ORIGINAL PAPER

Serum Leptin Levels in Patients with Alzheimer's Disease

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Abstract

Introduction: Leptin receptors have been identified in many peripheral tissues as well as the CNS including the hippocampus, which is particularly vulnerable in Alzheimer's disease (AD). Animal data shows that leptin may be implicated in the pathophysiology of AD. The aim of this study was to examine if there is any differences in serum leptin levels between patients with AD and normal controls.
Material and methods: Ninety patients with AD and 95 normal controls matched for age and gender were included. The diagnosis of Alzheimer dementia was based on standard criteria provided by the ICD-10 system.
Blood samples were frozen at -80^{o}C until analysis. Leptin levels were measured using a human leptin enzyme linked immunosorbent assay (ELISA) kit.
Differences in leptin levels were assessed between the two groups using the Mann–Whitney method. Linear regression analysis was also used to adjust for characteristics shown to be associated with leptin and cognitive decline.
Results: From the patients with AD, 74 were women and 16 were men (mean age 80.53±6.03, mean body weight 71.49±8.33) and from the control group 78 were women and 17 men (mean age 79.27±6.86, mean body weight 70.23±6.73). Linear regression revealed that the use of antipsychotic drugs was associated with serum levels of leptin ($p<0.001$). Serum leptin levels were, also, significantly lower in patients with AD compared to normal controls (17.89±23.59 in AD patients vs 26.82±17.77 in normal controls, $p<0.0001$, Mann-Whitney U).
Conclusion: Our study, in accordance with the findings of studies in animal models, provides evidence that leptin may be implicated in the pathophysiology of AD.

Keywords: Leptin, Alzheimer, sex

Introduction

Leptin is a protein that was originally discovered as an adipocyte-derived hormone controlling feeding behavior through receptors in the hypothalamus (Zhang et al 1994). Since its discovery, it has been shown that leptin has other important physiological roles in the control of fat storage or mobilization, the reproductive system, the immune system, bone homeostasis, insulin sensitivity (Leibel et al 1997,Schwartz et al 2000, Shimomura et al 1999) and neuronal activity and protection (Harvey 2007). Leptin receptors have been identified in peripheral tissues and in neurons in the brain, including the hippocampus (Garza et al 2008, Harvey 2007, Paulus et al 2005) which is particularly vulnerable in Alzheimer's disease (AD) (Terry and Davis 1980).

Several studies to date have addressed the correlation between reduced levels of circulating leptin and risk for AD. In the initial reports, only a limited number of cases were examined (Olson et al 1998, Power et al 2001) but have since been corroborated with larger patient populations. More interestingly, a negative correlation between leptin levels and severity of dementia has been observed (Ray and Wyss 2005). Furthermore, a large prospective study involving about 3,000 older persons followed over more than four years showed that those with the lowest leptin levels had a greater decline in their cognitive ability than those with the highest levels (Holden et al 2006, Holden et al 2009). Additionally, a large longitudinal analysis showed that central obesity in midlife increases the risk of dementia independent of diabetes and cardiovascular co-morbidities later in life (Whitmer et al 2008). Moreover, Lieb et al showed that higher leptin levels were associated with a lower risk of incident dementia and AD and with higher total cerebral brain volume and lower temporal horn volume (Lieb et al 2009). These observations indicate that leptin deficiency is common in AD (Merlo et al 2010) and is somehow associated with mid-life obesity, characterized by leptin "resistance" or "inefficiency.

The aim of this study was to investigate if the serum levels of leptin were decreased in patients with Alzheimer's disease.

Methods

Study population

A total of one hundred and eighty five participants were included in the study. Participants were stratified in two groups. The first group consisted of patients suffering from Alzheimer's disease (AD) (n=90) and the second group consisted of normal controls (n=95). The participants with AD were inpatients in neurological clinics. Informed consent of participation in this study was obtained from each participant except from cases when Alzheimer disease made it impossible. In such cases first degree relatives of the patient were informed and gave the consent. The ethics committee of the University Hospital of Larissa approved the study.

Measurements

The Mini Mental State Examination (MMSE) was administered to all participants. Clinical diagnosis of dementia was conducted by two geriatricians or two neurologists. The final diagnosis was made when full agreement between the two specialists obtained. A lot of patients with Alzheimer's disease were receiving chronic therapy with cholinesterase inhibitors and antipsychotics.

Venous blood samples were centrifigured at 3500 rpm for 5 minutes and aliquots of serum samples were stored at $-80^{o}C$ until further use. Serum was collected in the morning, between 8.00-9.30 after 12-14 h of fasting. Leptin levels were measured using a human leptin enzyme linked immunosorbent assay kit according to the manufacturer's instructions. Double measurements were performed. The reported value is the mean of two measurements per patient. Inter-assay variability of the assays was <10%.

Statistical analyses

Data analysis was conducted using commercial available personal computer software SPSS 15.0 (SPSS Inc., Chicago, IL). Normality assumption was checked using the Shapiro test. As departures from normality were significant, non-parametric methods were used.

The differences in the leptin concentrations between the two groups were assessed using the Mann-Whitney method. Linear regression analysis was also performed. The significant level was set at $p<0.05$

Results

The main demographic characteristics of both groups are reported in table 1.

Alzheimer disease was correlated positively with smoking (p=0.004). In detail, more patients with AD were active smokers than the normal controls, whereas more controls were ex-smokers than AD patients (figure 1).

Patients with AD used in a significant percent antipsychotic drugs (66.7%, figure 2).

The other demographic characteristics (age, weight and sex) did not show significant differences between the two groups.

Table 1. Main demographic characteristics in patients with Alzheimer's disease and normal controls.

	Alzheimer(N=90)	Controls(N=95)	p value
Age (y)	80.53 (6.03) [a]	79.27 (6.86) [a]	p=0,186 (ns) *
Weight (kgr)	71,49 (8.33) [a]	70.23 (6.73) [a]	p=0,262(ns) *
Smoking (%)	50	31.6	**p=0,004** **
Female Sex, No.(%)	74 (82.2)	78 (82.1)	p=0.983 **
Antipsychotic drugs No.(%)	60 (66.7)	0 (0)	**P<0.0001** **

a Values are mean (SD),* t-test, ** chi-square

Figure 1. Patients with Alzheimer's disease were current smokers in a greater percent than the normal controls.

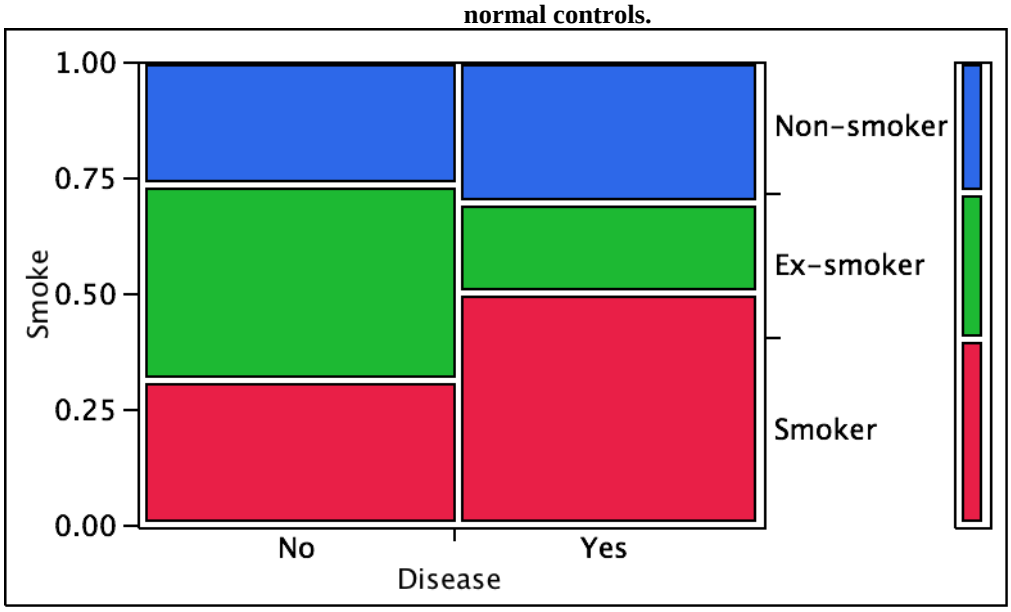
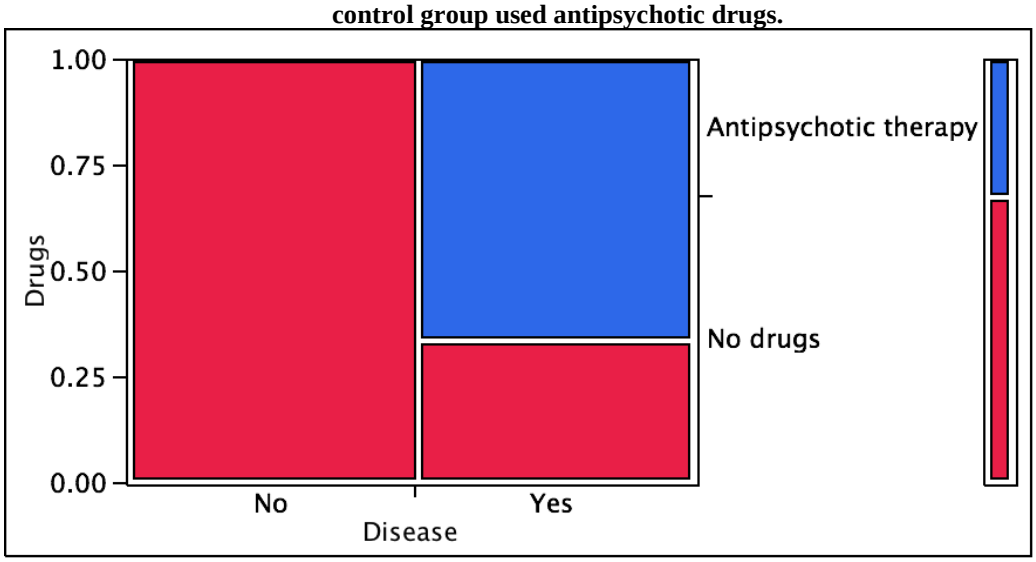


Figure 2. Patients with Alzheimer's disease often used antipsychotic drugs , while none of the control group used antipsychotic drugs.



Serum leptin levels

The distribution of the levels of leptin in both groups are presented in the tables 2 and 3 and in the figures 3 and 4.

Table 2. Levels of leptin in patients with AD

Quantiles

100.0%	**maximum**	**125**
99.5%		**125**
97.5%		**112.875**
90.0%		**44.9**
75.0%	**quartile**	**20.25**
50.0%	**median**	**10**
25.0%	**quartile**	**4**
10.0%		**2**
2.5%		**0.6375**
0.5%		**0.5**
0.0%	**minimum**	**0.5**

As it is demonstrated in the figures, leptin levels did not follow the normal distribution in both groups. Therefore, therefore we used the non-parametric test Mann Whitney in order to seek differences between the two groups. Mann-Whitney showed that patients with AD had statistically significant lower leptin levels than the normal controls ($p<0.0001$, figure 5).

Table 3. Leptin levels in normal controls.

Quantiles

100.0%	**maximum**	**95**
99.5%		**95**
97.5%		**78.8**
90.0%		**51.4**
75.0%	**quartile**	**37**
50.0%	**median**	**21**
25.0%	**quartile**	**14**
10.0%		**8.6**
2.5%		**4.4**
0.5%		**2**
0.0%	**minimum**	**2**

Linear regression analyses showed that this association was remained statistically significant after adjustment of other factors like age, sex, weight and smoking. The only factor which seemed to affect leptin was the use of antipsychotic drugs ($p<0.0001$, figure 6).

Figure 3. Distribution of the levels of leptin in patients with AD.

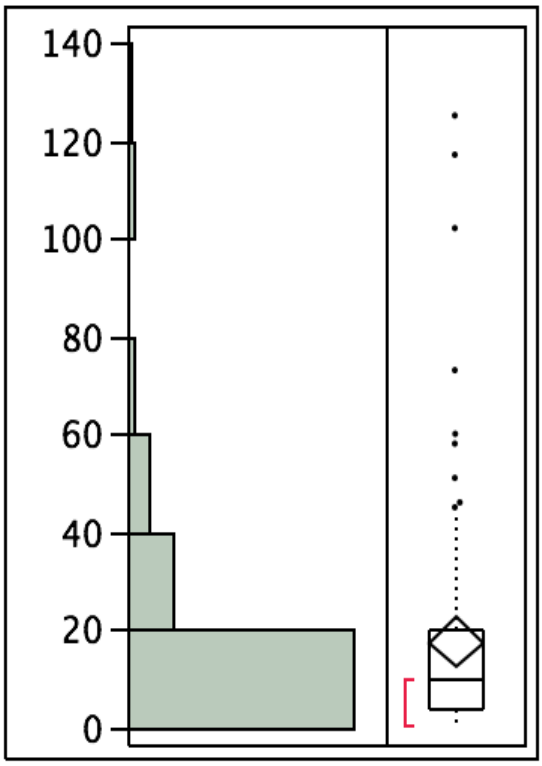
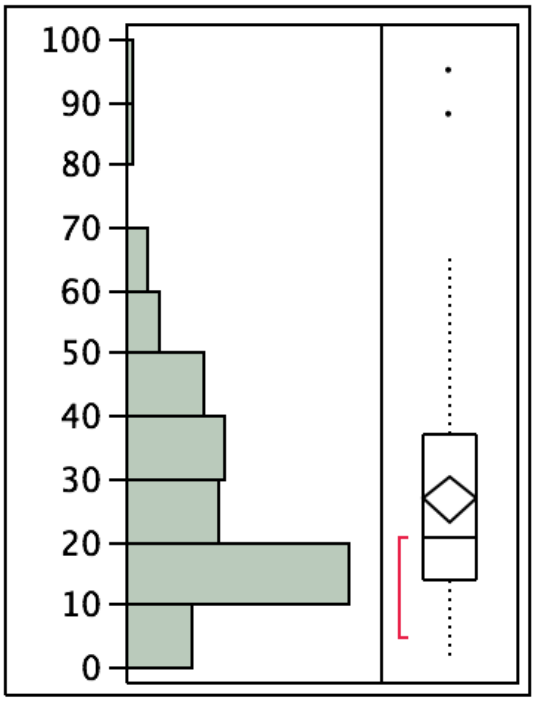


Figure 4. Distribution of leptin levels in normal controls.



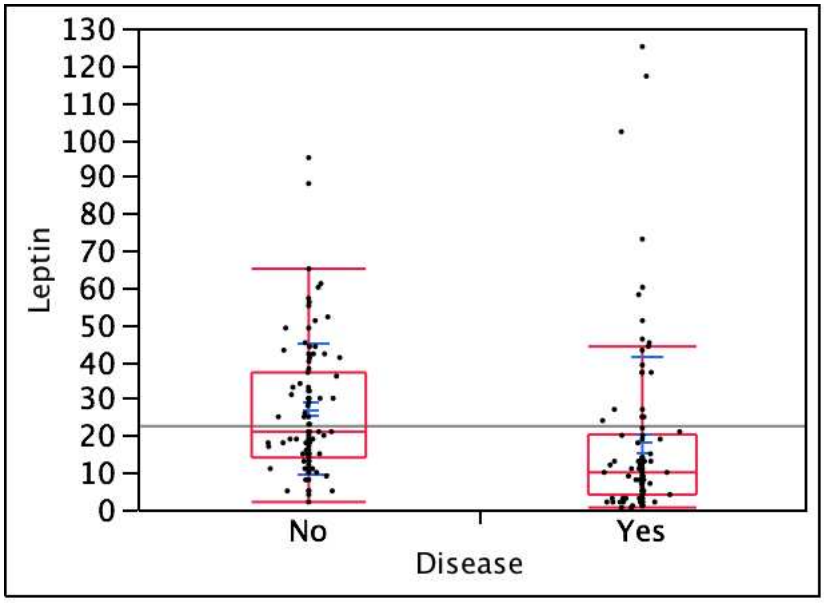


Figure 5. Association of Alzheimer's disease with leptin. Patients with AD had lower levels of leptin.

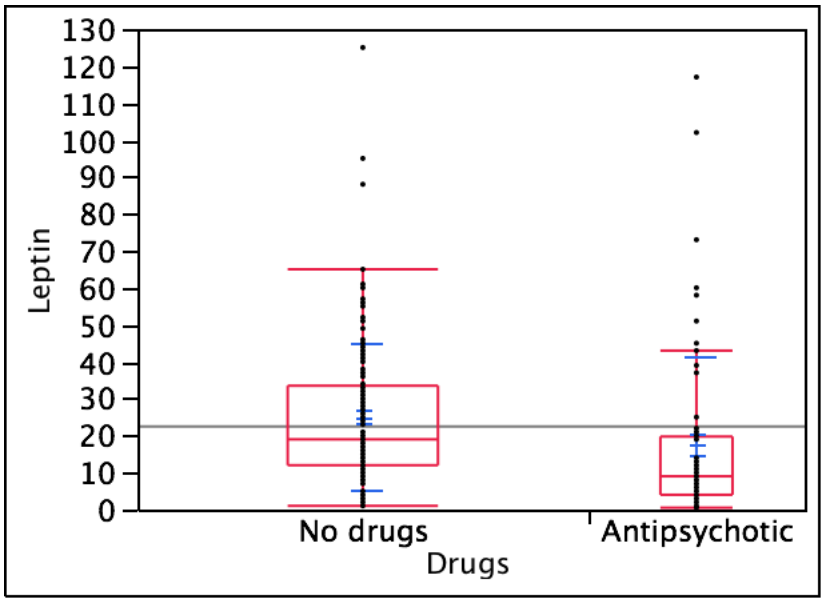


Figure 6. Leptin was associated negatively with the use of antipsychotic drugs.

Discussion

The main finding of our study is the association between AD and leptin. Patients with AD had lower levels of circulating leptin. The association of high leptin levels with protection from AD remained significant after adjustment for other factors like age, sex, weight and smoking. The only factor that could affect leptin levels, was antipsychotic drugs. The overall findings support the hypothesis that one possible reason for the presence of AD may be an acquired resistance to effects of leptin, including its neuroprotective effects (Tezapsidis et al 2009).

A growing body of evidence suggests that leptin has beneficial effects on brain development and function (Harvey et al 2005, Tang 2008). Leptin-deficient mice have a lower brain weight, an immature expression pattern of synaptic and glial proteins (Ahima et al 1999) and disrupted projection pathways within the hypothalamus (Bouret et al 2004), indicating that leptin is necessary for normal brain development. Furthermore, leptin appears to mediate structural and functional changes in the hippocampus and to improve memory function (Harvey et al 2006). Leptin receptors are present in the CA1 region of the hippocampus and leptin deficient or insensitive rats show reduced synaptic plasticity and poorer performance in spatial memory tasks. Leptin facilitates N-methyl-D aspartate receptor mediated conversion of short term potentiation to long term potentiation in the hippocampus (Shanley et al 2001) and also improves neuronal survival (Doherty et al 2008).

Leptin also has been shown to increase apolipoprotein E-dependent β-amyloid uptake into the cell and reduce brain extracellular concentrations of β-amyloid the major component of the neuritic plaques that are a histopathological hallmark of AD (Fewlass et al 2004). Leptin and insulin act in a dose-dependent and synergistic manner to decrease hyperphosphorylation of tau, the primary component of the neurofibrillary tangle, the second major histopathological hallmark of AD (Greco et al 2008). Most interesting is an observation that chronic leptin treatment improved memory performance in transgenic animal models of AD (Tezapsidis et al 2009, Greco et al 2010). Our results that patients with AD have lower leptin levels are consistent with these experimental results. Together these data support the concept that leptin exerts multiple functions in the brain, beyond those involved in food consumption and energy expenditure.

Concerning the limitations of our study, we have to mention the fact that a great percent of patients with AD were taking antipsychotic medications. The linear regression showed that antipsychotic drugs could affect the levels of leptin. It is noticeable that so many people were under this medication, because it exists a warning about the harmful consequences of these drugs to demented people. There are also publications which show that antipsychotic drugs, and especially the atypical ones, can provoke stroke or they can cause sudden death, probably because of prolongation of QTc. Moreover, they usually sedate demented people who become vulnerable to falls and hip fractures (Mittal et al 2011). Although, the side effects of antipsychotics can be lethal to this population, they are usually used to treat the disturbances of behaviour of people with dementia.

In conclusion, our study provides good evidence for an association between the circulating concentrations of leptin and the presence of AD. Although further research is required to address the precise cellular mechanisms underlying the reduced incidence of AD when leptin concentrations are high, it is possible that leptin may be a good indicator of susceptibility to AD in the elderly population.

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