

# Ghrelin is a possible new predictor associated with executive function in patients with type 2 diabetes mellitus

Siting Chen<sup>1</sup>, Xuyang Zuo<sup>1</sup>, Yuan Li<sup>1</sup>, Tian Jiang<sup>1</sup>, Nan Zhang<sup>1</sup>, Fang Dai<sup>1</sup>, Qiaoer Chen<sup>2</sup>, Qiu Zhang<sup>1\*</sup>

<sup>1</sup>Department of Endocrinology, The First Affiliated Hospital of Anhui Medical University, and <sup>2</sup>College of Stomatology, Anhui Medical University, Hefei, Anhui, China

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## \*Correspondence

Qiu Zhang

Tel.: +86-551-62923631

Fax: +86-551-62923631

E-mail address:

zhangqiu611@sina.com

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## ABSTRACT

**Aims/Introduction:** The aim of the present research was to study the ghrelin level, executive function and their possible association in patients with type 2 diabetes mellitus.

**Materials and Methods:** A total of 370 people were recruited between March 2015 and March 2016 in this study. Among them, 212 participants were patients with type 2 diabetes mellitus and 158 participants were included as the control group. Their blood sample was analyzed for the level of ghrelin and other clinical indexes. Cognitive function was measured by the Montreal Cognitive Assessment, and executive function was evaluated by the Wisconsin Card Sorting Test.

**Results:** In the type 2 diabetes mellitus group, age, years of education, duration of diabetes, fasting blood glucose, glycated hemoglobin, hypertension and waist-to-hip ratio were correlated with total Montreal Cognitive Assessment scores. No association was found between ghrelin level and total Montreal Cognitive Assessment score in patients with type 2 diabetes mellitus. However, ghrelin was found to be a significant predictor for executive function impairment measured by the Wisconsin Card Sorting Test in patients with type 2 diabetes mellitus.

**Conclusions:** The level of serum ghrelin might be a biomarker of executive function and become a strong predictor of executive function impairment in patients with type 2 diabetes mellitus. Ghrelin might have a potential protective effect against cognitive function impairment in type 2 diabetes patients.

## INTRODUCTION

The prevalence of diabetes mellitus is estimated to continue increasing. Type 2 diabetes mellitus, which accounts for 90% of patients with diabetes, is characterized by insulin resistance and relative insulin deficiency<sup>1</sup>. The population with diabetes mellitus was estimated to reach 366 million in 2030, and 90–95% will have type 2 diabetes mellitus<sup>2</sup>. Previous studies have suggested that diabetes mellitus is associated with the decline of cognitive function, and might be a risk factor of the development of mild cognitive impairment (MCI)<sup>3–5</sup>. MCI is considered to be a potential transitional stage between normal cognitive function and Alzheimer's disease, characterized by: (i) subjective memory complaint; (ii) objective memory impairment; (iii) normal mental status; (iv) intact activities of daily living; and (v) absence of dementia<sup>6</sup>. The conversion rate from

MCI to dementia was 11.65% per year<sup>7</sup>. Alzheimer's disease is the most common type of dementia among elderly patients, accounting for approximately 50–60% of elderly patients with dementia<sup>8</sup>. Recent studies also showed that diabetes mellitus is a strong risk factor for Alzheimer's disease<sup>9–11</sup>. The high risk of MCI and dementia in patients with diabetes mellitus points to the need to observe predictors of cognitive impairment. However, the increased risk of cognitive impairment in type 2 diabetes mellitus is not fully explained by conventional predictors.

Executive function, which is generally considered to be a higher-level function that integrates and controls more basic cognitive processes<sup>12</sup>, is an important part of cognitive function, and has been investigated among diabetic patients. A growing group of evidence has shown that patients with diabetes mellitus are associated with an increased risk of executive function impairment than those without diabetes mellitus<sup>13–16</sup>. It is now recognized that even in diabetes patients with normal cognition

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that was evaluated using conventional cognitive function assessment tools, impaired executive function might have occurred<sup>17</sup>. Therefore, the present study included executive function assessment as a major component of the cognitive function evaluation.

Ghrelin is a circulating peptide hormone mainly secreted from the stomach<sup>18</sup>. The effects of ghrelin are mediated through the GH secretagogue receptor, which is widely distributed in the human body<sup>19</sup>. In recent years, ghrelin administration has been shown to influence the peripheral metabolism, especially the carbohydrate and lipid metabolism<sup>20</sup>. A large number of publications studying the relationship between ghrelin and insulin resistance or diabetic states have shown a correlation between ghrelin and insulin resistance or diabetes mellitus<sup>21</sup>. In addition, ghrelin is linked to neuromodulation, neuroprotection, and memory and learning processes<sup>22</sup>. The neuroprotective capacity of ghrelin has been reported by increasing tolerance of the brain tissues to cerebral ischemia/reperfusion injury<sup>23</sup>. Furthermore, it was found in several studies based on animal models that ghrelin took part in modulating specific molecular intermediates involved in memory acquisition and consolidation by promoting synaptic plasticity, and was associated with improved memory and learning<sup>24–26</sup>.

However, the relationship between ghrelin and cognitive function in humans has rarely been investigated. In 2010, it was reported for the first time that the level of local brain ghrelin production in AD patients was reduced when compared with age-matched control group<sup>27</sup>. In addition, some studies carried out in different groups found that a higher level of serum ghrelin was related to better cognitive function using different assessment scales<sup>28,29</sup>, but only a small number of studies carried out on diabetic animals have suggested that ghrelin is associated with cognitive function in several aspects<sup>30,31</sup>. This is the first study to evaluate the relationship between ghrelin and cognitive function in type 2 diabetes patients.

## MATERIALS AND METHODS

### Participants

This was a cross-sectional study that started in March 2015 and ended in March 2016 in the first affiliated hospital of Anhui Medical University, Anhui, China. All participants were Chinese, and were asked to provide written informed consent according to a protocol approved by the research ethics committee. Patients with type 2 diabetes mellitus were recruited from people who sought treatment for type 2 diabetes mellitus in the endocrine outpatient clinic between March 2015 and March 2016. Type 2 diabetes mellitus was defined according to the World Health Organization 1999 criteria<sup>32</sup>. Another age-matched group of participants without diabetes mellitus was randomly selected from the physical examination center in the first affiliated hospital of Anhui Medical University, Anhui, China.

All participants who met the following exclusion criteria were excluded from the present study: (i) diabetic ketoacidosis or other acute diabetic complications in the past 3 months; (ii)

severe hypoglycemia in the past 1 month; (iii) severe heart failure, chronic renal failure, lung disease or had a history of infection of the central nervous system, stroke, cerebral hemorrhage, or other clinical evidence of central nervous damage; (iv) history of auditory disorders or visual disorders that might interfere with the test; (v) history of alcohol abuse or drug abuse; (vi) history of depression or other psychiatric illness; or (vii) use of possible or known cognition-impairment drugs in the past month.

### Ethical approval

All procedures carried out in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, and conformed to the provisions of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013). All participants were asked to provide informed consent.

### Demographic and clinical characteristics

These included documentation of demographic data, detailed physical examination and the results of blood sample analysis. The process of collecting information including age, years of education, duration of diabetes, insulin and drug use, smoking, history of hypertension, history of hyperlipidemia, diet, physical activity, and alcohol drinking from participants was completed by several experienced doctors by a face-to-face interview. Body mass index was calculated as bodyweight (kg) divided by the height squared (meters). Hypertension was defined as systolic blood pressure  $\geq 140$  mmHg, diastolic blood pressure  $\geq 90$  mmHg or previously diagnosed as hypertension and already taking antihypertensive medications. Blood samples were obtained after a 10-h overnight fast to determine glycated hemoglobin (HbA1c; %), fasting blood glucose (FBG), triglyceride, total cholesterol, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol and high-density lipoprotein cholesterol. Fasting ghrelin concentration was measured from plasma samples stored at  $-80^{\circ}\text{C}$ . The concentration levels were determined using the enzyme-linked immunosorbent assay kit. All procedures were carried out by experienced laboratory assistants.

### Cognitive function assessment

The Montreal Cognitive Assessment (MoCA) was developed as a tool to assess cognitive function and is considered to acquire high sensitivity and specificity to detect MCI<sup>33</sup>. In the present study, we applied the Beijing version of the MoCA, the most widely used version of the MoCA in mainland China<sup>34</sup>. It assesses different cognitive domains: visuospatial/executive function, naming, attention, abstraction, language, delayed memory and orientation. Total scores range from 0 to 30, and higher scores in each subscale indicate better cognitive function.

### Executive function assessment

The Wisconsin Card Sorting Test (WCST), first introduced by Grant and Berg<sup>35</sup>, is a measure of executive function that

assesses the ability to form abstract concepts, shift and maintain sets, and solve problems in response to feedback. The participants were provided with four cards that were different in color, form and number. Then they were presented with a deck of stimulus cards and required to match each card to one of the four cards that they thought the most suitable. Through the process, the computer provided feedback to the participant, indicating whether their choices were correct or incorrect. Therefore the participants had to infer the principles only through the limited feedback from the computer.

In the present study, we included the following indices as measurements of executive function: total categories achieved, total responses, total correct responses, correct response percentage (%), total errors, error percentage (%), perseverative responses, perseverative response percentage (%), perseverative errors, perseverative error percentage (%), non-perseverative errors, non-perseverative error percentage (%) and number of trials to complete the first category. These raw scores of WCST are recommended for clinical interpretation and can reflect executive function of participants<sup>36</sup>.

### Statistical analysis

All statistical analysis was carried out by Spss Software version 19.0 (SPSS Inc., Chicago, Illinois, USA). Data were given as mean  $\pm$  SD or number (percentage) when appropriate. Data

with normal distributions were analyzed by Student's *t*-test, and those with non-normal distributions were analyzed by the Wilcoxon rank-sum test. The  $\chi^2$ -test was used to compare categorical data. The predictors of the MoCA scores and WCST indices in the type 2 diabetes mellitus group were determined by carrying out a stepwise multivariate linear regression analysis. For all of the tests,  $P < 0.05$  was considered statistically significant in the present study.

### RESULTS

Between March 2015 and March 2016, 370 people who met the criteria agreed to participate in the present study and were recruited. A total of 212 patients with type 2 diabetes mellitus (57.30% of the total sample) and 158 people in the control group (42.70% of the total sample) completed our study.

Table 1 shows the demographic and clinical characteristics, and the ghrelin level of the study population. As shown in Table 1, type 2 diabetic patients had a higher body mass index, waist-to-hip ratio, FBG, HbA1c, high-density lipoprotein cholesterol, low-density lipoprotein ( $P < 0.001$ ), and a higher proportion of hypertension and hyperlipidemia than the control group. The level of plasma ghrelin was significantly lower in the type 2 diabetic group than that in the control group ( $2.85 \pm 1.59$  vs  $6.74 \pm 2.13$   $\mu\text{g/L}$ ,  $P < 0.001$ ).

**Table 1** | Demographic and clinical characteristics of participants with and without type 2 diabetes mellitus

| Characteristics                    | T2DM group ( $n = 212$ ) | Control group ( $n = 158$ ) | <i>P</i> -value |
|------------------------------------|--------------------------|-----------------------------|-----------------|
| Age (years)                        | 48.21 $\pm$ 7.61         | 47.03 $\pm$ 8.20            | 0.16            |
| Male, $n$ (%)                      | 128 (60.38%)             | 88 (55.70%)                 | 0.37            |
| Years of education                 | 11.92 $\pm$ 2.88         | 12.23 $\pm$ 3.17            | 0.34            |
| Duration of diabetes (years)       | 4.87 $\pm$ 3.92          | /                           | /               |
| Insulin use, $n$ (%)               | 46 (21.70%)              | /                           | /               |
| Oral antidiabetic drugs, $n$ (%)   | 185 (87.26%)             | /                           | /               |
| Smoking, $n$ (%)                   | 96 (45.28%)              | 75 (47.47%)                 | 0.68            |
| History of hypertension, $n$ (%)   | 139 (65.57%)             | 70 (44.30%)                 | <0.001          |
| History of hyperlipidemia, $n$ (%) | 131 (61.79%)             | 58 (36.71%)                 | <0.001          |
| Regular physical activity, $n$ (%) | 82 (38.68%)              | 75 (47.47%)                 | 0.09            |
| Regular diet, $n$ (%)              | 184 (86.79%)             | 139 (87.97%)                | 0.74            |
| Habitual alcohol drinking, $n$ (%) | 27 (12.74%)              | 17 (10.76%)                 | 0.56            |
| BMI ( $\text{kg/m}^2$ )            | 25.23 $\pm$ 3.01         | 23.01 $\pm$ 1.12            | <0.001          |
| Waist-to-hip ratio                 | 0.87 $\pm$ 0.04          | 0.84 $\pm$ 0.03             | <0.001          |
| FBG (mmol/L)                       | 9.27 $\pm$ 1.11          | 5.06 $\pm$ 0.41             | <0.001          |
| HbA1c (%)                          | 8.04 $\pm$ 1.63          | 5.13 $\pm$ 0.32             | <0.001          |
| TG (mmol/L)                        | 1.55 $\pm$ 0.73          | 1.42 $\pm$ 0.81             | 0.12            |
| TCH (mmol/L)                       | 4.65 $\pm$ 1.03          | 4.58 $\pm$ 0.86             | 0.46            |
| HDL-C (mmol/L)                     | 1.27 $\pm$ 0.35          | 1.61 $\pm$ 0.46             | <0.001          |
| LDL-C (mmol/L)                     | 2.77 $\pm$ 0.86          | 2.33 $\pm$ 0.44             | <0.001          |
| VLDL-C (mmol/L)                    | 0.45 $\pm$ 0.12          | 0.43 $\pm$ 0.12             | 0.12            |
| Ghrelin ( $\mu\text{g/L}$ )        | 2.85 $\pm$ 1.59          | 6.74 $\pm$ 2.13             | <0.001          |

Data are presented as  $n$  (%), mean  $\pm$  SD, as appropriate. BMI, body mass index; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T2DM, type 2 diabetes mellitus; TCH, total cholesterol; TG, triglyceride; VLDL-C, very low-density lipoprotein cholesterol.

Table 2 reports the results of the assessment of cognitive function (measured by MoCA) and executive function (measured by WCST) in the type 2 diabetes group and control group. Significant differences were observed in the total MoCA score ( $P < 0.001$ ) and seven subscales, namely, visuospatial/executive function ( $P < 0.001$ ), naming ( $P < 0.026$ ), attention ( $P < 0.001$ ), abstraction ( $P < 0.001$ ), language ( $P < 0.001$ ), delayed memory ( $P < 0.001$ ) and orientation ( $P < 0.047$ ) between the type 2 diabetes group and control group. Compared with the control group, the performance of the WCST in the type 2 diabetes group was worse. Higher scores of total categories achieved and correct response percentage indicate better executive function. Higher raw scores of other items indicate worse executive function. Details are shown in Table 2.

In the stepwise multivariate linear regression model carried out in the type 2 diabetes mellitus group, the independent variables included were ghrelin, age, years of education, duration of diabetes, smoking, hypertension, hyperlipidemia, physical activity, diet, alcohol drinking, body mass index, waist-to-hip ratio, FBG, HbA1c, triglyceride, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, insulin therapy and oral antidiabetic medications. As shown in Table 3, age, duration of diabetes, years of education, FBG, HbA1c, hypertension and waist-to-hip ratio was correlated with total MoCA scores. No

association was found between total MoCA score and ghrelin level of type 2 diabetes patients. Specifically, we analyzed the possible predictors of visuospatial/executive function and delayed memory subscales of the MoCA scales. In the stepwise multivariate linear regression model, ghrelin is a predictor of visuospatial/executive function (standardized  $\beta = 1.212$ ,  $P = 0.036$ ) and delayed memory (standardized  $\beta = 0.743$ ,  $P = 0.021$ ).

Predictors of executive function in the stepwise multivariate linear regression model for each of the indices of the WCST in

**Table 3** | Significant predictors of cognitive function in the Montreal Cognitive Assessment in a stepwise multivariate linear regression model in the type 2 diabetes mellitus group

|            | Predictors           | Standardized $\beta$ | P-value |
|------------|----------------------|----------------------|---------|
| MoCA score | Age                  | -0.146               | 0.002   |
|            | Years of education   | 0.203                | <0.001  |
|            | Duration of diabetes | -0.255               | 0.028   |
|            | FBG                  | -0.158               | 0.007   |
|            | HbA1c                | -0.304               | <0.001  |
|            | Hypertension         | -0.119               | 0.016   |
|            | Waist-to-hip ratio   | -0.217               | 0.013   |

FBG, fasting blood glucose; HbA1c, glycated hemoglobin; MoCA, Montreal Cognitive Assessment; T2DM, type 2 diabetes mellitus.

**Table 2** | Montreal Cognitive Assessment scores and Wisconsin Card Sorting Test indices of participants with and without type 2 diabetes mellitus

| Characteristic                            | T2DM group (n = 212) | Control group (n = 158) | P-value |
|---|----------------------|-------------------------|---------|
| <b>MoCA</b>                               |                      |                         |         |
| MoCA total score                          | 24.12 ± 2.28         | 27.36 ± 1.49            | <0.001  |
| Visuospatial/executive function           | 3.25 ± 0.88          | 4.13 ± 0.63             | <0.001  |
| Naming                                    | 2.57 ± 0.41          | 2.62 ± 0.15             | 0.026   |
| Attention                                 | 4.78 ± 0.43          | 5.88 ± 0.21             | <0.001  |
| Abstraction                               | 1.56 ± 0.37          | 1.91 ± 0.17             | <0.001  |
| Language                                  | 2.59 ± 0.31          | 2.95 ± 0.18             | <0.001  |
| Delayed memory                            | 3.65 ± 0.92          | 4.12 ± 0.74             | <0.001  |
| Orientation                               | 5.72 ± 0.24          | 5.75 ± 0.11             | 0.047   |
| <b>WCST</b>                               |                      |                         |         |
| Total categories achieved                 | 4.72 ± 1.63          | 5.89 ± 0.67             | <0.001  |
| Total responses                           | 121.68 ± 17.34       | 91.34 ± 21.59           | <0.001  |
| Total correct responses                   | 75.80 ± 10.56        | 72.38 ± 8.45            | <0.001  |
| Correct response percentage (%)           | 63.26 ± 13.75        | 79.24 ± 9.93            | <0.001  |
| Total errors                              | 41.30 ± 17.28        | 22.25 ± 16.59           | <0.001  |
| Error percentage (%)                      | 34.54 ± 12.90        | 23.34 ± 11.91           | <0.001  |
| Perseverative responses                   | 43.63 ± 12.01        | 31.65 ± 11.23           | <0.001  |
| Perseverative response percentage (%)     | 39.47 ± 7.83         | 29.69 ± 6.74            | <0.001  |
| Perseverative errors                      | 26.42 ± 11.87        | 11.36 ± 8.98            | <0.001  |
| Perseverative error percentage (%)        | 23.54 ± 7.29         | 12.14 ± 6.84            | <0.001  |
| Non-perseverative errors                  | 15.25 ± 7.62         | 9.68 ± 9.01             | <0.001  |
| Non-perseverative error percentage (%)    | 13.23 ± 5.33         | 10.24 ± 7.77            | <0.001  |
| No. trials to complete the first category | 26.76 ± 12.1         | 14.01 ± 8.29            | <0.001  |

Data are presented as mean ± SD, as appropriate. MoCA, Montreal Cognitive Assessment; T2DM, type 2 diabetes mellitus; WCST, Wisconsin Card Sorting Test.

the type 2 diabetes mellitus group are shown in Table 4. Ghrelin is a significant predictor associated with executive function in type 2 diabetes mellitus patients.

## DISCUSSION

A small amount of studies carried out on diabetic animals have suggested that ghrelin is associated with cognitive function in several aspects<sup>30,31</sup>. However, this is the first study to investigate the relationship between ghrelin and cognitive function in type 2 diabetic patients.

The role of ghrelin in type 2 diabetes mellitus patients has been studied by many experts. In 2003, Poykko *et al.*<sup>37</sup> first explored the fasting plasma ghrelin concentrations in a randomly selected sample of subjects, and found that low ghrelin is independently associated with type 2 diabetes mellitus, insulin concentration and insulin resistance. They speculated that the role of ghrelin might contribute to the pathogenesis of type 2 diabetes mellitus. Similarly, in the present study, the type 2 diabetes mellitus group had a lower level of serum ghrelin compared with the control group, and the difference was statistically significant ( $P < 0.001$ ). On the contrary, the result of a prospective study carried out by Bennett *et al.*<sup>38</sup> showed that no association between the baseline ghrelin level and the incidence of type 2 diabetes mellitus was found. Based on current knowledge, it is difficult to identify the exact mechanism regarding the association between low ghrelin concentration and the increased prevalence of type 2 diabetes. Several clinical studies have attempted to identify the relationship between serum ghrelin, glucose and insulin<sup>39–41</sup>, but the physiological interplay of them still remains unclear. Furthermore, most of the previous research concentrated on addressing the relationship between ghrelin and glucose metabolism, and insulin in the short-term. Studies on the long-term physiological effects of ghrelin on insulin and glucose metabolism are required.

It was reported in the present study that a decrease in cognitive function was found to be a significant problem in the type 2 diabetes mellitus population, with a mean MoCA score of  $24.12 \pm 2.28$  compared with  $27.36 \pm 1.49$  in the control group. As some recent research has shown<sup>42,43</sup>, possible mild cognitive deterioration has been observed in diabetic patients, which is compatible with the present results. In general, the majority of research assessing cognitive function in diabetic populations has focused only on overall performance of cognitive function, failing to offer an insight into different combinations in detail, which might lead to inaccurate consequences. Therefore, we took executive function, a sensitive domain of cognitive function, which might have been impaired when the cognitive function was normal<sup>17</sup> and might show decrement in the prediabetic stage<sup>44</sup>, into consideration. The results of the present study showed that the performance of executive function measured by the WCST in the type 2 diabetes mellitus group was lower than that in the control group. In accordance with our study, Watari *et al.*<sup>45</sup> reported in 2006 that there was a significant difference in executive function between the diabetic

and control group, which is similar to the result of the present study. In addition, Ruis *et al.*<sup>16</sup> found that patients in the early stage of type 2 diabetes mellitus performed significantly worse in executive function. Similarly, Berg *et al.*<sup>14</sup> concluded that type 2 diabetes mellitus is strongly associated with functioning of the brain, and type 2 diabetes mellitus increases memory deficit and reduces executive function.

In recent decades, many studies have shown that ghrelin-mediated signaling plays an important role in cognitive function, especially in memory function<sup>25,46</sup> and learning processes<sup>47,48</sup>, and have suggested that increasing ghrelin leads to better cognitive function. A few studies have investigated the relationship between ghrelin and cognitive function in diabetic animals. In a group of 36 streptozotocin diabetic rat models, Ma *et al.*<sup>30</sup> suggested that ghrelin improves cognitive function in streptozotocin-induced diabetic rats by improving the expressions of brain-derived neurotrophic factor and cyclic adenosine monophosphate response element binding protein, and by attenuating hippocampus neuronal apoptosis and the effects of ghrelin depend on the receptor of ghrelin, GHSR-1a, and the ERK1/2 pathway. Ghrelin was also reported to alleviate learning and memory dysfunction in diabetic rats, possibly by downregulating the expressions of DKK-1 and activating the WNT signaling pathways<sup>31</sup>.

The present results suggested that there was no significant association between overall cognitive function and serum ghrelin in the type 2 diabetes mellitus group, which is not consistent with previous studies of animals. Interestingly, in a study of serum ghrelin and cognitive function in a cohort of nondemented older adults, Spitznagel *et al.*<sup>49</sup> found that serum ghrelin was inversely associated with cognitive function in the sample. They concluded that alterations in ghrelin levels might precede cognitive decline associated with Alzheimer's disease as protection. In the present study, of note, the average MoCA score of the type 2 diabetes mellitus group was  $25.30 \pm 2.28$ , which means the type 2 diabetes patients in our study included both MCI and no MCI patients, and the relationship between cognitive function and ghrelin was investigated within them. Therefore, we speculate that the difference between the present results and previous studies might be attributable to the discrepancy of the role of ghrelin in the cognitive function of patients with MCI and patients with normal cognitive function. In addition, the present results reported that ghrelin concentration was remarkably positively correlated with executive function among the type 2 diabetes mellitus population using WCST as an assessment tool. Executive dysfunction is a sensitive indication of diabetic cognitive decline that might be impaired when cognitive function is normal<sup>17</sup>. In some studies, executive dysfunction was manifested in the early-stage of diabetes, and it might occur in the pre-diabetic stage<sup>44</sup>. Therefore, the level of serum ghrelin might be a biomarker of executive function and become a strong predictor of executive function impairment in patients with type 2 diabetes mellitus. Consistent with the findings from previous studies, the present results

**Table 4** | Significant predictors of executive function in the Wisconsin Card Sorting Test in a stepwise multivariate linear regression model in the type 2 diabetes mellitus group

| WCST                                      | Predictors           | Standardized $\beta$ | P-value |
|---|----------------------|----------------------|---------|
| Total categories achieved                 | HbA1c                | -0.231               | <0.001  |
|   | Years of education   | 0.415                | 0.003   |
| Total responses                           | Ghrelin              | -2.563               | 0.005   |
|   | Age                  | 0.423                | 0.024   |
|   | BMI                  | 0.537                | 0.018   |
|   | Years of education   | -1.078               | 0.004   |
| Total correct responses                   | HbA1c                | 1.346                | <0.001  |
|   | Ghrelin              | -1.015               | <0.001  |
| Correct response percentage (%)           | Ghrelin              | 1.007                | 0.013   |
|   | Age                  | -1.259               | 0.016   |
|   | Years of education   | 1.465                | <0.001  |
|   | HbA1c                | -2.136               | 0.006   |
| Total errors                              | Ghrelin              | -1.455               | <0.001  |
|   | Age                  | 0.176                | 0.023   |
|   | Duration of diabetes | 0.325                | 0.005   |
|   | Years of education   | -1.781               | <0.001  |
|   | HbA1c                | 2.104                | <0.001  |
|   | Hypertension         | 0.761                | 0.031   |
|   | Ghrelin              | -0.663               | 0.005   |
| Error percentage (%)                      | Age                  | 1.397                | 0.024   |
|   | Duration of diabetes | 0.963                | 0.012   |
|   | Years of education   | -0.789               | 0.008   |
|   | HbA1c                | 0.176                | <0.001  |
|   | Hypertension         | 0.674                | 0.017   |
|   | Ghrelin              | -2.031               | <0.001  |
|   | Years of education   | -1.169               | 0.003   |
| Perseverative responses                   | HbA1c                | 1.426                | <0.001  |
|   | Waist-to-hip ratio   | 2.312                | 0.012   |
|   | Ghrelin              | -1.906               | <0.001  |
|   | Years of education   | -0.384               | 0.002   |
|   | HbA1c                | 0.565                | <0.001  |
| Perseverative response percentage (%)     | Waist-to-hip ratio   | 1.981                | 0.041   |
|   | Ghrelin              | -1.253               | <0.001  |
|   | Duration of diabetes | 0.729                | 0.041   |
|   | Years of education   | -1.085               | 0.016   |
| Perseverative errors                      | HbA1c                | 1.873                | <0.001  |
|   | Ghrelin              | -0.412               | <0.001  |
|   | Duration of diabetes | 0.294                | 0.002   |
|   | Years of education   | -0.367               | 0.037   |
|   | HbA1c                | 0.753                | <0.001  |
| Perseverative error percentage (%)        | Waist-to-hip ratio   | 1.276                | 0.019   |
|   | Ghrelin              | -0.132               | 0.004   |
|   | Age                  | 0.478                | 0.028   |
|   | Years of education   | -0.365               | 0.002   |
| Non-perseverative errors                  | Hypertension         | 0.709                | 0.014   |
|   | Ghrelin              | -0.343               | 0.019   |
|   | Age                  | 0.127                | 0.028   |
|   | Years of education   | -0.659               | 0.004   |
| Non-perseverative error percentage (%)    | Hypertension         | 0.682                | 0.006   |
|   | Ghrelin              | -0.873               | <0.001  |
|   | Age                  | 0.164                | 0.048   |
| No. trials to complete the first category | Years of education   | -1.009               | <0.001  |
|   | HbA1c                | 1.275                | <0.001  |
|   | Ghrelin              | -0.873               | <0.001  |

BMI, body mass index; HbA1c, glycated hemoglobin; T2DM, type 2 diabetes mellitus; WCST, Wisconsin Card Sorting Test.

implied that ghrelin might have a potential protective effect against cognitive function impairment in patients with diabetes.

There were several limitations to the present study. First, as we applied the MoCA and WCST as the only assessment tools to evaluate the cognitive and executive function of the participants in our study, more accurate results would be obtained if more evaluation methods were included. Second, the relationships between ghrelin and cognitive function, and executive function, respectively, were studied among a type 2 diabetes population that included people with both MCI and no MCI. More research is required to study the relationship between ghrelin, cognitive function and executive function in the MCI and no MCI group separately. The third limitation of the present study was its cross-sectional nature, and further studies of longitudinal data are required to extend the results.

In conclusion, ghrelin concentration is positively associated with executive function in patients with type 2 diabetes mellitus. The level of serum ghrelin might be a biomarker of executive function and become a strong predictor of executive function impairment in patients with type 2 diabetes mellitus. Ghrelin might have a potential protective effect against cognitive function impairment in type 2 diabetes patients.

## DISCLOSURE

The authors declare no conflict of interest.

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