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Plasma BDNF is reduced among middle-aged and elderly women with impaired insulin function: Evidence of a compensatory mechanism

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Abstract

Brain-derived neurotrophic factor (BDNF) plays a regulatory role in neuronal differentiation and synaptic plasticity and has been linked to glucose regulation and cognition. Associations among plasma BDNF, cognition, and insulin function were explored. Forty-one participants with impaired insulin function (IIF), ranging from insulin resistance to type 2 diabetes mellitus (T2DM), were matched with 41 healthy controls on gender, age, education, and IQ. Participants received complete medical, neurological, psychiatric, and neuropsychological evaluations. IIF individuals had significantly lower plasma BDNF levels than controls, particularly females, and higher BDNF levels were associated with poorer explicit memory in IIF females, suggesting that higher levels within this group may reflect the body's efforts to respond to damage. After accounting for age, education, and HbA1c, BDNF significantly predicted 13.1%–23.5% of the variance in explicit memory in IIF women. These findings suggest that BDNF elevations within diseased groups may not always be a marker of health.

Keywords

BDNF; memory; cognition; insulin resistance; type 2 diabetes gender

1. Introduction

Brain-derived neurotrophic factor (BDNF) is a neuronal growth factor that plays a regulatory role in neuronal differentiation, synaptic plasticity, and apoptosis (Chiaramello et al., 2007; Lang, Hellweg, Seifert, Schubert, & Gallinat, 2007; Szatmari, Kalita, Kharebava, & Hetman, 2007). BDNF is found in high concentrations in the central nervous system (CNS) and the

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periphery, where it is stored in platelets and released into plasma (Fujimura et al., 2002). Peripheral BDNF crosses the blood brain barrier by a high-capacity saturable transport system (Pan, Banks, Fasold, Bluth, & Kastin, 1998). BDNF levels in CSF reportedly parallel those measured in plasma during brain development (Karege, Schwald, & Cisse, 2002), suggesting that peripheral BDNF levels reflect BDNF levels in the CNS.

BDNF has been associated with metabolic processes and disease states. Elevations have been linked to exercise in both animals and humans (Soya et al., 2007; Berchtold, Chinn, Chou, Kesslak, & Cotman, 2005; Rojas Vega et al., 2006), while reductions have been implicated in Alzheimer's disease (Laske et al., 2006), Huntington's disease (Ciammola et al., 2007), schizophrenia (Gama et al., 2007), and depression (Piccinni et al., 2008).

BDNF affects glucose metabolism and possibly insulin sensitivity. BDNF reduces serum glucose, insulin, and HbA1c levels when injected into diabetic rats, possibly improving insulin sensitivity (Tonra et al., 1999). However, there are inconsistent findings on BDNF and glucose metabolism interactions in humans: individuals with T2DM had decreased plasma BDNF, independent of obesity (Krabbe K.S. et al., 2006), while others reported increased serum BDNF in T2DM, associated with obesity (Suwa et al., 2006). Some physiological characteristics and medications may affect BDNF levels, although evidence is again inconsistent. In non-T2DM patients, body mass index (BMI) was negatively associated with plasma BDNF (Lommatzsch et al., 2005), yet it has also been positively correlated with plasma and serum BDNF (Suwa et al., 2006). Additionally, cholesterol lowering drugs (statins) reportedly increase BDNF levels (Wu et al., 2008; Chen et al., 2005).

Finally, gender may impact BDNF levels. Women were found to have higher plasma and whole-blood BDNF than men, which may be affected by body weight (Lommatzsch et al., 2005; Trajkovska et al., 2007). This parallels well-established gender differences in vascular health biomarkers that are related to cognition such as HDL and C-reactive protein (Seidell et al., 1991; Klimis, Gnardellis, & Trichopoulou, 2000; Siqueira et al., 2008; Lakoski et al., 2006).

There is good evidence that insulin sensitivity and BDNF may affect cognition and neural integrity in both animals and humans. Insulin and its receptor have been linked to learning and memory (van der Heide, Ramakers, & Smidt, 2006). Impaired glucose regulation in humans, ranging from insulin resistance to T2DM, has deleterious effects on the hippocampus and memory function (Gold et al., 2007; Convit, Wolf, Tarshish, & de Leon, 2003). BDNF is highly concentrated in the hippocampus, and is linked to long-term potentiation (LTP) and memory (Alonso et al., 2005; Kesslak, So, Choi, Cotman, & Gomez-Pinilla, 1998). Treatment with recombinant BDNF ameliorated deficits in BDNF knock-out mice with decreased long-term potentiation in the hippocampus (Patterson et al., 1996). Conversely, recent findings show that chronic BDNF over-expression in mice may impair learning and short-term memory (Cunha et al., 2009). Although not studied extensively, changes in peripheral BDNF levels have been related to cognition in humans. Sustained increases in serum BDNF levels after intense exercise correlated with better short-term learning (Winter et al., 2007). Because insulin sensitivity and BDNF may both affect brain function, we sought to explore the association between BDNF and cognition in individuals with IIF.

Our study sought to evaluate plasma BDNF levels in middle-aged and elderly individuals with IIF (i.e. insulin resistance or T2DM) relative to age-, gender-, and IQ-matched controls with no evidence of insulin resistance. Furthermore, we sought to examine associations between plasma BDNF levels and cognitive functioning among individuals with IIF. Given that both insulin and BDNF affect the hippocampus, we hypothesized that BDNF levels would be positively associated with cognitive performance. Our group has described specific reductions

in explicit memory performance among individuals with IIF (Convit et al., 2003; Gold et al., 2007), demonstrating medium to large effect sizes. Therefore we were particularly interested in explicit memory as our primary measure of cognitive function. We also examined the relationship between explicit memory and HbA1c, which reflects quality of peripheral glucose regulation and provides an indirect measure of insulin function, allowing us to evaluate T2DM and insulin resistant individuals in the same analyses. To our knowledge, this is the first study to examine the associations between plasma BDNF levels and memory performance in individuals with impaired insulin function.

2. Results

2.1 Demographic variables and descriptors

As expected, the control and IIF groups differed significantly in BMI, glucose, insulin, QUICKI, and HbA1c (all *p's* < 0.010, see Table 1). In addition, IIF and non-insulin resistant groups differed significantly in plasma BDNF levels; an independent samples *t*-test revealed that the controls (5.03 ± 1.74) had significantly higher levels of BDNF than IIF individuals (3.85 ± 1.32; $p = 0.024$). Correlations showed that neither statin use ($r = 0.072$, $p = 0.521$) nor BMI ($r = -0.140$, $p = 0.209$) were significantly associated with BDNF across the entire sample.

Given that women reportedly have higher plasma BDNF levels than men, and that women may be more vulnerable to inflammation which accompanies insulin resistance (Thorand et al., 2006), we wanted to ascertain whether group differences in plasma BDNF were influenced by gender. When we conducted separate analyses by gender, we found that women in the control group had significantly higher plasma BDNF levels than women with IIF (see Table 2). However, no such differences emerged for the men (controls: 4.06 ± 1.31, IIF: 3.68 ± 1.61; $p = 0.415$). These distinct patterns for men and women held true even after accounting for statin use and T2DM diagnosis. Since no associations between insulin function and plasma BDNF existed for the men, we restricted subsequent analyses to the female groups. As expected, women with IIF differed significantly from control women on glucose, insulin, QUICKI, HbA1c, and BMI (Table 2).

2.2 Relationship between plasma BDNF and Cognitive Performance

IIF women scored lower on cognitive tests than control women. These findings were specific to tests of explicit memory (Table 3) with medium to large effect sizes on all but one test. This is consistent with our findings from prior work, in which individuals with T2DM were found to have lower scores on explicit memory and smaller hippocampal volumes (Convit et al., 2003;Gold et al., 2007)..

Correlational analyses revealed that higher levels of BDNF were associated with lower explicit memory scores in the female IIF group (r range from −0.482 to −0.342, p's = 0.034 to 0.140), whereas positive associations were found for the controls (r range from 0.230 to 0.016, p's = 0.330 to 0.945). In an attempt to understand the significance of higher BDNF values among the IIF women with poorer memory performance, we conducted separate regression analyses for the control and IIF groups to determine the collective impact of BDNF and HbA1c on memory performance. We also compared the association between BDNF and memory performance between groups, in order to further explore the possibility that group membership moderated these relationships. Results from hierarchical regression model for the IIF group revealed that after accounting for age and education (block I), HbA1c accounted for some of the variance in measures of explicit memory (block II in table 4) while BDNF accounted for a greater amount (block III; see Table 4). When the regressions were repeated controlling for statin use (entered prior to BDNF), statins did not significantly account for the variance in any memory tests. BDNF as the third block still explained a significant amount of the variance

(9.2–23.6%, *p*'s = 0.003–0.039), with the exception of the WMS-R general score (Statin: 12.7%, $p = 0.059$, BDNF: 9.2%, $p = 0.081$). When the regression model was run with the control group, BDNF did not predict a significant amount of the variance in any cognitive domains (data not shown).

Finally, we ran ANCOVAs to examine the effect of the interaction between BDNF and group membership on cognitive performance. We found that BDNF was indeed inversely related to immediate and delayed explicit memory performance in the IIF group compared to controls, and unrelated to performance on other cognitive tests (see Table 5). While not every test in this domain achieved significance, the effect sizes were medium to large. Thus, among the women in our sample, BDNF values were significantly associated with insulin function and performance on most memory tests.

3. Discussion

The goals of this study were to evaluate whether middle-aged and elderly individuals with IIF have alterations in plasma BDNF levels relative to age-, gender-, IQ- and education-matched individuals with no evidence of insulin resistance and to examine the relationship between BDNF levels and cognitive functioning among individuals with IIF. Furthermore, we examined the impact of gender on these relationships *post hoc*.

We found that individuals with IIF had lower BDNF levels in comparison to matched non-insulin-resistant controls. This is in line with the literature, generally showing decreased BDNF levels in disease states affecting the brain, such as Alzheimer's Disease, depression, schizophrenia, and Huntington's Disease (Laske et al., 2006; Piccinni et al., 2008; Gama et al., 2007; Ciammola et al., 2007), and further adds to the notion of BDNF as a potential marker of neural integrity.

Although BDNF scores were lower overall among women with IIF, IIF women with higher levels of BDNF exhibited the poorest memory performance, whereas the opposite was true in the control group where higher BDNF levels were associated with better memory performance. These findings suggest that although individuals with IIF have lower BDNF levels as a group, higher levels *within* this group may reflect the body's efforts to respond to a state of damage. We suggest that systemic increases in BDNF may reflect a compensatory response, a position derived from previous literature. Research has shown that BDNF is released from the platelets into plasma when it is needed for repair (Fujimura et al., 2002), suggesting that BDNF levels may rise in response to damage or injury. For instance, it has been demonstrated that BDNF secretion from brain endothelial cells increases in response to hypoxia (Wang, Ward, Boswell, & Katz, 2006). Additionally, endothelial dysfunction is documented in insulin resistance (Tousoulis, Tsarpalis, Cokkinos, & Stefanadis, 2007). Thus, BDNF may be increased in women with severe insulin resistance as a compensatory response to insulin resistance-related endothelial dysfunction. Perhaps with advancing levels of insulin resistance, which have been associated with brain damage (Convit et al., 2003; Gold et al., 2007; Convit, 2005), higher levels of BDNF are produced in an attempt to compensate for this damage. Similar results have been found in other disease conditions. For example, BDNF levels are elevated in the early clinical stages of Alzheimer's disease but decrease as the disease progresses (Laske et al., 2006), perhaps because the system is overwhelmed by the disease. Therefore, although BDNF is associated with beneficial effects, elevations within diseased groups may not always be a marker of health as typically assumed.

We propose that BDNF elevations may have different functions depending on the underlying conditions of the brain environment. When neurologically normal individuals receive BDNF exogenously, or when BDNF levels are increased endogenously due to healthy lifestyle

activities (e.g. exercise), these increases are expected to have a neurotrophic effect. However, in our sample with impaired insulin function, we suggest that BDNF increases may be proportional to the degree of damage (in this case reflected by impaired memory performance). Further, while these BDNF-based compensatory increases likely have a beneficial effect, they are clearly not sufficient to overcome the negative impact of impaired insulin function on the brain.

It is still unclear why results varied by gender. One potential explanation is that estrogen enhances BDNF up-regulation. Estrogen can trigger neurotrophins and may increase BDNF levels (Gibbs, 1999). Rodent studies have documented the presence of both estrogen receptors and BDNF mRNA within the same hippocampal neurons during development, particularly in field CA3. Gonadectomies significantly decreased BDNF mRNA, which reversed following estrogen replacement, suggesting that estrogen is involved in early BDNF regulation (Solum & Handa, 2002). Estrogen also increases MAPK and P13K pathway activity, both of which are part of the BDNF signal transduction cascade and linked to learning and memory (Prokai & Simpkins, 2007; McCusker et al., 2006).

We did not establish menopausal status nor did we collect estrogen levels among our female participants given that we had not anticipated finding differential associations between BDNF and insulin function by gender group. However, the average age for female study participants was 59, therefore it is likely that the majority of women included in the study were postmenopausal. Nonetheless, without specific data on this, we cannot determine what impact this might have on BDNF levels. Future research should explore the potential influence of these factors. Other possible limitations of this study are the relatively modest sample size, the number of analyses conducted and the possibility of Type I error. However, this study has several major strengths: all the BDNF assays were run simultaneously with the same assay kit and diluted to the same standard curve. Groups were also very well matched on age, education, and IQ.

In addition to obtaining information on menopausal status or measuring estrogen levels, future studies should also measure whole-blood BDNF (since this would include platelets, which store BDNF and may represent BDNF reserve) in addition to plasma levels (which may contain only the bio-available BDNF). Future studies should also aim to relate BDNF levels to MRI-based brain measurements directly. Further pursuit of this area is indicated not only because of the interesting associations reported here, but also the emerging links between impairments in peripheral glucose regulation and hippocampal compromise, a key brain region in learning and memory and one of the first areas affected in conditions that impact the brain

4. Experimental Procedure

4.1 Subjects

Study participants were selected from volunteers recruited through collaborating endocrinologists, internet advertisements, and our current studies of normal aging. Individuals with significant neurological, psychiatric, or medical disorders (except dyslipidemia, T2DM, or hypertension) were excluded from the study. Individuals were also excluded if they were not fluent in English, had significant missing cognitive data, or had extreme outliers in their cognitive performance. Forty-one participants with T2DM or insulin resistance short of diabetes (21 women and 20 men) met these criteria. They were compared with 41 healthy controls with normal insulin regulation. Controls were selected from a larger pool of normal individuals so as to match the IIF group on gender, age, education, and IQ. All participants were community residing individuals between the ages of 43 and 79 with at least a high school education. All participants included were non-demented as reflected by Mini Mental State Exam (MMSE) scores of 26/30 or higher. Participants provided written, informed consent and

were compensated for their time. The study was approved by the New York University School of Medicine Institutional Board of Research Associates.

4.2 Impaired Insulin function (IIF) group participants

Individuals included in the IIF group met criteria for either T2DM or insulin resistance, and were not being treated with insulin or insulin secretagogues. Individuals met criteria for T2DM if they had a prior diagnosis of T2DM, fasting glucose levels greater than 125 mg/dL on two separate occasions, or a 2 hour glucose level greater than 200 mg/dL during a 75 g oral glucose tolerance test (OGTT). Individuals met criteria for insulin resistance if they were non-diabetic and had a quantitative insulin-sensitivity check index (QUICKI) < 0.35 (Chen, Sullivan, & Quon, 2005a). The QUICKI incorporates both fasting glucose and insulin levels and is a useful measure of insulin sensitivity that has been validated against clamp assessments (Chen, Sullivan, & Quon, 2005b). Of the 41 individuals in this group, 18 had T2DM and 23 had insulin resistance short of diabetes. In this first study of BDNF and cognition among individuals with insulin resistance we combined those with IR and those with T2DM based on the fact that they were indistinguishable on BDNF and cognition. This provided with groups of sufficient size to test our hypotheses. Seventeen of the 41 IIF individuals (42%) had previously been diagnosed with hypercholesterolemia and had been prescribed cholesterol-lowering medications (statins).

4.3 Control group participants

Control participants were part of a larger ongoing study of aging, and were selected to match the IIF group on gender, age, education, and IQ (see table 1). Control participants met inclusion criteria if they had a QUICKI ≥ 0.35, which indicates insulin function within the normal range. Five of the 41 control individuals (12%) had previously been diagnosed with hypercholesterolemia and had been prescribed statin medications.

4.4 Medical evaluation

Participants received complete medical and neurological exams. A list of current medications was obtained, and height and weight were measured to compute BMI. Fasting blood samples were drawn at 9:00 am during a regular study visit. Complete fasting blood analyses were performed including glucose, insulin, HbA1c, and lipid panels.

4.5 Plasma BDNF Measurements

Fasting blood samples was drawn at 9:00 am through a 19 or 21 gauge needle and placed on ice immediately. Within a few minutes of drawing, they were spun down in a refrigerated centrifuge (4C) for 10 minutes at 1,500 rpm. Aliquots of plasma were then placed into polypropylene tubes and frozen at −80C. A 0.25 cc aliquot, the third of 4 aliquots obtained (top to bottom of the plasma sample) was used for plasma BDNF analyses at a later time. Plasma samples were batch processed in duplicate to assess plasma BDNF levels using ELISA (BDNF Emax ImmunoAssay System, Promega, Madison, WI) according to the manufacturer's instructions. The assay is highly specific for BDNF with negligible cross-reactivity (less than 3%) when spiked with other neurotrophic factors (NGF, NT-3, NT-4/5) at concentrations as high as 100 ng/ml. Briefly, 96 well plates (Costar, Corning, NY) were coated with anti-BDNF pAb, sealed and incubated at 4C overnight. The following morning, plates were washed and blocked, followed by placement of plasma specimens diluted 100 fold in the supplied sample buffer solution. All subsequent incubations were performed on a rotating surface at 400 cycles/min. followed by vigorous washings. After 2 hours of incubation, a second anti-BDNF mAb was applied to sample wells and again incubated for 2 hours. Horseradish peroxidase-coupled secondary Ab was then applied, incubated for 1 hour, followed by washing and then exposure to substrate for 10 minutes with termination of the reaction by 1N HCl. Absorption level at

450 nm was determined on a spectrophotometer 96 well plate reader (Versamax 380plus, Molecular Devices Corp., Sunnyvale, CA). To avoid the effects of inter-assay variation, all patient specimens were evaluated simultaneously, with results generated from a concurrently run standard curve. The standard curve included duplicate wells containing BDNF in concentrations ranging from 500 pg/ml to 7.8 pg/ml, along with negative controls containing the sample buffer only, and corresponds to an effective range of 0.78 ng/ml to 50 ng/ml.

4.6 Neuropsychological and psychiatric evaluations

All neuropsychological tests administered were standardized tests that have been described in detail elsewhere (Lezak, 2004). Briefly, the immediate explicit (recent) memory domain consisted of the sum score of trials 1–5 from the California Verbal Learning Test (CVLT) (Delis, Kramer, Kaplan, & Ober, 1987), the immediate paragraph score from the Guild (Gilbert, 1970), and the general memory raw score from the WMS-R (Wechsler, 1987), which includes both verbal and visual memory subtests. The delayed explicit memory domain consisted of the short delay free recall scores from the CVLT, the delayed paragraph score from the Guild test, and the delayed memory raw score from the WMS-R, comprised of both verbal and visual memory delayed subtests. The executive functioning domain consisted of the total scores for letters F, A, and S from the Controlled Oral Word Association Test (COWAT) (Benton, Hamsher, Varney, & Spreen, 1983), and the total excess moves from the Tower of London Test (Davis, Bajszar, & Squire, 1994). The attention domain consisted of the total score from the Digit Symbol Substitution Test (DSST) (Uiterwijk, 2001), and the attention raw score from the WMS-R. General intelligence was assessed using scores from the Shipley Institute of Living Scale to estimate Full Scale IQ (FSIQ) from the Wechsler Adult Intelligence Scale – Revised norms (WAIS-R) (Zachary, 1940).

4.7 Statistical analyses

D'Agostino-Pearson test of normality indicated that BDNF values were not normally distributed $K^2 = 18.122$, $p = 0.000$). Therefore, a square root transformation was performed, and assumptions of normality were met ($K^2 = 0.380$, $p = 0.827$). Independent samples t-tests were used to examine group differences in demographic variables and plasma BDNF. Analysis of variance (ANOVA) tests and analysis of covariance (ANCOVA) tests were also used to examine group differences in plasma BDNF and cognition. Two linear regression models were used to examine cognitive domains: Model 1- age, gender, and education (block I), HbA1c (block II), and plasma BDNF (block III) were entered. Model 2 - blocks II and III were inverted. Linear regressions were used to determine the amount of variance in cognitive domains predicted by HbA1c and plasma BDNF, respectively. We accounted for age, gender, education, and HbA1c, all of which have been related to cognitive test performance. In our sample, age, BMI, and statin use were not associated with plasma BDNF, however, linear regressions were rerun controlling for these variables in order to rule out any potential confounds. ANCOVA analyses were used to examine the interaction between plasma BDNF and group membership on cognitive performance.

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Table 1

Description of control and impaired insulin function (IIF) groups

	Control (N = 41)	IIF (N = 41)	*p*
Age (years)	61.59 ± 8.31	61.14 ± 7.64	0.796
Education (years)	15.90 ± 2.12	16.20 ± 2.01	0.532
No. women/men	21/20	21/20	N/A
BDNF (ng/ml)	4.56 ± 1.60	3.85 ± 1.32	**0.024**
Glucose (mg/dl)	82.59 ± 1.28	90.74 ± 9.69	**0.002**
Insulin* (μIU/ml)	5.87 ± 1.85	13.30 ± 4.03	**0.000**
QUICKI*	0.38 ± 0.02	0.33 ± 0.01	**0.000**
HbA1c (%)	5.32 ± 0.41	6.14 ± 0.80	**0.000**
BMI (kg/m^2)	25.11 ± 3.38	30.52 ± 6.14	**0.000**
IQ	113.19 ± 9.07	111.73 ± 10.38	0.506
Hamilton score	1.85 ± 2.34	2.49 ± 2.17	0.219
No. diagnosed with T2DM	N/A	18	N/A
No. on T2DM medication	N/A	15	N/A
No. on statin medication	5	17	N/A

*Individuals with T2DM excluded

Table 2

Description of control and impaired insulin function (IIF) women groups

	Control (N = 21)	IIF (N = 21)	*p*
Age (years)	58.64 ± 6.31	59.95 ± 7.32	0.533
Education (years)	15.95 ± 2.20	15.52 ± 2.41	0.552
BDNF (ng/ml)	5.03 ± 1.74	4.01 ± 0.98	**0.025**
Glucose (mg/dl)	80.24 ± 8.61	94.50 ± 9.30	**0.001**
Insulin* (μIU/ml)	5.23 ± 1.63	13.02 ± 4.74	**0.000**
QUICKI*	0.38 ± 0.02	0.33 ± 0.01	**0.000**
HbA1c (%)	5.29 ± 0.43	6.42 ± 0.83	**0.000**
BMI (kg/m^2)	25.11 ± 3.38	30.52 ± 6.14	**0.000**
IQ	112.41 ± 6.17	109.52 ± 9.99	0.268
Hamilton score	2.19 ± 2.52	2.78 ± 2.16	0.438
No. diagnosed with T2DM	N/A	11	N/A
No. on T2DM medication	N/A	9	N/A
No. on statin medication	1	7	N/A

*Individuals with T2DM excluded

Table 3

Cognitive performance in women by group

	Control (N = 21)	IIF (N = 21)	Cohen's *d*	F	*p*	*Partial* η^2
Immediate Explicit Memory				3.630	**0.022**	**0.237**
CVLT Trials 1–5	62.86 ± 7.39	61.83 ± 12.24	0.07			
Immediate Guild Paragraph	8.45 ± 2.83	6.31 ± 1.91	0.89			
WMS-R General Score	147.24 ± 12.41	132.67 ± 25.39	0.73			
Delayed Explicit Memory				2.980	**0.045**	**.203**
CVLT Short Delay Free Recall	14.00 ± 2.00	12.32 ± 4.19	0.51			
Delayed Guild Paragraph	11.10 ± 3.66	8.13 ± 2.66	0.93			
WMS-R Delayed Score	90.30 ± 9.87	80.21 ± 17.41	0.71			
Executive Functioning				0.763	0.475	.050
Verbal Fluency	48.44 ± 17.83	44.00 ± 13.00	0.29			
Tower of London excess moves	6.69 ± 6.00	10.13 ± 10.32	−0.41			
Attention				0.489	0.617	.026
DSST	57.25 ± 7.80	58.70 ± 9.17	−0.02			
WMS-R Attention Score	69.20 ± 14.73	66.80 ± 10.82	0.19			

Table 4

Linear regression model predicting explicit memory in women with impaired insulin function (IIF)*

	I (Age/Education)			II: HbA1c			III: BDNF		
	R^2	β	*p*	ΔR^2	β	*p*	ΔR^2	β	*p*
CVLT Trials 1–5	.547	−1.050/1.096	.000/0.097	.001	.869	.636	.207	−.5.364	**.002**
Imm. Guild Paragraph	.230	−.002/.297	.961/.093	.129	−.903	.092	.23.5	−.990	**.013**
WMS-R General	.223	−1.114/1.789	.071/.368	.196	−12.512	**.034**	.143	−9.901	**.043**
CVLT Short Delay	.304	−.218/.474	.032/.130	.064	−1.142	.202	.194	−.1.189	**.017**
Del. Guild Paragraph	.437	−.075/.556	.192/.011	.075	−.916	.111	.131	−.962	**.033**
WMS-R Delay	.383	−.980/2.020	.009/084	.192	−8.611	**.013**	.132	−6.578	**.020**

*The column headings represent the order of blocks. Change in R^2 from previous block (ΔR^2), β-value, and *p* value for ΔR^2 are shown.

Table 5

ANCOVA examining the interaction between BDNF and group membership on cognitive performance in women.

	F	*p*	Partial η^2
Immediate Explicit Memory	2.805	0.055	**0.203**
CVLT Trials 1–5	7.731	**0.009**	**0.181**
Immediate Guild Paragraph	1.689	0.202	0.046
WMS-R General Score	5.289	**0.028**	**0.131**
Delayed Explicit Memory	2.240	0.102	**0.169**
CVLT Short Delay Free Recall	7.005	**0.012**	**0.167**
Delayed Guild Paragraph	1.776	0.191	0.048
WMS-R Delayed Score	3.986	0.054	**0.102**
Executive Functioning	0.004	0.996	0.000
Verbal Fluency	0.002	0.965	0.000
Tower of London ex. moves	0.004	0.948	0.000
Attention	0.244	0.785	0.014
DSST	0.129	0.721	0.004
WMS-R Attention Score	0.204	0.654	0.006