.007
C-peptide	-0.3600	0.003
HOMA-R	-0.3423	0.005
HOMA-F	-0.2448	0.049

Table 4. Significant Spearman rank correlations of leptin levels in AD patients and controls.

Leptin in AD patients	r_s	P value
RBANS	0.3529	0.041
FAB	0.3959	0.019
BMI	0.7150	<0.001
BAI	0.8331	<0.001
Triacylglycerols	0.3471	0.038
C-peptide	0.3981	0.020
HbA1c	0.3621	0.028
Leptin in controls		
BMI	0.4807	<0.001
BAI	0.7015	<0.001
Insulin	0.5274	<0.001
C-peptide	0.5155	<0.001
HOMA-R	0.5012	<0.001
HOMA-F	0.4332	<0.001

i) Adiponectin levels negatively correlated with the MMSE score in AD patients, which means that higher adiponectin levels correlated with lower cognitive function. A negative correlation of low molecular weight adiponectin with MMSE was found also by Baranowska-Bik (Baranowska-Bik *et al.* 2018), and this is in accordance with the conclusions of studies indicating high adiponectin levels in AD (Ma *et al.* 2016).

Table 5. Significant Spearman rank correlations of adipsin levels in AD patients and controls.

Adipsin in AD patients	r_s	P value
Age	0.5003	0.002
AD duration	0.3859	0.022
BMI	0.3616	0.033
Adipsin in controls		
Age	0.3685	0.003
BMI	0.2767	0.026
HDL-cholesterol	-0.3012	0.015
Glucose	0.2529	0.042

ii) Leptin correlated positively with the RBANS and FAB neuropsychological tests, which supports the neuroprotective role of leptin and its function in memory (Forny-Germano *et al.* 2018).

iii) We revealed higher adipsin levels in AD women, and they positively correlated with age and AD duration. Not much is known about adipsin and its role in AD pathogenesis. There is one study confirming its passage through the blood-brain barrier and associations with inflammation processes (Schmid *et al.* 2016). Adipsin could play a role in the pathogenesis of AD *via* the modulation of the brain inflammation that accompanies AD pathogenesis (Holscher 2019).

iv) We did not reveal any significant differences in serum adiponectin and leptin levels between AD patients and elderly controls, both without histories of diabetes mellitus. Similarly to several other studies (Warren *et al.* 2012, Teunissen *et al.* 2015), we can conclude that adipokines are not suitable serum/plasma biomarkers for AD. However, the association of adiponectin and leptin levels with cognitive decline and AD is very controversial in the literature and needs to be elucidated.

There are studies that have reported lower adiponectin and higher leptin plasma levels in obese and T2DM patients that are prone to cognitive deficits and even dementia and AD (Arnoldussen *et al.* 2014). Lower serum adiponectin as an early marker of cognitive decline was also reported in postmenopausal women (De Franciscis *et al.* 2017). On the other hand, there is a growing body of evidence supported by large meta-analyses (Ma *et al.* 2016, Khemka *et al.* 2014, Van Himbergen *et al.* 2012, Gilbert *et al.* 2018) describing a dramatic rise in adiponectin (especially in

women) and decrease in leptin levels in patients with AD. Some researchers have tried to explain this adiponectin paradox, i.e. its neuroprotective and anti-neurodegenerative vs. neurodegenerative activities, by the adaptation of adiponectin to an insulin-resistant state or by adiponectin resistance *per se* (Waragai *et al.* 2017) and then by the hypothesis of amyloidogenic evolvability (Waragai *et al.* 2020).

The role of adipokines is much more complex than expected, and we have to go back to the physiology of ageing and „metabolic" pathophysiology of AD (Chen *et al.* 2019, Kang *et al.* 2017). Even „physiological" aging is associated with a progressive decline in physiological functions that subsequently leads to the development of age-related disorders such as diabetes, cardiovascular disease, cancer etc. (Kirkland 2013). Aging also plays a critical role in neurodegenerative disease and is an important cause of AD. Aging is accompanied by mitochondrial dysfunction, cellular senescence, metabolic declines, adipose tissue dysfunction, insulin resistance, chronic sterile inflammation, and dysregulated nutrient sensing (Stout *et al.* 2017, Cedikova *et al.* 2016).

Obesity, T2DM and AD share many pathological metabolic features, similarly to ageing *per se,* but they occur earlier and are more pronounced. Systemic and brain insulin resistance seems to be the common denominator of the metabolic dysregulation connecting the periphery with the brain (Arnold *et al.* 2018, Holscher 2019). Insulin is not only a hormone that regulates blood glucose levels but it is also an important growth factor that regulates neuronal growth, repair and functions. It regulates synaptic activity in the brain and the integrity of neuronal networks (Ferrario *et al.* 2018). Insulin signaling is tightly connected with adiponectin and leptin signaling, and is counteracted by pro-inflammatory signaling (Wang *et al.* 2019, De La Monte *et al.* 2019). Impairments could lead to neurodegeneration, cognitive decline and AD. AD is usually accompanied with serum hyperinsulinemia (also seen in our study), which probably compensates for the brain insulin resistance. Moreover, positron emission tomography (PET) scans of people at high risk for developing AD have detected decreases in the rate of glucose metabolism decades before the appearance of AD symptoms (Kuehn 2020).

The other link between the periphery, specifically white adipose tissue, and the CNS are adipokines. There is evidence that adipokines, especially the most-studied adiponectin and leptin, also play an important role in brain functioning and cognitive decline (Forny-Germano *et al.* 2018, Waragai *et al.* 2017, Gilbert *et al.* 2018, Chen *et al.* 2019).

Although our study did not confirm significantly elevated adiponectin levels or decreased leptin levels in patients with AD as found in meta-analysis (Ma *et al.* 2016), we did see a similar trend, especially in women. This result may be due to the fact that most patients were enrolled in the study at an early stage of the disease and have negative history of T2DM, which could more rapidly deteriorate the mutual insulin-adipokine signaling. However, these AD patients with the same insulin sensitivity (HOMA-R) had higher serum insulinemia compared to controls, which may indirectly indicate insulin resistance in the brain (Arnold *et al.* 2018).

This study has several limitations. Firstly, the control group was significantly younger than the AD patient group. However, only adipsin but not adiponectin and leptin correlated with age in our study. Secondly, it is known that adipokines can cross the blood-brain barrier, and the situation in the brain itself is unknown. Measurements of adipokines in cerebrospinal fluid were not included in this study.

In summary, from the published data we can see parallels in the risk factors and pathogenic mechanisms of obesity, T2DM, metabolic syndrome and early cognitive decline. These diseases are associated with increasing insulin resistance and gradually decreasing plasma adiponectin levels and increasing leptin levels, probably due to developing leptin resistance. However, only overt AD is associated with increases in plasma adiponectin and declines in leptin levels. This breaking point in adipokine levels seems to be crucial in AD pathogenesis. Discrepancies in the published data concerning adipokines could be related to study designs (mainly cross-sectional studies) and the heterogeneity of groups of patients before/after this "breaking point".

It will be a challenge to find the factors that influence this switch into the AD pathogenesis, as they may include critical brain insulin resistance, physical inactivity, high fat diet, mental stress, environmental metabolic disruptors, genetic background etc., which alone or in interactions overcome their thresholds. Dysregulation of the brain adipokines probably must precede the AD diagnosis many years before, because weight-loss and under-nutrition are also associated with AD risk (Sergi *et al.* 2013, Gillette Guyonnet *et al.* 2007),

and disturbed eating behaviors and reductions in body weight may be seen nearly two decades before the diagnosis of AD (Barrett-Connor *et al.* 1996). These changes in body composition are probably due to hypothalamic changes in insulin-adipokine signaling.

Longitudinal studies are needed to monitor adipokine levels during the adult lifetime of persons at risk of AD in comparison with subjects without familial histories of AD, obesity and T2DM, together with extensive screening of potential risk factors influencing the break point leading to disease.

Conflict of Interest

There is no conflict of interest.

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