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Chronic Administration of Ellagic Acid Improved the Cognition in Male Middle-aged Overweight

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Abstract

This study aims to investigate whether ellagic acid has beneficial effects on cognitive deficits in middle-aged overweight individuals and the possible mechanism. A total of 150 middle-aged male participants, including 76 normal weight and 74 overweight, aged between 45 to 55 years were recruited for this study. Both normal weight and overweight participants were administered either 50 mg ellagic acid or placebo cellulose daily for 12 weeks. Blood lipids, peripheral brain-derived neurotrophic factor (BDNF) and saliva cortisol were assessed on the last day of the procedure to investigate the effects induced by ellagic acid. The results revealed that ellagic acid treatment improved the levels of blood lipid metabolism with a 4.7% decline in total cholesterol, 7.3% decline in triglycerides, 26.5% increase in high-density lipoprotein and 6.5% decline in low-density lipoprotein. Additionally, ellagic acid increased plasma BDNF by 21.2% in the overweight group and showed no effects on normal weight participants. Moreover, the increased saliva cortisol level in overweight individuals was inhibited by 22.7% in a 12-week ellagic acid treatment. Also, compared with placebo, overweight individuals who consumed ellagic acid showed enhanced cognitive function as measured by the Wechsler Adult Intelligence Scale-Revised and the Montreal cognitive assessment. To the best of our knowledge, this is the first report showing that ellagic acid prevents cognitive deficits through normalization of lipid metabolism, increase in plasma BDNF level and reduction of saliva cortisol concentration. These results indicate that ellagic acid has a potential to restore cognitive performance related to mild age-related declines.

Key words: Ellagic Acid, Midlife Overweight, Cognition, Lipoprotein, Cholesterol, Brain-Derived Neurotrophic Factor, Cortisol
Introduction

Being overweight in middle age increases the risk for cognitive decline and is especially associated with increased dementia risk in old age (Gorospe and Dave, 2007; Rosengren et al., 2005; Whitmer et al., 2008; Hassing et al., 2009). There is growing evidence suggesting that high blood cholesterol, especially high cholesterol concentrations as well as reduced high-density lipoproteins (HDL) cholesterol level in midlife are associated with cognitive impairment and dementia in later life (EPOD Evaluation, 2001; Kaffashian et al., 2011; Kivipelto et al., 2001; Whitmer et al., 2005). A recent cross-sectional study in aging women revealed that increased HDL level was significantly associated with better verbal learning and memory performance, suggesting a protective effect of HDL on cognitive function in the aging brain (Bates et al., 2017). In addition, serum lipoprotein levels, including higher total cholesterol (TC) and low-density lipoproteins (LDL), have been reported to be associated with cognitive impairment in postmenopausal women without dementia (Yaffe et al., 2002). Also, several meta-analyses have found that green tea, which has the property of lowering plasma TC and LDL levels, is also associated with decreased risk of cognitive decline in the elderly (Ma et al., 2016; Yuan et al., 2017). Moreover, consistently, physical exercise training with weight loss was found to produce the most positive effects on cognitive function in older adults (Ortmeyer et al., 2017; Saez de Asteasu et al., 2017).

Stress-induced, especially chronic stress-induced, activation of the hypothalamic-pituitary-adrenal (HPA) axis and the release of glucocorticoid hormones (cortisol in humans; corticosterone in rodents), has been linked with overweight and obesity. For instance, a large-scale study, including 5077 male and female participants, found that chronic psychosocial stressors resulted in high total energy intake and overweight, suggesting that stress control may be an effective strategy in obesity prevention (Isasi et al., 2015). Also, a prospective community cohort study, 6-month follow-up, showed that higher cortisol and chronic stress were predictive of more food cravings and greater future weight gain (Chao et al., 2017). Accordingly, it has been proposed that cortisol responsiveness may be used as a marker to identify individuals at risk of weight gain and subsequent obesity (Hewagalamulage et al., 2016).
Available evidence showed that glucocorticoid hormones and stressful events induce negative effects on cognitive performance (Roozendaal, 2002; de Quervain et al., 2009). The cognitive deficits triggered by chronic stress are generally believed to lead to the long-lasting strain on hippocampal function and morphology (McEwen, 2001; Sapolsky, 2000). The hippocampus is a key brain region for learning and memory and is also sensitive to long-term stress and high glucocorticoids-induced damage (Ohl et al., 2000; Kim et al., 2006). Also, chronic stress not only impaired the memory, but also decreased hippocampal synaptic plasticity and reduced brain-derived neurotrophic factor (BDNF) (Smith et al., 1995; Nibuya et al., 1999). In addition, it has been shown that greater duration of exercise was associated with significant increase in BDNF concentration in the peripheral blood and improved memory and spatial cognition in healthy adults (Dinoff et al., 2017; Rogge et al., 2017). Therefore, interventions that focus on the upregulation of BDNF have been considered to be potential approaches for the reversal of stress-induced cognitive impairment.

Ellagic acid is a low-molecular-weight polyphenol derived from fruits (pomegranates, cranberries, vacciniums, broccoli), vegetables (vacciniums, broccoli), and nuts (walnut, terminalia fruit)(de Oliveira, 2016). Ellagic acid exhibits analgesic characteristics and induces anti-inflammatory responses in various mammalian tissues (Liu et al., 2017). Previous evidence showed that ellagic acid, which is the active compounds of the medicine derived from the terminalia fruit, exerted significant neuroprotective effect on the middle cerebral artery occlusion/reperfusion (MCAO/R) in rats (Liu et al., 2017). These results reveal new insights about the underlying protective mechanisms of ellagic acid in brain cells. Although ellagic acid has been identified as a possible neuroprotective agent, there has been minimal in vivo research conducted to explore this possibility. Accordingly, whether ellagic acid produces beneficial effects on cognitive deficits in middle-aged overweight individuals and the possible mechanism involved remain to be explored. For the purpose of this investigation, we will focus on lipid metabolism, neurotrophic factors and the stress hormone cortisol to determine the potentially beneficial effect of ellagic acid on cognitive deficits in middle-aged subjects.

**Experimental procedures**
Study design

This study was a 12-week, randomized, double-blind, placebo-controlled clinical trial. Participants were recruited through online advertisements between March and December 2016 in Qiqihar China. The informed written consent form was individually signed by each participant. Both normal weight and overweight participants were randomly and equally allocated into two groups (placebo and ellagic acid). Both ellagic acid and placebo capsules were packed in identical containers labelled with the participant’s code and allocated according to the order of enrolment of the participant in the study. The trial protocol was approved by the Human Research Ethics Committee at Qiqihar Medical University (No. Q201615).

Participants

A total of 150 male participants aged 45-55 years with a BMI of 18.5-24.9 for the normal weight control group (76 individuals) and a BMI of 25-29.9 for the overweight group (74 individuals) were enrolled in the study. All the participants are nonsmokers without diabetes, cardiovascular disease, hypertension, cancer, psychotic disorder, bipolar disorder, and any substance abuse or dependence disorder. Age is a key factor that induces cognitive decline, so the age range is strictly limited in the current study. The overweight status also affected the cognitive function. Accordingly, we enrolled the participants with the onset of overweight within 1 to 6 years and with stable weight (±2.5 kg) during the last 3 months.

Interventions

Placebo (cellulose) and ellagic acid (98%) capsules were prepared in the lab of the National Research Center of Chinese Minority Medicine of Minzu University of China and were identical by High Pressure Liquid Chromatography (HPLC) analysis. Ellagic acid was given in a 50 mg dose administered in a capsule containing the ellagic acid. Participants were directed to take one capsule, once daily with or without food for 12 weeks.

Outcomes

Bodyweight and waist circumference measurement

Bodyweight (kg), height (m) and waist circumference (cm) of each participant was measured at 8:00-10:00 am in the morning without breakfast at baseline level and at the end of the experiment. BMI was calculated as weight divided by height $^2$ (kg/m$^2$).
Blood collection

Venous blood samples were collected at the start of week 0 as baseline level and at the end of week 16. Subjects arrived at the experimental center in the morning after overnight fast. For the BDNF measurement, the blood was collected 1 h after breakfast. Subjects rested for 15 minutes before blood samples were drawn. Blood samples were withdrawn by venipuncture into vacuum tubes containing ethylene diamine tetraacetic acid (EDTA). All the blood samples were centrifuged at 4°C for 10 minutes at 3,000g, and aliquots of plasma were stored at −80°C until further analysis.

Plasma lipid

Plasma total cholesterol (TC), HDL cholesterol, and triglycerides (TG) concentrations were measured in duplicate using an oxidase method on a COBAS analyzer (Roche Diagnostics System, Tokyo, Japan), and LDL cholesterol was estimated using the Friedewald formula (Friedewald et al., 1972). The TC/HDL and LDL/HDL ratios were calculated by dividing TC and LDL by HDL, respectively.

Measurement of plasma BDNF levels

Plasma BDNF levels were measured using an ELISA assay Kit, according to the manufacturer’s instructions (Fitzgerald, No. 55Rm1556; Fitzgerald, MA, USA) and a previous report (Ruiz de Azua et al., 2013). Briefly, plasma samples (diluted 1:100) and serial dilutions of the BDNF standards were incubated for 24 h in 96-well immunoassay plates coated with mouse anti-human BDNF monoclonal antibody at 4°C. The plates were then washed and a biotinylated mouse anti-human BDNF monoclonal antibody (1:1000) was added to each well and incubated for 3 h at room temperature. After washing, a streptavidin-enzyme conjugate (1:1000) was added and incubated at room temperature for 1 hour. After washing, a substrate solution was added to the wells to initiate a reaction, which was stopped after 15 min by adding the stop solution (HCl). The amount of BDNF was determined immediately by measuring absorbance at 450 nm using a microplate reader. The standard curve demonstrated a direct relationship between optical density and the BDNF concentration.

Measurement of salivary cortisol levels
Saliva was collected from each participant using Salivette collection devices (Sarstedt, Nümbrecht, Germany) after the final behavioral measurement. The samples were stored at 4°C for 1–4 days and then frozen at -20°C. Free cortisol levels were detected using a radioimmunoassay kit (Immuno-Biological Laboratories, Furui Company, Beijing, China). The intra-assay and inter-assay coefficients of variation were less than 5% and 10%, respectively.

**Cognitive assessment**

All subjects underwent comprehensive neuropsychological tests, including assessment of intelligence, memory, learning and executive function. The Wechsler Adult Intelligence Scale-Revised (WAIS-R) was used to assess general cognitive competence, indicated as the IQ value (Wechsler, 2008). Additionally, the Chinese version of the Montreal cognitive assessment (MoCA), which is a one-page 30-point test, was administered in 10 minutes according to its developer (Nasreddine et al., 2005). The detailed procedure of MoCA is as follows: Multiple aspects of executive functions are assessed using an alternation task (1 to A to 2...E; 1 point), a phonemic fluency task (more than 11 words in 1 minute; 1 point), and a two-item verbal abstraction task (2 points). The short-term memory recall task (5 points) involves two learning trials of five nouns and delayed recall after approximately 5 minutes. Visuospatial abilities are assessed using a clock-drawing task (11:10; 3 points) and a three-dimensional cube copy (1 point). Attention, concentration, and working memory are evaluated using a sustained attention task (target detection using tapping; 1 point), a serial subtraction task (100-7-7-7-7-7; 3 points), and digits forward and backward (21854 and 742; 1 point each). Language is assessed using a three-item confrontation naming task with low-familiarity animals (lion, camel, rhinoceros; 3 points), repetition of two syntactically complex sentences (I just know that Ming Li is the one who did me a favor today; When the dog is in the room, the cat is always hiding under the couch; 2 points), and the aforementioned fluency task. Finally, orientation to time and place is evaluated (6 points).

**Statistical analysis**

Data are presented as the mean ± SD. The statistical analyses of cognition, blood and salivary measurements in the ellagic acid and placebo-treated subjects were performed using one- or two-way analysis of variance (ANOVA). Values of $P<0.05$ were considered statistically significant.
Results

The baseline characteristics of participants

We initially recruited 165 participants, each of which received a physical examination, routine hematologic and biochemical tests, urine toxicology measurements, and an electrocardiogram (ECG) to detect unstable medical illness or substance use. Eventually, 15 subjects were excluded for not meeting the inclusion criteria, and the remaining 150 subjects were divided into two groups according to their BMI: normal weight (BMI: 18.5-24.9, n=76) and overweight (BMI 25-29.9, n=74) as shown in the diagram of the experimental procedure in Figure 1. There were no significant differences in age and education between the groups. The cognitive function in both normal weight and overweight adults as measured by both WAIS-R IQ and MoCA baseline scores.

Chronic ellagic acid treatment improved lipid metabolism in middle-aged overweight individuals

Since abnormal blood lipids were reported in cognitive decline of overweight adults (Kivipelto et al., 2001; Whitmer et al., 2005), we investigated the effect of ellagic acid on lipid metabolism regulation in both normal weight and overweight conditions. The results showed that the TC, TG and LDL levels, as well as the TC/HDL and LDL/HDL values, were significantly increased, while the HDL level was decreased in overweight adults compared to normal weight participants (Figure 2A-F). However, ellagic acid reduced TC (P<0.01), TG (P<0.01), LDL (P<0.01), TC/HDL (P<0.01) and LDL/HDL (P<0.01), and increased HDL (P<0.01) in overweight individuals compared with placebo at the end of 12-week treatment, followed by Post hoc analysis. Ellagic acid did not alter the levels of TC, TG, LDL, HDL, TC/HDL and LDL/HDL in normal weight group, suggesting that the improved blood lipids levels are associated with lower bodyweight.

Chronic ellagic acid treatment increased plasma BDNF in middle-aged overweight individuals

In order to validate the possible link between the effect of ellagic acid on cognitive function and its regulatory effect on peripheral BDNF, we compared the level of serum BDNF in the participants treated with the placebo and those treated with ellagic acid in both the normal weight
and overweight groups. The two-way ANOVA test revealed a significant effect of overweight
(F_{1,138}=93.42, P<0.01), ellagic acid (F_{1,138}=16.82, P<0.01), and the interaction between overweight
and ellagic acid on the BDNF level (F_{1,138}=25.29, P<0.01) (Figure 3). Decreased BDNF level was
found in blood samples from overweight subjects compared to normal weight subjects (P<0.01).
However, ellagic acid treatment increased plasma BDNF in overweight adults (P<0.01), whereas
it had no effects on BDNF levels in the normal weight groups.

Chronic ellagic acid treatment decreased saliva cortisol in middle-aged overweight
individuals
The saliva cortisol was used to evaluate the effects of 12-week ellagic acid treatment on stress
regulation. We found that the saliva cortisol level was significantly increased in overweight
individuals compared with normal weight participants. Conversely, the increased cortisol was
inhibited by 12-week ellagic acid treatment in the overweight group relative to the placebo-treated
overweight adults. The two-way ANOVA test indicated a significant effect of overweight
(F_{1,138}=125.29, P<0.01), ellagic acid (F_{1,138}=28.27, P<0.01), and the interaction between
overweight and ellagic acid (F_{1,138}=48.54, P<0.01) (Figure 4). These findings revealed that the
activated HPA function in overweight individuals could be restored by chronic administration of
ellagic acid, as indicated by the reduced saliva cortisol concentrations.

Chronic ellagic acid treatment improved cognitive performance in middle-aged overweight
individuals
Aiming to determine whether chronic ellagic acid administration could reverse the cognitive
decline, we measured the cognitive performance with the WAIS-R IQ and MoCA scores. The
results revealed that 12-week ellagic acid treatment could reverse the overweight-induced
cognitive decline in both the WAIS-R test (Figure 5A) and MoCA task (Figure 5B). The two-way
ANOVA test revealed a significant effect of overweight (F_{1,138}=19.62, P<0.01), ellagic acid
(F_{1,138}=4.19, P<0.05), and the interaction between overweight and ellagic acid (F_{1,138}=6.36,
P<0.05), on the WAIS-R test scores (Figure 5A). For the MoCA scores, the two-way ANOVA test
also showed a significant effect of overweight (F_{1,138}=23.71, P<0.01), ellagic acid (F_{1,138}=6.51,
$P<0.05$), and the interaction between overweight and ellagic acid ($F_{1,138}=23.19$, $P<0.01$) (Fig. 5B).

No differences in the WAIS-R IQ and MoCA scores were observed between the placebo and ellagic acid groups in normal weight subjects (Figure 5A, B).

**Discussion**

In this study, we aimed at elucidating the putative effects of ellagic acid on cognitive improvements in middle-aged overweight individuals. Our data clearly demonstrate that: (1) Chronic administration of ellagic acid can restore the cognitive decline to that level seen in normal weight individuals of the same age; (2) Chronic ellagic acid treatment improved lipid metabolism in middle-aged overweight individuals; (3) Chronic ellagic acid treatment increased plasma BDNF and decreased saliva cortisol, and these effects are associated with normal cognitive performance. These data provided new support for the clinical improvement of cognition achieved by 12-week treatment of middle-aged overweight individuals with ellagic acid.

Cognitive impairment is common and a leading cause of disability in late life as demonstrated by an intermediate state of cognitive function among the changes observed in aging. The lack of targeted pharmacological treatments for cognitive impairment represents an unmet need and an opportunity for pharmaceutical development. There are few pharmacological studies suggesting the potential beneficial effects of ellagic acid on cognitive disorder. In the current study, we confirm the available evidence indicating that ellagic acid consumption can enhance mild cognitive impairment in middle-aged overweight individuals. The significant beneficial effect of ellagic acid on cognitive function encouraged us to further characterize the specific mechanism whereby neurocognition was enhanced by ellagic acid in middle-aged overweight males.

Our results may be particularly important for middle-aged overweight subjects as these individuals are at high risk of developing cognitive decline. Increasing evidence indicates that serum cholesterol is linked to age-related cognitive decline. Specifically, higher total serum cholesterol in mid-life predicts worse cognitive function later in life (Solomon et al., 2009). The increased cholesterol, such as LDL, accelerates the impact of age on neural networks and consequently produces deleterious effects on the brain health (Spielberg et al., 2017). It has been
shown that ellagic acid protects against atherogenic signaling induced by oxidized LDL through inhibition of reactive oxygen species (ROS) generation (Ou et al., 2010). Furthermore, ellagic acid also exhibits neuroprotective effects against oxidative damage in diabetic rats (Uzar et al., 2012). The incidence of cognitive impairment is closely linked to oxidative stress. Ellagic acid has potential for the treatment of brain injury induced by ROS. Our finding confirmed that oral ellagic acid decreased LDL and TC, TG in middle-aged overweight participants. However, whether the cognitive changes induced by chronic ellagic acid in middle-aged overweight is directly associated with the normalization of blood lipids remains unknown and still needs further investigation.

BDNF plays an important role in depression and cognitive impairment, and is also associated with energy homeostasis (Brunoni et al., 2008; Shimada et al., 2014). In addition, high fasting serum BDNF concentrations have been reported in women with obesity (Suwa et al., 2006). To further investigate the possible mechanisms involved in ellagic acid-induced improvement of cognitive ability in middle-aged overweight males, we measured the level of peripheral BDNF in the participants. BDNF has been suggested to increase within 1 hour after food intake (Bariohay et al., 2005). Since it is believed that the postprandial elevation of circulating BDNF, but not the fasting level, might be associated with obesity in men (Lee et al., 2016), we collected the blood samples 1 h after food intake. To better understand the changes in serum BDNF after ellagic acid treatment in overweight subjects, we assessed the difference in serum BDNF concentration between individuals receiving the placebo and those receiving ellagic acid. The levels of BDNF in peripheral blood has been found to reflect the level in the central nervous system (Lebrun et al., 2006; Pan et al., 1998). Accordingly, the increased serum BDNF concentrations may possibly be attributed to the improvement of cognition and reduction of bodyweight after ellagic acid administration.

The relationship between cortisol level and overweight is widely established. Studies have reported elevated cortisol level, resulting from an over activated HPA axis, in patients with central obesity (Pasquali et al., 2006; Mussig et al., 2010) and a positive association of cortisol with visceral fat accumulation (Marin et al., 1992). Notably, we found here that the saliva cortisol levels were significantly increased in overweight individuals compared with normal weight subjects. On the other hand, the increased cortisol was inhibited by the 12-week ellagic acid
treatment in the overweight group compared to placebo-treated overweight adults. Our data suggest an additional effect via cortisol, which is known to increase bodyweight. We hypothesize that the decrease in bodyweight induced by treatment with ellagic acid might be explained by the permissive effect of glucocorticoids on catecholamines. Moreover, the cortisol level might be a predictor of the response to the treatment with ellagic acid in overweight individuals suffering from cognitive decline.

**Conclusions**

In conclusion, our study first suggests that chronic treatment with ellagic acid has a promising potential to attenuate or prevent mild age-related cognitive decline by normalizing lipid metabolism, increasing the level of plasma BDNF and reducing the saliva cortisol concentration. These results provide important insights into the relationship between middle-aged overweight and poor cognitive performance, and encourage the development of novel interventions to improve and prevent cognitive deficits and related disorders in midlife. However, the current data suggested that ellagic acid can only improve mild age-related cognitive decline but not severe or pathological cognitive decline. Therefore, ellagic acid may be useful as a therapeutic agent for reducing cognition deficits in middle-aged overweight individuals and possibly other obesity-related diseases.

**Competing interests**

The authors declare that they do not have any conflicts of interest.

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References


Figure Captions:

**Figure 1.** Diagram of the experimental procedure.

**Figure 2.** Effects of ellagic acid on the blood lipids in middle-aged overweight adults. Ellagic acid reduced TC (A), TG (B), LDL (C), TC/HDL (D) and LDL/HDL (E), and increased HDL (F) in overweight individuals compared with placebo at the end of the 12-week treatment. Data are presented as the mean ± SD. n=33-38 per group. *P<0.01 compared with normal weight placebo treatment; #P<0.01 compared with overweight placebo treatment. TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein, LDL, low-density lipoprotein.

**Figure 3.** Effects of ellagic acid on the plasma BDNF level in middle-aged overweight adults. Data are presented as the mean ± SD. n=33-38 per group. *P<0.01 compared with normal weight placebo treatment; #P<0.01 compared with overweight placebo treatment.

**Figure 4.** Effects of ellagic acid on the saliva cortisol level in middle-aged overweight adults. Data are presented as the mean ± SD. n=33-38 per group. *P<0.01 compared with normal weight placebo treatment; #P<0.01 compared with overweight placebo treatment.

**Figure 5.** Effects of ellagic acid on the cognitive function in middle-aged overweight adults. Cognition was measured by WAIS-R IQ (A) and MoCA (B) scores. Data are presented as the mean ± SD. n=33-38 per group. *P<0.01 compared with normal weight placebo treatment; #P<0.01 compared with overweight placebo treatment. WAIS-R IQ, Wechsler Adult Intelligence Scale-Revised IQ; MoCA, Montreal Cognitive Assessment.