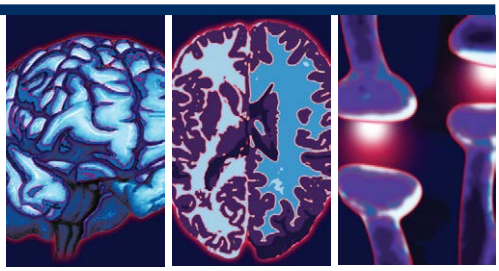

Ghrelin Levels in Elderly, Diabetic Patients with Mild Cognitive Impairment

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Abstract

Objective:

The etiology of cognitive impairment in diabetes is unknown, but probably associated with many factors. One of the recent hypotheses suggests that ghrelin could be involved in cognitive impairment in diabetic patients. It has been reported that ghrelin signaling occurs in the hippocampus and improves memory and spatial learning. The aim of this study was to evaluate serum levels of ghrelin in elderly, diabetic patients with and without mild cognitive impairment (MCI) and to determine the predictors of having MCI in elderly diabetics.

Methods:

The cross-sectional study was conduct among 276 elderly subjects with type 2 diabetes. 87 patients with MCI and 189 controls were selected according to the criteria proposed by the MCI Working Group of the European Consortium on Alzheimer's Disease (using the Montreal Cognitive Assessment: MoCA score). Data of biochemical parameters and biomarkers were collected. The serum levels of ghrelin were assessed using ELISA kit.

Results:

Serum levels of ghrelin were significantly lower in patients with MCI compared to controls. In MCI patients serum ghrelin levels were positively correlated with MoCA score and negatively correlated with HbA1c and BMI. The logistic regression models revealed that variables which increased the likelihood of diagnosis of MCI in elderly diabetic patients were: lower levels of ghrelin, higher levels of HbA1c, hypertension, previous cardiovascular disease, increased number of co-morbidities, and less years of formal education.

Conclusion:

In summary, serum levels of ghrelin were decreased in MCI elderly diabetic patients compared to controls and associated with poor MoCA score. The results indicated that lower ghrelin levels may be a risk factor for a cognitive impairment in diabetic, elderly patients. Further prospective larger studies are needed to confirm the role of this marker in the progression to dementia.

Keywords:

Elderly, Ghrelin levels, Mild cognitive impairment, Type 2 diabetes

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Introduction

Diabetes is a major public health problem with established cross-sectional and longitudinal relationships with cognitive impairment and dementia. There is a high prevalence of mild cognitive impairment (MCI) in individuals with type 2 diabetes mellitus (T2DM) [1,2]. A meta-analysis of longitudinal studies reported that individuals with diabetes had 1.21-fold significantly increased odds of having Alzheimer's disease, 1.51-fold higher risk for any dementia and 1.21 higher risk for mild cognitive impairment compared to non-diabetic individuals [3]. Another large study revealed that diabetes in midlife was associated with a 19% greater cognitive decline over 20 year period [4]. Aging itself has also an independent impact on greater risk of cognitive decline. The prevalence of MCI in subjects aged 70 years and older was around 14-18% [5]. Despite the clear link between cognitive impairment and diabetes disorders in epidemiologic studies, relatively little is known about the mechanism that underlies this association. A lot of studies had suggested that cognitive dysfunction in diabetes could be connected with severe hypoglycemia or persistent hyperglycemia, depression, inflammation, an increased prevalence of macrovascular and microvascular disease [6,7]. All these vascular etiology factors can interfere with other neurological changes in the aging brain and worsen the cognition [8].

One of recent hypotheses suggested that a common background of cognitive impairment, ageing and diabetes is a low-grade activation of the inflammatory system. The chronic inflammation could be provoked and sustained by changes in the gut microbiota composition. This phenomenon is called "gut-brain axis" and it incorporates bidirectional communication between the central nervous system and the gastrointestinal system [9,10]. Strong objective evidence of a linkage between gut bacteria and brain function comes from several human and animal studies [11-13]. Both dysbacteriosis in the gut and higher systemic inflammation in diabetic population can lead to the intestinal barrier and blood brain barrier dysfunction. Thus harmful substances such as advanced glycation end products, pathogens, endoxin with easy access to neurons, can cause neuroinflammation and the development of cognitive decline [10].

Recently, some researchers have suggested a potential role for ghrelin in cognitive dysfunction in diabetic patients [14,15]. Ghrelin is a hormone predominantly produced by the gastrointestinal tract, whereas some small amounts are derived from the brain [16]. It is responsible for weight gain through appetite control, energy balance and increasing food intake providing the hypothalamus with information on the amount of body fat [17,18]. Ghrelin signaling occurs in the areas in the brain - hippocampus and the ventral tegmental area which regulate mood and reward. One study showed that ghrelin signaling improves spatial learning and memory by increasing dendritic spine synapse density and long-term potentiation [19]. A lot of studies proved the protective cardiovascular effects of this protein [20]. Ghrelin lower blood pressure through dilating arteries, decrease the sympathetic activity of the autonomous nervous system and thus lower heart rate and vassal tone. Some researchers found that ghrelin levels are decreased in patients with coronary atherosclerosis [21], hypertension [22], and metabolic syndrome [23], coronary heart disease [24], diabetic subjects with advanced carotid atherosclerosis [25]. As little literature data is available concerning ghrelin levels in elderly diabetic subjects with MCI, the aims of the study were twofold: Firstly, evaluate levels of serum ghrelin in elderly patients with T2DM with and without MCI and secondly, identify the factors (including ghrelin levels) associated with MCI in elderly patients with T2DM.

Material and Methods

■ Participants and setting

This cross-sectional study was performed at out-patient diabetology clinic affiliated with the university hospital no 1 in Lodz, Poland from November 2013 to February 2014. A brief screening for recruitment was conducted by the investigators to identify potential participants. We included patients aged 65 and over with diabetes type 2 diagnosed minimum 1 year earlier, subjects who had been able to understand and cooperate with study procedures. The number of cases in the area during the study period determined the sample size. Exclusion criteria were: diagnosed depression or dementia, use of possible or known cognition-impairing drugs in the previous 3 month, presence of neoplasm, constant alcohol or substance abuse, severe visual, mobility, or motor coordination impairment, history of head trauma, inflammatory or infectious brain disease, severe neurological or psychiatric illness.

276 participants were recruited from the study previously described elsewhere [1].

■ Study design

The first step of the research included complete physical examination, blood pressure measurements, height and weight assessment and morning blood draw after a 10-12 hour overnight fast. After eating a breakfast capillary glucose level was measured to ensure that subjects were not hypoglycemic at the time of cognitive testing.

The second step included a standardized interview and cognitive testing took place in a private area in the clinic.

■ Instruments

Demographic forms and the detailed medical history of diabetes type 2 were taken and included: age, sex, years of education, duration of T2DM, smoking habit, the treatment for diabetes, number of comorbitidies, presence of diabetic complications: nephropathy, retinopathy, neuropathy, cardiovascular disease (CVD), stroke, hyperlipidemia and hypertension. All variables and possible risk factors were recorded in a standardized interview. Height and weight were measured and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Systolic and diastolic blood pressure was measured after a rest for at least 10 minutes with a sphygmomanometer in sitting position. The diagnosis of hypertension was established based on blood pressure levels measured at the study visit (≥ 140/90 mmHg) or a prior diagnosis of hypertension and current treatment with antihypertensive medications. Hyperlipidemia was defined as an untreated triglycerides 1.7 mmol/l or/and serum LDL cholesterol level 2.6 mmol/l or use of any lipid lowering agent.

■ Biochemical analyses

In all patients, peripheral venous blood samples were collected after overnight fast. Glycosylated hemoglobin (HbA1c), fasting blood glucose (FBG), total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels were measured in a centralized laboratory. Serum ghrelin levels were assessed using ELISA kit (EIAab, Wuhan, China) according to the instructions of the manufacturer. Minimum detectable concentration was 15.6 pg/ml.

■ Neuropsychological assessment

All participants underwent a neuropsychological assessment. The Montreal Cognitive Assessment (MoCA) was administrated to evaluate the cognitive function [26].

The MoCA test consisted of 8 domains: visuospatial/executive reasoning, memory, naming, abstraction attention, language and orientation skills, which were aggregated for a maximum score of 30. The normal score for MoCA score is ≥ 26, with one point added if the subject had fewer than 12 years of formal education. The MoCA is best screening tool for detecting MCI in the elderly, diabetic patients [27]. The diagnosis of MCI was established according to 2006 European Alzheimer's Disease Consortium criteria [28,29]. These criteria are currently available standard test and include absence of dementia. In epidemiological studies the cut-off points for MoCA scores (19/30) are recommended for the diagnosis of 'dementia'. Subjects who had MoCA score 19 and fewer were excluded from the study as dementia and sent to psychiatrist for further care. The criteria mentioned above included also absence of major repercussions on daily life. Katz Basic Activities of Daily living (BADL) and Lawton Instrumental Activities of Daily Living (IADL) questionnaires were performed to collect information on daily activities [30,31].

Depressed mood was ascertained from the 30-item Geriatric Depression Scale (GDS) [32]. A score between 0 to 9 has been found as normal, scores between 10-19 has been found as depressive symptoms. Scores ≥ 20 are indicative of the presence of severe depression and these subjects were sent to psychiatrist for further clinical diagnosis.

To evaluate the association of ghrelin levels with MCI 276 elderly subjects with T2DM were selected into groups: 87 patients with MCI and 189 patients without MCI as a control.

■ Ethics

The study was carried out in full compliance with the guidelines of good clinical practice of the world assembly declaration of Helsinki and was approved by the university ethical committee. Each patient was assigned a number by which he was identified to keep his privacy. The purpose, nature, and potential risks of the experiments were fully explained to the participants. Written informed consent was taken from all enrolled patients at the beginning of the study.

■ Statistical analyses

Statistical analyses were performed using Statistica 10.0 (StatSoft, Poland, Krakow). This study was designed to detect significant changes between the diabetic patients with MCI and control (diabetic patients without MCI). The mean prevalence of MCI in diabetic patients is 14-31 % [1,2] thus with prediction of MCI in this study about 25%, using a two tails test with power of 90% and $\alpha = 0.05$ a calculated minimal sample size of 60 for diabetic MCI positive patients was required to yield a statistically significant result [http://www.gpower.hhu.de/].

Shapiro-Wilk test of normality was applied. The descriptive statistics for the continuous variables were tested using the Student's t or the Mann Whitney-U tests and for the categorical variables using the χ^2, and whenever applicable. To evaluate the correlations between ghrelin levels and selected variables we calculated Pearson correlation coefficients for normally distributed variables and Spearman rank correlation coefficients for nonnormally distributed variables. Simple logistic regression model was done in order to select so-called independent factors which increase the selection risk MCI in elderly patients with type 2 diabetes. To evaluate the association of ghrelin levels with MCI, we constructed multivariable logistic regression model to assess whether circulating ghrelin was independently associated with MCI. To "optimize" the multivariable model, a stepwise approach was used (backward elimination with Wald criteria). Odds ratios (OR) were computed and presented with the 95% interval of confidence (CI). A P value of less than 0.05 was considered statistically significant. Data are shown as means ± SD.

Results

■ General description of MCI subjects and controls

The demographic, clinical and biochemical characteristics of the study group are presented in **Tables 1 and 2**. The results of the χ^2 test indicated patients with MCI were significantly more likely to be diagnosed with CVD, hyperlipidemia, hypertension, retinopathy and nephropathy **(Table 1)**. Furthermore, the Mann–Whitney U test and T-test showed that patients with MCI were older with less years of education, had a longer duration of diabetes, higher number of co-morbidities, higher level of HbA1c and triglycerides, lower level of HDL cholesterol and lower MoCA score **(Table 2)**. Lastly, no significant differences were found between the groups in sex, smoking habit, stroke, neuropathy, type of the treatment, presence of depressive symptoms, BMI, levels of total cholesterol and LDL cholesterol ($p > 0.05$).

■ Ghrelin levels in MCI subjects and controls

Serum levels of ghrelin were significantly decreased in patients with MCI compared to controls ($p<0.001$) **(Table 2)**. As expected, in the group of patients with MCI serum ghrelin level was negatively correlated with BMI ($r=-0.395$, $p<0.001$), with HbA1c level ($r=-0.318$, $p<0.001$) and with triglycerides ($r=-0.19$, $p=0.001$). Furthermore, serum ghrelin concentration was highly correlated with MoCA score ($r=0.554$, $p<0.001$). A positive but weak correlation was found between this parameter and HDL cholesterol level. The results are presented in **Table 3 (Table 3)**.

■ Logistic regression models

The univariate logistic regression models revealed that variables which increased the likelihood of having been diagnosed with MCI in elderly patients with type 2 diabetes were: diagnosis of CVD, hypertension, hiperlipidaemia, nephropathy, retinopathy, increased number of co-morbidities, older age and less years of education, longer duration of diabetes, higher levels of HbA1c and triglycerides, lower levels of ghrelin and HDL cholesterol **(Table 4)**. Finally we constructed a multivariate logistic regression model to determine the predictors of MCI. Independent factors associated with MCI were as follows: lower levels of ghrelin, higher levels of HbA1c, diagnosis of CVD and hypertension, increased number of co-morbidities and less years of education **(Table 5)**.

Discussion

Serum ghrelin levels of elderly diabetic patients with MCI were significantly decreased compared to controls. Human studies about protective role of ghrelin in cognitive dysfunction in diabetes are poor. In one recently published study the authors found also lower levels of ghrelin in T2DM patients with MCI compared to those with healthy cognition [9]. Other researchers found that ghrelin is an important predictor of executive function impairment in patients with type 2 diabetes [10]. Both studies mention above

Table 1: Demographic and clinical characteristics of type 2 diabetic elderly patients.

	Type 2 diabetes with MCI (n=87)	Type 2 diabetes without MCI (n=189)	χ^2	P value
Sex, male/female	34/53	93/96	2.46	0.12
Smoked tobacco regularly	26 (29.8%)	67 (35.4%)	0.83	0.36
Previous CVD*	71 (81.6%)	38(20.1%)	94.3	<0.001
Stroke	7 (8.04%)	7 (3.7%)	2.33	0.13
Previous HA/ use of HA drugs*	80 (91.95%)	138 (73.01%)	18.3	<0.001
Hiperlipidemia*	81 (93.1%)	132(69.8%)	12.87	<0.001
Retinopathy*	61 (70.1%)	60 (31.7%)	35.6	<0.001
Nephropathy*	43 (49.4%)	54 (28.5%)	11.37	0.007
Neuropathy	20 (22.9%)	36 (19.04%)	0.57	0.45
OAD	71 (81.6%)	151(79.8%)	0.11	0.74
Insulin	42 (48.2%)	88 (46.5%)	0.07	0.79
Presence depressive symptoms %	25 (28.7%)	57 (30.2%)	0.06	0.81

*significance, $p<0.05$; χ^2 test was used to test for significant differences between patients with MCI and those without MCI (controls), CVD - cardiovascular disease, HA- hypertension, OAD- oral anti-diabetic drug, MCI – mild cognitive impairment

Table 2: Characteristics and biochemical parameters of type 2 diabetic elderly patients.

	Type 2 diabetes with MCI (n=87)	Type 2 diabetes without MCI (n=189)	Z/t	P value
Age (years)*	75.7 ± 4.6	72.6 ± 4.6	-4.96	<0.001
Education-years*	9.7 ± 1.8	12.0 ± 2.2	7.97	<0.001
Duration of T2DM (years)*	11.25 ± 6.3	7.51 ±5.85	-5.96	<0.001
BMI (kg/m^2)	30.4 ± 3.59	29.6 ± 3.68	-1.92	0.054
Ghrelin (pg/mL)*	239.03 ± 47.09	297.83 ± 39.18	10.85	<0.001
HbA1c (%)*	7.73±0.71	7.01±0.54	-7.5	<0.001
CHOL (mmol/L)	10.31±2.2	10.29±1.71	-0.5	0.61
LDL (mmol/L)	6.01±1.64	6.08 ±1.73	-0.2	0.86
TG (mmol/L)*	10.59±2.68	9.22±1.84	-6.6	<0.001
HDL (mmol/L)*	2.3 ± 0.6	2.67±0.42	6.34	<0.001
Co-morbidity (n)*	7.07 ± 3.22	3.55 ± 2.33	-8.15	<0.001
MoCA score*	21.6 ± 1.5	27.4 ± 1.3	13.34	<0.001

Data are mean – SD values

*significance, $p<0.05$; Mann-Whitney U test (Z), or t test (t) were used to test for significant differences between patients with MCI and those without MCI (controls)

DM – diabetes mellitus, BMI – body mass index, HbA1c - glycosylated hemoglobin, CHOL - total cholesterol;; HDL - high-density lipoprotein cholesterol; LDL - low density lipoprotein cholesterol; TG – triglycerides; MoCA - Montreal Cognitive Assessment

were performed in much younger population than our study, which the first examine serum ghrelin levels in elderly subjects. These results are consistent with some data come from animal studies, which suggest that ghrelin can affect memory function. Kang *et al.* [33] examined the effect of central acylated-ghrelin and DES-acetylated ghrelin (native ghrelin) on memory function and glucose metabolism in an experimentally induced Alzheimer's disease (AD) rat model. They reported that central acylated ghrelin prevented the deterioration of memory function and propose that ghrelin could be investigated for cognitive and metabolic benefits, especially in patients with early symptoms of memory impairment. Another study showed that ghrelin agonist improved spatial learning in the mice with induced an Alzheimer's disease and therefore it might improve cognition via a central nervous system mechanism involving insulin signaling in hippocampus [34]. Jiao *et al.* [35]

Table 3: Relationship of serum levels of ghrelin with other clinical indicators in the elderly, diabetic patients with MCI.

	Ghrelin r	p
MoCA score*	0.554	<0.001
BMI*	-0.395	<0.001
HbA1c*	-0.318	<0.001
CHOL (mmol/l)	0.008	0.89
LDL (mmol/l)	0.07	0.23
TG (mmol/l)*	-0.19	0.001
HDL (mmol/l)*	0.25	<0.001

*significance, $p<0.05$; r-correlation coefficient

BMI – body mass index, HbA1c - glycosylated hemoglobin, CHOL - total cholesterol;; HDL - high-density lipoprotein cholesterol; LDL - low density lipoprotein cholesterol; TG – triglycerides; MoCA - Montreal Cognitive Assessment

Table 4: Assessment results of the risk of having MCI in a simple logistic regression model in the elderly patients with T2DM.					
Variables analyzed	ß	SE of ß	p value	OR	95% CI
Sex: female	0.2	0.1	0.12	1.2	0.49-1.59
Smoked tobacco regularly	0.12	0.1	0.36	0.8	0.6-1.16
Previous CVD*	1.43	0.16	P<0.001	4.19	3.03-5.81
Stroke	0.41	0.27	0.14	1.5	0.87-2.58
Previous HA/ use of HA drugs*	0.88	0.22	P<0.001	2.41	1.55-3.76
Hiperlipidemia*	0.72	0.21	0.001	2.01	1.35-3.12
Retinopathy*	0.8	0.14	P<0.001	2.24	1.7-2.96
Nephropathy*	0.44	0.13	0.001	1.56	1.2-2.03
Neuropathy	0.11	0.01	0.45	1.12	0.82-1.53
OAD	0.1	0.03	0.74	1.11	0.58-2.14
Insulin	0.25	0.06	0.79	1.07	0.64-1.78
Presence depressive symptoms %	0.03	0.01	0.8	0.96	0.73-1.27
Age (years)*	0.137	0.03	P<0.001	1.15	1.08-1.22
Education-years*	-0.639	0.09	P<0.001	0.53	0.44-0.63
Duration of T2DM (years)*	0.097	0.02	P<0.001	1.1	1.05-1.15
BMI (kg/m^2)	0.058	0.03	0.1	1.06	0.98-1.13
Ghrelin (pg/mL)*	-0.033	0.004	P<0.001	0.968	0.96-0.98
HbA1c (%)*	1.69	0.23	P<0.001	5.47	3.45-8.67
CHOL (mmol/L)	0.01	0.003	0.95	1.01	0.99-1.01
LDL (mmol/L)	0.01	0.004	0.74	1.01	0.99-1.01
TG (mmol/L)*	0.02	0.004	P<0.001	1.02	1.01-1.02
HDL (mmol/L)*	-0.09	0.018	P<0.001	0.91	0.87-0.94
Co-morbidity (n)*	0.426	0.05	P<0.001	1.53	1.37-1.71
Abbreviations: ß: regression coefficient; CI: confidence interval for odds ratio; OR: odds ratio; SE: standard error; *significance, p<0.05 T2DM – diabetes type 2, CVD - cardiovascular disease, HA- hypertension, BMI – body mass index, CHOL - total cholesterol; CRP - C-reactive protein; HbA1c - glycosylated hemoglobin; HDL-C - high-density lipoprotein cholesterol; LDL-C - low density lipoprotein cholesterol;					

Table 5: Assessment results of the risk of having MCI in a multivariable logistic regression model in the elderly patients with T2DM.					
Variables analyzed	ß	SE of ß	p value	OR	95% CI
HbA1c (%)*	0.88	0.37	0.017	2.42	1.17-5.01
Education-years*	-0.47	0.12	P<0.001	0.62	0.48-0.80
Previous HA/ use of HA drugs*	1.83	0.81	0.024	6.26	1.28-30.7
Previous CVD*	2.17	0.47	P<0.001	8.76	3.42-22.4
Co-morbidity (n)*	0.20	0.08	0.01	1.23	1.05-1.44
Ghrelin (pg/mL)*	-0.03	0.006	P<0.001	0.97	0.95-0.98
Abbreviations: ß: regression coefficient; CI: confidence interval for odds ratio; OR: odds ratio; SE: standard error; *significance, p<0.05 HbA1c - glycosylated hemoglobin, HA- hypertension, CVD - cardiovascular disease					

summarized the effects of ghrelin on regulating brain advanced function. They suggested that ghrelin could regulate neuronal activity, increased synaptic transmission and synaptic plasticity, promote synapse formation and enhances long-term potentiation to regulate memory and cognition. Human studies revealed that mRNA levels of ghrelin are decreased in patients with Alzheimer's disease, which is characterized by progressive memory loss and cognitive decline [36]. On the other hand ghrelin is reported to have neuroprotective, anti-apoptotic and anti-inflammatory function and therefore it increases neuronal survival [35]. The anti-inflammation effect of ghrelin is played via reducing the microglial activation and inflammatory cytokines release in inflammatory reaction. In addition, ghrelin plays neuroprotective role for attenuating blood–brain barrier disruption [35]. It has been hypothesized in previous studies that low-grade chronic inflammation is associated with MCI or with cognitive decline [37,38].

We found in our study that in the group of patients with MCI serum ghrelin level was highly correlated with MoCA score. In agreement with our results other authors had also confirmed that association [9]. In one study ghrelin was positively correlated with executive function assessed by the Wisconsin Card Sorting Test, although it wasn't related to MoCA score in

T2DM patients. The executive function could be a sensitive indication of diabetic cognitive decline among diabetes population. The author proposed that serum ghrelin level might be a biomarker of executive function in patients with type 2 diabetes mellitus [10].

Expectedly, we found significant but negative relationships between serum ghrelin levels BMI, HbA1c level and triglycerides. Many studies confirmed that sustained hyperglycemia, indicated by elevated HbA1c, is an independent risk factor for the cognitive impairment [39]. Other researchers also noticed that ghrelin levels in obesity patients was lower compared to age-matched controls health people [40], and they are negatively correlated with BMI [9,41].

Finally, we found that lower serum ghrelin levels were the independent factor associated with MCI in elderly diabetic patients in multivariate analysis. Higher levels of HbA1c, diagnosis of CVD and hypertension, increased number of co-morbidities and less years of education - all these factor are proven to contribute to cognitive impairment in diabetes [1,6]. Decreased ghrelin levels could be another predictor of diagnosis of MCI in elderly diabetic population.

Although our study provides important insights into the pathophysiology of cognition dysfunction in elderly diabetic patients; it does have limitations.

Firstly, the study population was relatively small, and the findings should be interpreted with caution.

Secondly, this investigation was limited to patients with diabetes, and therefore, an association between ghrelin levels with other parameters in subjects without diabetes should also be assessed.

Thirdly, the study wasn't designed as longitudinal investigation. Our findings indicate only an association between the coexistence of diabetes and MCI and the biochemical alterations found in this study. Further study is required to investigate the precise mechanisms underlying lower ghrelin levels and coexisting cognitive impairment in diabetic patients.

Conclusions

In summary, elderly diabetic patients with MCI were found to have lower serum ghrelin compared to controls. The multivariable logistic regression models revealed that variables which increased the likelihood of having been diagnosed with MCI were: lower levels of ghrelin, higher levels of HbA1c, diagnosis of CVD and hypertension, increased number of co-morbidities and less years of education. Various pathophysiological mechanisms like lower ghrelin levels**,** hyperglycemia, increased inflammation**,** oxidative stress, vacular pathology and some neurodegenerative disorders may underlie comorbid cognitive dysfunction and diabetes. Therefore further prospective larger studies are needed which can provide potential directions for research, treatment and prevention of these conditions.

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References

1. Gorska-Ciebiada M, Saryusz-Wolska M, Ciebiada M, *et al.* Mild cognitive impairment and depressive symptoms in elderly patients with diabetes- prevalence, risk factors and co-morbidity. *J. Diabetes. Res* 179648(1), 1-7 (2014).
2. Luchsinger JA, Reitz C, Patel B, *et al.* Relation of diabetes to mild cognitive impairment. *Arch. Neurol* 64(1), 570-575 (2007).
3. Cheng G, Huang C, Deng H, *et al.* Diabetes as a risk factor for dementia and mild cognitive impairment: a metaanalysis of longitudinal studies. *Intern. Med. J* 42(5), 484-491 (2012).
4. Rawlings AM, Sharrett AR, Schneider AL, *et al.* Diabetes in midlife and cognitive change over 20 years: a cohort study. *Ann. Intern. Med* 161(1), 785-793 (2014).
5. Petersen RC, Roberts RO, Knopman DS, *et al.* Mild cognitive impairment: ten years later. *Arch. Neurol* 66(12), 1447-1455 (2009).
6. Strachan MW, Reynolds RM, Marioni RE, *et al.* Cognitive function, dementia and type 2 diabetes mellitus in the elderly. *Nat. Rev. Endocrinol* 7(1), 108-14 (2011).
7. Gorska-Ciebiada M, Saryusz-Wolska M, Borkowska A, *et al.* Serum levels of inflammatory markers in depressed elderly patients with diabetes and mild cognitive impairment. *PLoS. One* 10(3), e0120433 (2015).
8. Munshi MN. Cognitive dysfunction in older adults with diabetes: what a clinician needs to know. *Diabetes. Care* 40(1), 461-467 (2017).
9. Solas M, Milagro FI, Ramírez MJ, *et al.* Inflammation and gut-brain axis link obesity to cognitive dysfunction: plausible pharmacological interventions. *Curr. Opin. Pharmacol* 37(1), 87-92 (2017).
10. Xu Y, Zhou H, Zhu Q. The Impact of Microbiota-Gut-Brain Axis on Diabetic Cognition Impairment. *Front. Aging. Neurosci* 9(1), 106 (2017).
11. Tillisch K, Labus J, Kilpatrick L, *et al.* Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology* 144 (1), 1394-1401 (2013).
12. Bajaj JS, Ridlon JM, Hylemon PB, *et al.* Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am. J. Physiol. Gastrointest. Liver. Physiol* 302(1), G168-G175 (2012).

13. Chunchai T, Thunapong W, Yasom S, *et al.* Decreased microglial activation through gut-brain axis by prebiotics, probiotics, or synbiotics effectively restored cognitive function in obese-insulin resistant rats. *J. Neuroinflammation* 15(1), 11 (2018).

14. Huang R, Han J, Tian S, *et al.* Association of plasma ghrelin levels and ghrelin rs4684677 polymorphism with mild cognitive impairment in type 2 diabetic patients. *Oncotarget* 8(9), 15126-15135 (2017).

15. Chen S, Zuo X, Li Y, *et al.* Ghrelin is a possible new predictor associated with executive function in patients with type 2 diabetes mellitus. *J. Diabetes. Investig* 8(3), 306-313 (2016)

16. Carlini VP, Varas MM, Cragnolini AB, *et al.* Differential role of the hippocampus, amygdala, and dorsal raphe nucleus in regulating feeding, memory, and anxiety-like behavioral responses to ghrelin. *Biochem. Biophys. Res. Commun* 313(1), 635-641 (2004).

17. van der Lely AJ, TschopM, HeimanML, *et al.* Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocr. Rev* 25(1), 426-457 (2004).

18. Williams J, Mobarhan S. A critical interaction: leptin and ghrelin. *Nutr. Rev* 61(11), 391-393 (2003).

19. Diano S, Farr SA, Benoit SC, *et al.* Ghrelin controls hippocampal spine synapse density and memory performance. *Nat. Neurosci* 9(1), 381-398 (2006).

20. Zhang G, Yin X, Qi Y, *et al.* Ghrelin and cardiovascular diseases. *Curr. Cardiol. Rev* 6(1), 62e70 (2010).

21. Vörös K, Prohászka Z, Kaszás E, *et al.* Serum ghrelin level and TNF-α/ghrelin ratio in patients with previous myocardial infarction. *Arch. Med. Res* 43(7), 548-554 (2012).

22. Yano Y, Toshinai K, Inokuchi T, *et al.* Plasma des-acyl ghrelin, but not plasma HMW adiponectin, is a useful cardiometabolic marker for predicting atherosclerosis in elderly hypertensive patients. *Atherosclerosis* 204(1), 590e594 (2009).

23. Kotani K, Sakane N, Saiga K, *et al.* Serum ghrelin and carotid atherosclerosis in older Japanese people with metabolic syndrome. *Arch. Med. Res* 37(1), 903e906 (2006).

24. Kadoglou NP, Lampropoulos S, Kapelouzou A, *et al.* Serum levels of apelin and ghrelin in patients with acute coronary syndromes and established coronary artery disease - KOZANI STUDY. *Transl. Res* 155(1), 238e246 (2010).

25. Kadoglou NP, Sailer N, Moumtzouoglou A, *et al.* Visfatin (nampt) and ghrelin as novel markers of carotid atherosclerosis in patients with type 2 diabetes. *Exp. Clin. Endocrinol. Diabetes* 118(2), 75-80 (2010).

26. Nasreddine ZS, Phillips NA, Bedirian V, *et al.* The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment, *J. Am. Geriatr. Soc* 53(4), 695-699 (2005).

27. Alagiakrishnan K, Zhao N, Mereu L, *et al.* Montreal Cognitive Assessment is superior to standardized Mini-Mental Status Exam in detecting Mild Cognitive Impairment in the middle-aged and elderly patients with type 2 diabetes mellitus. *Biomed. Res. Int* 2013(1), 186106 (2013).

28. Petersen RC. Mild cognitive impairment as a diagnostic entity *J. Intern. Med* 256,3:183-194 (2004).

29. Portet F, Ousset PJ, Visser PJ, *et al.* Mild cognitive impairment (MCI) in medical practice: a critical review of the concept and new diagnostic procedure. Report of the MCI Working Group of the European Consortium on Alzheimer's Disease. *J. Neurol. Neurosurg. Psychiatry* 77(6), 714-718 (2006).

30. Katz S, Downs TD, Cash HR, *et al.* Progress in development of the index of ADL. *Gerontologist* 10(1), 20-30 (1970).

31. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist* 9(3)179-86 (1969).

32. Yesavage J, Brink T, Rose T. Development and validation of a geriatric depression screening scale: A preliminary report. *J. Psychiatr. Res* 7(1), 37-49 (1983).

33. Kang S, Moon NR, Kim DS, *et al.* Central acylated ghrelin improves memory function and hippocampal AMPK activation and partly reverses the impairment of energy and glucose metabolism in rats infused with β-amyloid. *Peptides* 2015 71(1), 84-93.

34. Kunath N, van Groen T, Allison DB, *et al.* Ghrelin agonist does not foster insulin resistance but improves cognition in an Alzheimer's disease mouse model. *Sci. Rep* 19(5),11452 (2015).

35. Jiao Q, Du X, Li Y, *et al.* The neurological effects of ghrelin in brain diseases: Beyond metabolic functions. *Neurosci. Biobehav. Rev* 73(1), 98-111 (2017).

36. Gahete MD, Rubio A, Cordoba-Chacon J, *et al.* Expression of the ghrelin and neurotensin systems is altered in the temporal lobe of Alzheimer's disease patients. *J. Alzheimers. Dis* 22(1), 819-828 (2010).

37. Gorska-Ciebiada M, Saryusz-Wolska M, Borkowska A, *et al.* Serum Soluble Adhesion Molecules and Markers of Systemic Inflammation in Elderly Diabetic Patients with Mild Cognitive Impairment and Depressive Symptoms. *Biomed. Res. Int* 2015(1), 826180 (2015).

38. Schuitemaker A, Dik MG, Veerhuis R, *et al.* Inflammatory markers in AD and MCI patients with different biomarker profiles. *Neurobiol. Aging* 30(1),1885-1889 (2008).

39. Ryan CM, Geckle M. Why is learning and memory dysfunction in Type 2 diabetes limited to older adults? *Diabetes. Metab. Res. Rev* 16(1), 308-315 (2000).

40. Wali P, King J, He Z, *et al.* Ghrelin and obestatin levels in children with failure to thrive and obesity. *J. Pediatr. Gastroenterol. Nutr* 58(1), 376-381 (2014).

41. Nakahara T, Harada T, Yasuhara D, *et al.* Plasma obestatin concentrations are negatively correlated with body mass index, insulin resistance index, and plasma leptin concentrations in obesity and anorexia nervosa. *Biol. Psychiatry* 2008; 64(1), 252-255.