

# Combined exercise training-induced improvements in executive functions are related to baseline plasmatic levels of Brain-Derived Neurotrophic Factor (BDNF) in middle-aged adults and older with Type 2 Diabetes Mellitus

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## Research Article

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# Abstract

**Background:** Type 2 Diabetes Mellitus (T2DM) affects many cognitive functions and aerobic plus resistance exercise training, named combined training (CT), by improving metabolic control may mitigate or reverse the cognitive impairment T2DM-related. Brain-derived neurotrophic factor (BDNF) plays a substantial role in cognitive functions. However, the effects of CT on BDNF levels of T2DM subjects are poorly known.

**Aim:** This study analyzed the effects of 8 weeks of CT on circulating BDNF levels and assessed whether plasmatic BDNF levels were related to CT-induced improvements in executive functions and long-term memory of T2DM subjects.

**Methods:** Thirty-five ( $63 \pm 8$  years old) T2DM subjects of both sexes entered into CT ( $n = 17$ , thrice-weekly during 8 weeks) or control group (CONT,  $n = 18$ ). Executive functions (measured through Trail making test, Stroop color task, and Digit Span), long-term memory (measured through the simplified version of Taylor Complex Figure Test), and blood samples were evaluated pre- and post-intervention.

**Results:** Pre-CT plasma BDNF levels were positively related to CT-induced improvements on executive functions composite z-score ( $r = 0.71$ ), inhibitory control ( $r = 0.58$ ) and cognitive flexibility ( $r = 0.56$ ), but not to long-term memory. Plasma BDNF levels were not statistically changed (pre-CT:  $179 \pm 88$  pg/ml; post-CT:  $148 \pm 108$  pg/ml; pre-CONT:  $163 \pm 71$  pg/ml; post-CONT:  $141 \pm 84$  pg/ml,  $p > 0.05$ ).

**Conclusion:** Higher pre-training BDNF levels might be a potentializing factor of the training-induced improvements on executive functions, independently of the training-alterations in resting BDNF levels of T2DM subjects.

## Introduction

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder that impairs several metabolic pathways participating in insulin signaling, hence dysregulating glucose metabolism (DeFronzo et al. 2015). Impaired glucose metabolism affects several organic tissues, and the brain is one of them. The negative changes in brain functioning and structure by T2DM induced some complications, as the increased risk for mild cognitive impairment (MCI) and dementia in nearly 20% and 50%, respectively (Cheng et al. 2012). Furthermore, these T2DM-related cognitive dysfunctions are progressive and accelerated, as type 2 diabetic subjects with MCI are likely to develop dementia almost three years before those without MCI (Ma et al. 2014).

From a healthcare perspective, adequate levels of cognitive functions are crucial to the T2DM treatment regimen, including blood glucose monitoring, medication adherence, reducing stress, adopting healthy behaviors, caring for both skin and feet. Poor diabetes self-care management (e.g. blood glucose monitoring and self-management of prescribed drug therapy), low diabetes knowledge, and higher help care needs are observed in those with the lowest cognitive screening scores in a sample of older adults

with T2DM (Sinclair et al. 2000). Furthermore, older adults with T2DM and MCI are less likely to adhere to exercise and diet (Feil et al. 2012). T2DM subjects have shown impaired performance in tasks assessing some neurocognitive domains, such as executive functions and long-term memory (Palta et al. 2014; Sadanand et al. 2016). Low levels of executive function, somehow, increase the risk of older adults developing frailty and disability (Johnson et al. 2007). Otherwise, higher executive function scores may preserve functionality. T2DM subjects with the highest memory scores are more likely to adhere to prescribed drug therapy (Vedhara et al. 2004), which, in turn, can improve metabolic control and prevent T2DM-related outcomes. Thus, therapeutic strategies focused on improving cognitive functions may modify the course of T2DM, preventing or mitigating the progression of cognitive damages, hence contributing to the maintenance of T2DM subjects' cognitive health and functionality.

The planned exercise training program is a promising and low-cost therapy in preventing and treating many diseases, including cardiovascular diseases, certain types of cancer, and T2DM (Booth et al. 2012). Moreover, exercise training also has central effects since midlife physical exercise lowers 40% the odd for developing MCI in late-life (Geda et al. 2010). Exercise training improved the long-term memory of cognitively normal older adults and executive functions of healthy and older adults with MCI (Sanders et al. 2019). In adults and older with T2DM, exercise training also enhances some cognitive functions (Silveira-Rodrigues et al. 2021). However, the underpinning mechanisms involved in exercise training-induced cognitive improvement in T2DM subjects needs to be better understood.

The Brain-derived Neurotrophic Factor (BDNF), a protein of the neurotrophins family, that when binds to its most abundant receptor, tropomyosin receptor kinase B (TrkB), exerts its central function by triggering neurons survival, growth, and improving their resistance to damage (Rozanska et al. 2020). Due to its actions in modulating synaptic transmission, synaptic plasticity, neurogenesis, learning, and memory processes, BDNF is assumed as a biomarker that reflects mnemonic symptoms in several pathologic conditions (Miranda et al. 2019). Several cross-sectional studies reported lower circulating BDNF levels in T2DM subjects than non-T2DM controls (Geroldi et al. 2006; Krabbe et al. 2007; Fujinami et al. 2008; Zhen et al. 2013; Sun et al. 2018). Indeed, the lower serum BDNF levels in adults and older with T2DM subjects were not only accompanied by poor attentional and long-term memory performance (Zhen et al. 2013) but also increased by at least 50% the risk for developing MCI in this population (Sun et al. 2018). For these reasons, BDNF appears to play a relevant role in T2DM-related cognitive dysfunctions.

BDNF participates in the muscle-brain crosstalk since is released by skeletal muscle and other tissues after an exercise bout hence increasing their circulating and central levels (Walsh and Tschakovsky 2018). Only single (Rasmussen et al. 2009) or twice aerobic exercise bouts (Neeper et al. 1996) raises the mRNA BDNF expression in the prefrontal cortex and hippocampus, and an accumulating body of evidence suggests that BDNF modulates some of the exercise effects on cognition (Intlekofer et al. 2013; Tang et al. 2017). In humans, due to the inability to assess brain BDNF mRNA expression, current studies often assess the serum or plasma circulating BDNF levels since these levels are strongly correlated to brain BDNF levels (Sartorius et al. 2009). As it crosses the blood-brain barrier through a rapid saturable

neurotrophic role (Pan et al. 1998). In T2DM subjects (Brinkmann et al. 2017) and other populations (Szuhany et al. 2015), circulating BDNF levels increase after a single exercise bout. However, the effects of exercise training (repeated exercise bouts) on circulating BDNF of T2DM subjects need to be better understood. A long-term exercise training program failed to demonstrate significant changes in serum BDNF of adults and older with T2DM (Swift et al. 2012). Moreover, to the best of our knowledge, the relationship between BDNF levels and training-induced cognitive improvements remains unknown. Thus, considering that BDNF and its receptors TrkB are expressed in the prefrontal cortex and hippocampus (Neeper et al. 1996; Rasmussen et al. 2009), brain areas that encompass the executive functions and long-term memory, it is tempting to suggest that BDNF levels can mediate the training-induced improvements in these cognitive functions.

Therefore, this study aimed to analyze the effects of 8 weeks of combined (aerobic and resistance) exercise training on circulating BDNF levels of T2DM subjects and whether the pre-training BDNF levels or training-induced changes in BDNF levels were related to training-induced improvements on executive functions and long-term memory. It was hypothesized that BDNF levels would be related to the exercise training improvements in cognitive functions of middle-aged and older adults with T2DM.

## Material And Methods

### Ethical care

The study protocol followed Helsinki's Declaration and was approved by the Research ethics committee and was registered in Brazilian Register of Clinical Trials (register no. RBR86hfhz5). Volunteer's recruitment was performed by phone contact between February and May/17.

### Subjects

Thirty-five subjects signed a consent form to participate in this study after an explanation of the experimental procedures. Subjects were aged between 50–79 years, had up to 2 years of T2DM diagnosis, were literate, had normal or corrected visual and auditory functions, were physically inactive during the last three months and were absent of musculoskeletal conditions that would not allow physical training (See sample characteristics in Table 1). Were adopted as exclusion criteria those an attendance rate of less than 60% of the total of 24 CT training sessions. Seventeen subjects entered combined training (CT) and eighteen in control (CONT) groups. Four subjects left the study (one in CT for personal reasons; and three in CONT, one for lower limb fracture in daily living activity, another two for the impossibility to be contacted by phone number). In two participants of CONT, although cognitive tests were performed, it was not possible to assess BDNF levels. Therefore, 31 participants were included in the cognitive analysis, and 29 in BDNF levels analysis (Fig. 1).

Table 1  
Pre-training sample characteristics (Mean  $\pm$  SD or absolute and relative value)

Parameter	CT ( <i>n</i> = 16)	CONT ( <i>n</i> = 15)	<i>p</i> value
Age (years)	63.9 $\pm$ 7.7	63.3 $\pm$ 7.8	0.98
Time of T2DM diagnosis (years)	11.3 $\pm$ 7.7	14 $\pm$ 10.1	0.50
HOMA-IR	3.8 $\pm$ 2.8	4.3 $\pm$ 2.7	0.34
Fructosamine (mmol/ml)	249 $\pm$ 61	259 $\pm$ 54	0.65
Six-minutes walk test (m)	527 $\pm$ 88	516 $\pm$ 80	0.72
Education time (years)	9.4 $\pm$ 4.7	8.9 $\pm$ 5.1	0.53
MoCA score	20.3 $\pm$ 5.4	21.8 $\pm$ 3.9	0.70
Cognitively normal (MoCA score > 25)	5 (31%)	5 (33%)	-
Mild cognitive impairment (MoCA 19–25)	7 (44%)	7 (47%)	-
Dementia (MoCA score < 18)	4 (25%)	3 (20%)	-
Sex (females)	10 (63%)	14 (93%)	-
<i>MetS NCEP-ATP III criteria</i>			
HHD (SAP: <135, DAP: <85mmHg)	8 (50%)	10 (67%)	-
WC (W: >88, M: >102cm)	11 (69%)	13 (87%)	-
TG (> 150 mg/dl)	15 (94%)	13 (87%)	-
HDL-c (W: >50, M: >40 mg/dl)	5 (31%)	4 (27%)	-
FG (> 110 mg/dl)	15 (94%)	14 (93%)	-
Above than 3 factors	12 (75%)	14 (93%)	-
<p>Note: CONT: control group, CT: Combined training group, DAP: Diastolic arterial pressure, FG: Fasting glucose, HDL-c: High-density lipoprotein cholesterol, HHD: Hypertensive heart disease, HOMA-IR: Homeostatic model assessment, MetS: Metabolic syndrome, MoCA: Montreal Cognitive Assessment, SAP: Systolic arterial pressure, TG: Triglycerides, WC: Waist circumference, <i>NCEP-ATP III criteria</i>: National cholesterol education program adult treatment panel III (Expert Panel on Detection, Evaluation 2001).</p>			

## Experimental design

Pre-training data were assessed in three moments, interspaced by at least 48h. On day one, the volunteers were cognitively screened and answered a questionnaire with daily living information, health status, occupation, and physical activity level. At the second occasion, blood samples and blood pressure were assessed. On day three, cognitive tasks and the six-minutes walk test were performed. After these

Loading [MathJax]/jax/output/CommonHTML/jax.js combined training and CONT-group was guided to maintain

their life routine. At least 72 hours after the last training session, post-training blood samples and cognitive tasks were assessed. All the volunteers should avoid intense physical activity, caffeinated or alcoholic beverages ingestion, and to maintain their habitual food and water ingestion in the 24 hours before the experimental procedures.

## Combined training program (CT)

The CT-protocol follows recommendations of the American Diabetes Association for exercise in subjects with T2DM (Colberg et al. 2016) and consisted of thrice-weekly non-consecutive sessions for 8 weeks when alternate increments in the exercise volume or exercise intensity conducted after each two-week microcycle. Exercise protocol are detailed in (Silveira-Rodrigues et al. 2021).

## Executive functions and data transformation

Executive functions are the suite of processes that underlie goal-directed self-regulatory behaviors (Diamond 2013). The three components of the executive functions' core are cognitive flexibility (that refers to creative processes and comprises the ability to alternate the perspective of analysis of a task or situation); inhibitory control (that encompasses the ability of attention, behavior, and emotion control to mitigate the intervention of distractors), and working memory (that includes the ability to hold information in mind and work mentally as math reasonings or reorder a task list, as an example) (Diamond 2013).

We used one different task to assess each executive function component. Cognitive flexibility was assessed through the Trail making test (TMT), inhibitory control by the Stroop color task (SCT), and working memory by Digit Span (DS). The interference score (Nagamatsu et al. 2012) was adopted to measure the executive function performance of each domain, subtracting the values of the incongruent trial from the neutral trial. The cognitive flexibility score was assessed by TMT-A minus TMT-B. Inhibitory control score through composite z-score of SCT-congruent and SCT-incongruent (total time + (2 \* mean time per word \* error number)). Working memory through DS-reverse minus DS-direct. Executive function composite z-score represents the mean of cognitive flexibility, inhibitory control, and working memory z-scores. If the volunteer lost or was unable to perform any task, the arithmetic means of the other task parameters were determined as the individual composite z-score.

The cognitive task parameters were z-score transformed as adopted previously (Espeland et al. 2017) through the equation:  $Z = \frac{x - X}{\sigma}$ , where x: individual value, X: sample mean and  $\sigma$ : group standard deviation. Considering that in TMT and SCT the lower time for answering these tasks represents better performance, then, the interference z-scores were multiplied by -1 (Nagamatsu et al. 2012).

## Long-term memory

Memory refers to the capacity of retaining and manipulating acquired previous information through neural plasticity (Squire 2004). The simplified version of the Taylor Complex Figure Test (Paula et al. 2016) a task involving visuospatial abilities and episodic memory recall was employed to assess long-

term memory. Individuals' scores were obtained through the difference between the immediate copy score and the delayed 30min-copy score. In this case, lower values represented better performance.

## Blood Samples and biochemical analysis

Samples were collected 72h after the last familiarisation session (pre-training) and last CT-session (post-training). Blood samples were collected in heparinized tubes in the morning and after 12h of fasting. After collection, samples were stored in styrofoam until centrifugation (10 min, 3500rpm, and 4°C) for plasma separation. After that samples were stored at -80°C in aliquots of 100 µl until analysis. Plasma BDNF concentrations were quantified by sandwich ELISA specific kit (DY248 DuoSet, R&D Systems™, MN, USA) according to the manufacturer's instructions. A single plate was blocked for 3 h in reagent diluent (1% bovine serum albumin (BSA)/ phosphate-buffered saline (PBS)) and incubated for nearly 12 h with 100 µl of samples at 4° C. Samples were diluted 1:64 to fit standard curve range (detection range: 23-1500 pg/ml). The baseline blood glucose, lipids, and fructosamine were quantified by the enzymatic colorimetric technique using specific kits (Gold Analisa™, MG, BR). Plasma insulin was quantified by chemiluminescence (Siemens Centaur™, NY, USA). Metabolic syndrome was assumed considering a previously reported NCEP-ATP III criteria (see Expert Panel on Detection, Evaluation, 2001) and 75% of the CT and 93% of CONT subjects attained three or more criteria for determining metabolic syndrome presence.

## Sample size calculation and a posteriori power

Sample size calculation was performed in G\*Power v3.0.10 (Universität Kiel, KI, GER) based on the effect of combined training on executive function parameters (Liu-Ambrose et al. 2010). Considering the changes induced by experimental conditions in the two independent groups were determined: (1)  $\alpha = 5\%$ ; (2) power  $(1-\beta) = 0,7$ ; (3) allocation rate of 1:1 and (4) aimed *Cohen's d* = 0.8 (large effect size) in a one-tailed test (considering that CT improves executive functions). Thereby, a minimum sample size of 16 subjects was obtained, adjusted for 17 subjects after sample loss consideration. *A posteriori* power was calculated for BDNF levels using G\*Power v3.0.10.

## Statistical analysis

Normality and homoscedasticity were tested with *Shapiro-Wilk's* and *Levene's* tests, respectively. All variables were normally distributed, allowing parametric analysis. Sample characteristics and plasmatic BDNF levels were presented in mean  $\pm$  standard deviation (SD). *Student's t-test* for independent samples (two-tailed) compared the changes in executive function composite z-score and interference scores in both groups after 8-weeks. *Two-way* ANOVA with repeated measures was used to compare the BDNF levels between time (pre vs. post-training) and group (CT vs. CONT). *Three-way* ANOVA was performed to compare the BDNF between time (pre vs. post-training), group (CT vs. CONT), and age (CT vs. CONT). Simple linear regression analyses were used to determine the relationship between plasmatic BDNF levels with CT-induced changes in executive functions and long-term memory. Additionally, *Cohen's d* was calculated:  $(x^1 - x^2) / (\sigma \text{ pooled})$ , where  $x^1$  was the pre-training mean,  $x^2$  the post-training mean value, and  $\sigma$

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studied parameters was classified according to the effect size (Cohen's  $d$ ) as trivial if  $d < .2$ , small:  $d = .2$  to  $.5$ ), medium  $d = .5$  to  $.8$ , and large  $d > .8$ . Data were analyzed in GraphPad Prism v5.0 (GraphPad Software Inc., CA, USA). The significance level was 5%.

## Results

Plasmatic BDNF levels (pre-CT:  $179 \pm 88$  pg/ml; post-CT:  $148 \pm 108$  pg/ml; pre-CONT:  $163 \pm 71$  pg/ml; post-CONT:  $141 \pm 84$  pg/ml) didn't show main effects for time ( $F_{(1,53)} = 2.64$ ;  $p = 0.12$ ), group ( $F_{(1,53)} = 0.08$ ;  $p = 0.78$ ) or interaction ( $F_{(1,53)} = 0.32$ ;  $p = 0.58$ ). A posteriori power ( $1-\beta$ ) of BDNF analysis was 0.23 (Fig. 2). Considering that training-effect on BDNF levels may be modulate by age (Leckie et al. 2014), we tested the subjects above ( $n = 8$  for both CT and CONT groups) and below 65 years old ( $n = 8$ , 5 in CT and CONT groups). No main effects or interaction ( $F > 1.16$ ,  $p > 0.29$  for all) were observed in plasmatic BDNF levels neither in older adults (pre-CT:  $180 \pm 106$  pg/ml; post-CT:  $155 \pm 113$  pg/ml; pre-CONT:  $187 \pm 110$  pg/ml; post-CONT:  $145 \pm 69$  pg/ml) nor in middle-aged adults (pre-CT:  $177 \pm 74$  pg/ml; post-CT:  $141 \pm 109$  pg/ml; pre-CONT:  $148 \pm 31$  pg/ml; post-CONT:  $150 \pm 59$  pg/ml).

The CT improved executive functions (Fig. 3), as demonstrated by the higher reductions in composite executive function z-score (CT:  $0.38 \pm 0.58$  z, CONT:  $-0.46 \pm 0.70$  z,  $d = 1.31$ ,  $t = 3.59$ ,  $df = 28$ ,  $p = 0.001$ ). Also, CT showed higher changes than control group in the interference scores of the inhibitory control (CT:  $-0.10 \pm 0.20$  seg, CONT:  $0.12 \pm 0.31$  seg,  $d = 0.87$ ,  $p = 0.03$ ), and working memory (CT:  $-0.33 \pm 0.98$  seg, CONT:  $0.05 \pm 0.91$  seg,  $d = 0.40$ ,  $p = 0.03$ ), but not for cognitive flexibility although higher effect size there was obtained (CT:  $-0.45 \pm 0.76$  seg, CONT:  $0.19 \pm 0.48$  seg,  $d = 0.96$ ,  $p = 0.15$ ). Otherwise, the changes on long-term memory z-score were not different between groups ( $d = 0.22$ ,  $t = 0.56$ ,  $df = 25$ ,  $p = 0.56$ ) as described in previous published research (Silveira-Rodrigues et al. 2021).

Interestingly, pre-training BDNF levels were correlated ( $r = 0.71$ ,  $r^2 = 50\%$ ;  $p < 0.01$ ; 95% CI: 0.33–0.89;  $d = 0.62$ ; a *posteriori* power: 0.71) to CT-induced changes on composite executive function z-score (Fig. 4). Additional analysis was performed considering the three areas of the executive functions separately. Pre-training BDNF levels was related to inhibitory control ( $r = 0.58$ ;  $p = 0.02$ ) and cognitive flexibility ( $r = 0.56$ ;  $p = 0.04$ ). However, there was no correlation between pre-training BDNF levels and CT-induced changes in working memory z-score ( $p = 0.31$ ) and long-term memory z-score ( $p = 0.55$ ).

Additionally, we tested whether the CT-induced change on BDNF levels (post minus pre-CT) are related to composite executive function z-score ( $p = 0.20$ ), inhibitory control ( $p = 0.30$ ), cognitive flexibility ( $p = 0.33$ ), working memory z-score ( $p = 0.51$ ) or long-term memory z-score ( $p = 0.77$ ) but no significant correlations were found for all.

## Discussion

The main finding of our study was that CT-induced improvements in composite z-score of executive function were positively correlated with pre-training plasma BDNF levels in T2DM subjects, even without

alterations in plasmatic BDNF levels elicited by the 8-week in those subjects. It suggests that sedentary T2DM subjects with the highest plasmatic BDNF levels may be the best responders in improving some domains of their executive functions after a short-term CT exercise program. Conversely, long-term memory appears not to be related to pre-training plasmatic BDNF concentrations.

Despite recent progress in the T2DM diagnosis and treatment, little attention is given to the chronic complications which impair the quality of life and self-management of T2DM. Growing and novel evidence focuses on the development of course-modifying therapies to counteracts T2DM-related cognitive dysfunction (Biessels and Despa 2018). A recent meta-analysis highlights the trifling amount of published studies regarding the exercise training effects on cognitive outcomes of T2DM subjects (Cooke et al. 2020). Our results show that CT induced positive effects on executive functions of T2DM adults and older. Executive Functions have been related to general health (see Diamond, 2013) (Diamond 2013), and in T2DM subjects it has not been different (Sadanand et al. 2016). Several health aspects as self-management of T2DM, lower hospitalization number (Sinclair et al. 2000), adherence to diet and exercise (Feil et al. 2012), depending on cognitive health preservation. Furthermore, executive functions were a better predictor of functional decline and mortality of older women than global cognition (Johnson et al. 2007). Enhance the executive functions by exercise training can induce other health benefits, for example, the 1-year resistance training improvements in executive function contributed to the maintenance of physical activity over the following year (Best et al. 2014). However, the studies exploring concomitantly the mechanisms underlying the training-induced cognitive-benefits in T2DM subjects are scarce.

BDNF is considered a promising biomarker for early identifying T2DM subjects with a higher risk to develop MCI (Sun et al. 2018). By modulating synaptic plasticity and neurogenesis, BDNF may induce positive cognitive effects (Intlekofer et al. 2013). In this regard, it has been speculated that BDNF can also induce positive effects on the cognition of T2DM subjects (Rozanska et al. 2020). However, according to a recent meta-analysis (Jamali et al. 2020), the effects of exercise training in BDNF levels of adults and older with T2DM are still scarce and inconclusive (Jamali et al. 2020). Tang et al. (2017) investigated the effects of exercise training on the memory of diabetic rats, revealing that resistance training improves not only the learning performance of rats but also upregulates the expression of BDNF mRNA and its receptor TrkB in the hippocampus, suggesting that BDNF also seems to be involved in training-induced cognitive benefits in the face of metabolic alterations present in diabetes.

The present study failed to demonstrate significant changes in BDNF levels between the trained and control group. Evidence reveals that an exercise training program (lasting from a week to months) has a lower capability to increase the circulating BDNF levels compared to a single bout of exercise (Szuhany et al. 2015). In T2DM subjects, similar results are reported, when a single bout of aerobic exercise increases circulating BDNF levels (Brinkmann et al. 2017), whereas nine months of aerobic, resistance, or combined training didn't increase serum BDNF in 30–75 years old T2DM subjects (Swift et al. 2012). Furthermore, older adults with glucose intolerance, aerobically trained for six months also didn't have their plasma BDNF levels altered (Baker et al. 2010). Thus, the present study advances to current knowledge by

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of our knowledge, that an 8-week short-term combined

(aerobic plus resistance) does not significantly change resting plasmatic BDNF levels in sedentary middle-aged and older T2DM subjects.

In an unprecedented way, we showed that the pre-training plasmatic BDNF levels had a positive correlation with CT-induced improvements in executive function (composite z-score) in middle-aged and older T2DM subjects. Rasmussen et al. (2009) reported that a single bout of prolonged aerobic exercise at low to moderate intensity briefly raised the plasmatic BDNF levels in humans, but one hour after exercise returned to pre-exercise levels. However, when analyzing the brain mRNA BDNF expression in the mice, a long-lasting increasing response was found in this gene expression. Unlikely plasmatic BDNF levels, an evident increase in the BDNF mRNA expressed in the prefrontal cortex and hippocampus occurred immediately after prolonged aerobic exercise in mice (Rasmussen et al. 2009). Interestingly, the peak of BDNF mRNA expression in both brain regions occurred around two hours after exercise cessation and remained upregulated for the next 24h (Rasmussen et al. 2009). In another study, after three weeks of voluntary exercise, brain BDNF mRNA expression increased, which was paralleled to memory improvements in mice (Intlekofer et al. 2013). However, when the BDNF synthesis is blocked, the exercise-induced improvements in mice's memory didn't occur, suggesting that the cognitive amelioration was BDNF-dependent (Intlekofer et al. 2013). Likewise, higher serum BDNF levels were accompanied by higher increases in functional connectivity in the parahippocampus and middle temporal gyrus of middle-aged and older adults that performed 12 months of a training program focusing on flexibility, toning, and balance (Voss et al. 2013). Taken together, all these results endorse that the repeated transitory elevation of circulating BDNF levels after a single bout of physical exercise is a substantial contributor to the exercise-induced cognitive benefits, even without changing the circulating BDNF levels at rest. Therefore, it is possible that in our study the higher plasmatic BDNF basal levels can potentially favor the CT-induced cognitive improvements of the T2DM subjects.

A possible extrapolation of our findings deals with the potential existence of an optimal window of opportunity that can boost up the exercise-induced improvements on executive functions in T2DM subjects. As it is known, T2DM subjects show lower basal BDNF levels than non-diabetic controls (Geroldi et al. 2006; Krabbe et al. 2007; Fujinami et al. 2008; Zhen et al. 2013; Sun et al. 2018), and this neurotrophic profile (*i.e.* inferred by BDNF levels) seems to be related to the metabolic disruption observed in the subjects (Geroldi et al. 2006; Levinger et al. 2008; Hristova 2013). For example, those with higher risk factors for metabolic syndrome showed higher plasmatic BDNF levels than those with the lowest risk factors (Levinger et al. 2008). Also, higher BDNF levels are reported in the early stages whereas lower BDNF levels are reported in the late stage of T2DM (Geroldi et al. 2006; Hristova 2013). Another study with animals shows that BDNF modulates glucose metabolism. The exogenous intracerebroventricular administration of BDNF inhibited hepatic gluconeogenesis, hence, relieving hyperglycemia in the T2DM murine model, even without explaining the exact neuronal mechanisms involved (Meek et al. 2013). Akin, in our investigation, 75% of the sample studied attained multiple criteria for metabolic syndrome (Expert Panel on Detection, Evaluation 2001). *The Neurotrophic Theory* proposed by Hristova (2013) postulated that the early raises in BDNF levels observed in subjects with a higher risk factor for metabolic syndrome

Loading [MathJax]/jax/output/CommonHTML/jax.js the initial T2DM pathophysiology impairments comprising

altered glucose metabolism and insulin signaling. Thence, we might suggest that the highest pre-training circulating BDNF levels in the T2DM subjects of our study may have provided an advantage in obtaining the exercise-induced improvements on executive functions.

When dissociated, the subcomponents of executive functions in our study were differently related to pre-training plasma BDNF levels. Higher inhibitory control and cognitive flexibility were positively correlated to higher pre-training BDNF levels. However, the exercise-induced improvements in working memory and episodic long-term memory were unrelated to pre-training BDNF levels. Contrasting with our findings, BDNF was crucial to memory development in a rodent model (Intlekofer et al. 2013). Also, in T2DM subjects, a previous study reported that serum BDNF levels correlated to episodic and semantic memory (Zhen et al. 2013). Indeed, memory is a complex system with some subdivisions, encompassing ultrarapid, short-term, and long-term memories. Long-term memory consists of storing information for late use and are separated into declarative and non-declarative memories. Declarative memory, in turn, is separated into semantics, which comprises ideas and concepts not drawn from personal experience, and episodic memory that is dependent on a specific event (Squire 2004). Working memory is one of the three core executive functions, composed of verbal and visuospatial working memory (Diamond 2013). The main difference between working and short-term memory is their component of manipulating information in the mind (Diamond 2013). In our study, both visuospatial episodic and verbal working memories were not significantly related to circulating pre-training BDNF levels. However, two main aspects should be considered. Firstly, animal studies measure the expression of the brain BDNF mRNA, which is impossible in human in-vivo studies. This might be relevant since, in response to an exercise session, the increases in brain BDNF levels last longer than those observed in plasma (Rasmussen et al. 2009). Secondly, our study assessed long-term memory by the adapted Taylor Complex Figure test, which only assesses episodic visuospatial abilities. Also, the working memory test used only recognizes the verbal component of working memory. Thus, should be included in future investigations the assessment of other memory components, including semantic and visuospatial memories. This fact would explain more properly the relationship between circulating baseline BDNF levels and putative exercise-induced memory improvements.

This study has some limitations. Although the sample size was calculated based on executive function variables, a *posteriori* power reached between plasmatic BDNF levels pre and post-intervention was 23%, suggesting a considerable likelihood of type II error occurrence. It can be due to the age and cognitive performance heterogeneity of our sample. Also, this study used a commercial enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems™), that does not recognize proBDNF, a molecule that antagonizes mature BDNF in their actions in the central nervous system. Further, most of the study participants underwent medication therapy, which might have modulated plasmatic BDNF levels. Future studies may consider larger sample size and other metabolic pathways to clarify this differential effect of exercise training under distinct baseline BDNF levels.

## Conclusion

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The magnitude of exercise-induced improvements in executive functions can be dependent on pre-training BDNF levels in T2DM subjects independently of significant training-induced changes in resting plasma BDNF levels.

## Declarations

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### Data availability:

Data are available online as supplemental material.

### Compliance with ethical standards

**Conflict of interests:** Nothing to declare;

**Ethical Standards:** Approved in the Human Research Ethics Committee of the Federal University of Minas Gerais (no. 2.067.044/66804817.8.0000.5149), and registered in the Brazilian Register of Clinical Trials (no. RBR86hfvz5)

**Consent to participate:** All individuals included in the current study were fully informed and gave their written consent to participate and for the data to be published.

**Consent for publication:** Not applicable

**Data availability:** Data are available.

**Author contribution (CRediT author statement):**

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**JGSR:** Conceptualization, Data Curation, Validation, Formal Analysis, Investigation, Methodology, Project Administration, Software, Writing – Original Draft Preparation and Writing – Review & Editing. **NGHMN:** Conceptualization, Formal Analysis, Validation, Writing – Original Draft Preparation and Writing – Review & Editing. Writing – Review & Editing. **LOF:** Conceptualization, Data Curation, Formal Analysis, Validation, Writing – Original Draft Preparation and Writing – Review & Editing, Writing – Review & Editing. **DSP:** Formal Analysis, Writing – Original Draft Preparation and Writing – Review & Editing. Writing – Review & Editing. **DDS:** Data Curation, Conceptualization, Formal Analysis, Validation, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Review & Editing.

## Abbreviations

ANOVA: Analysis of Variance

BDNF: Brain-derived Neurotrophic Factor

CONT: Control group

CT: Combined training

MCI: Mild cognitive impairment

TrkB: tropomyosin receptor kinase B

T2DM: Type 2 Diabetes Mellitus

## References

Baker LD, Frank LL, Foster-Schubert K, et al (2010) Aerobic Exercise Improves Cognition for Older Adults with Glucose Intolerance, A Risk Factor for Alzheimer's Disease. *J Alzheimer's Dis* 22:569–579.

<https://doi.org/10.3233/JAD-2010-100768>

Best JR, Nagamatsu LS, Liu-Ambrose T (2014) Improvements to executive function during exercise training predict maintenance of physical activity over the following year. *Front Hum Neurosci* 8:1–9.

<https://doi.org/10.3389/fnhum.2014.00353>

Biessels GJ, Despa F (2018) Cognitive decline and dementia in diabetes mellitus: mechanisms and clinical implications. *Nat Rev Endocrinol* 14:591–604. <https://doi.org/10.1038/s41574-018-0048-7>

Booth FW, Roberts CK, Laye MJ (2012) Lack of Exercise Is a Major Cause of Chronic Diseases. In: *Comprehensive Physiology*. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp 1143–1211

Brinkmann C, Schäfer L, Masoud M, et al (2017) Effects of Cycling and Exergaming on Neurotrophic preliminary Investigation. *Exp Clin Endocrinol Diabetes*

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125:436–440. <https://doi.org/10.1055/s-0043-103967>

Cheng G, Huang C, Deng H, Wang H (2012) Diabetes as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies. *Intern Med J* 42:484–491.

<https://doi.org/10.1111/j.1445-5994.2012.02758.x>

Colberg SR, Sigal RJ, Yardley JE, et al (2016) Physical activity/exercise and diabetes: A position statement of the American Diabetes Association. *Diabetes Care* 39:2065–2079.

<https://doi.org/10.2337/dc16-1728>

Cooke S, Pennington K, Jones A, et al (2020) Effects of exercise, cognitive, and dual-task interventions on cognition in type 2 diabetes mellitus: A systematic review and meta-analysis. *PLoS One* 15:1–18.

<https://doi.org/10.1371/journal.pone.0232958>

DeFronzo RA, Ferrannini E, Groop L, et al (2015) Type 2 diabetes mellitus. *Nat Rev Dis Prim* 1:15019.

<https://doi.org/10.1038/nrdp.2015.19>

Diamond A (2013) Executive Functions. *Annu Rev Psychol* 64:135–168.

<https://doi.org/10.1146/annurev-psych-113011-143750>

Espeland MA, Lipska K, Miller ME, et al (2017) Effects of Physical Activity Intervention on Physical and Cognitive Function in Sedentary Adults With and Without Diabetes. *J Gerontol A Biol Sci Med Sci* 72:861–866. <https://doi.org/10.1093/gerona/glw179>

Expert Panel on Detection, Evaluation and T of HBC in A (2001) Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA J Am Med Assoc* 285:2486–2497. <https://doi.org/10.1001/jama.285.19.2486>

Feil DG, Zhu CW, Sultzer DL (2012) The relationship between cognitive impairment and diabetes self-management in a population-based community sample of older adults with Type 2 diabetes. *J Behav Med* 35:190–199. <https://doi.org/10.1007/s10865-011-9344-6>

Fujinami A, Ohta K, Obayashi H, et al (2008) Serum brain-derived neurotrophic factor in patients with type 2 diabetes mellitus: Relationship to glucose metabolism and biomarkers of insulin resistance. *Clin Biochem* 41:812–817. <https://doi.org/10.1016/j.clinbiochem.2008.03.003>

Geda YE, Roberts RO, Knopman DS, et al (2010) Physical Exercise, Aging, and Mild Cognitive Impairment. *Arch Neurol* 67:. <https://doi.org/10.1001/archneurol.2009.297>

Geroldi D, Minoretti P, Emanuele E (2006) Brain-derived neurotrophic factor and the metabolic syndrome: More than just a hypothesis. *Med Hypotheses* 67:195–196. <https://doi.org/10.1016/j.mehy.2006.02.001>

- Hristova MG (2013) Metabolic syndrome - From the neurotrophic hypothesis to a theory. *Med Hypotheses* 81:627–634. <https://doi.org/10.1016/j.mehy.2013.07.018>
- Intlekofer KA, Berchtold NC, Malvaez M, et al (2013) Exercise and sodium butyrate transform a subthreshold learning event into long-term memory via a brain-derived neurotrophic factor-dependent mechanism. *Neuropsychopharmacology* 38:2027–2034. <https://doi.org/10.1038/npp.2013.104>
- Jamali A, Shahrbanian S, Morteza Tayebi S (2020) The Effects of Exercise Training on the Brain-Derived Neurotrophic Factor (BDNF) in the Patients with Type 2 Diabetes: A Systematic Review of the Randomized Controlled Trials. *J Diabetes Metab Disord* 19:633–643. <https://doi.org/10.1007/s40200-020-00529-w>
- Johnson JK, Lui L-Y, Yaffe K (2007) Executive Function, More Than Global Cognition, Predicts Functional Decline and Mortality in Elderly Women. *Journals Gerontol Ser A Biol Sci Med Sci* 62:1134–1141. <https://doi.org/10.1093/gerona/62.10.1134>
- Krabbe KS, Nielsen AR, Krogh-Madsen R, et al (2007) Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. *Diabetologia* 50:431–438. <https://doi.org/10.1007/s00125-006-0537-4>
- Leckie RL, Oberlin LE, Voss MW, et al (2014) BDNF mediates improvements in executive function following a 1-year exercise intervention. *Front Hum Neurosci* 8:1–12. <https://doi.org/10.3389/fnhum.2014.00985>
- Levinger I, Goodman C, Matthews V, et al (2008) BDNF, metabolic risk factors, and resistance training in middle-aged individuals. *Med Sci Sports Exerc* 40:535–541. <https://doi.org/10.1249/MSS.0b013e31815dd057>
- Liu-Ambrose T, Nagamatsu LS, Graf P, et al (2010) Resistance training and executive functions: A 12-month randomized controlled trial. *Arch Intern Med* 170:170–178. <https://doi.org/10.1001/archinternmed.2009.494>
- Ma F, Wu T, Miao R, et al (2014) Conversion of mild cognitive impairment to dementia among subjects with diabetes: A population-based study of incidence and risk factors with five years of follow-up. *J Alzheimer's Dis* 43:1441–1449. <https://doi.org/10.3233/JAD-141566>
- Meek TH, Wisse BE, Thaler JP, et al (2013) BDNF action in the brain attenuates diabetic hyperglycemia via insulin-independent inhibition of hepatic glucose production. *Diabetes* 62:1512–1518. <https://doi.org/10.2337/db12-0837>
- Miranda M, Morici JF, Zanoni MB, Bekinschtein P (2019) Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological Brain. *Front Cell Neurosci* 13:1–25. <https://doi.org/10.3389/fncel.2019.00363>

- Nagamatsu LS, Handy TC, Hsu CL, et al (2012) Resistance training promotes cognitive and functional brain plasticity in seniors with probable mild cognitive impairment. *Arch Intern Med* 172:666–8. <https://doi.org/10.1001/archinternmed.2012.379>
- Neeper SA, Gómez-Pinilla F, Choi J, Cotman CW (1996) Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Res* 726:49–56. [https://doi.org/10.1016/0006-8993\(96\)00273-9](https://doi.org/10.1016/0006-8993(96)00273-9)
- Palta P, Schneider ALC, Biessels GJ, et al (2014) Magnitude of cognitive dysfunction in adults with type 2 diabetes: A meta-analysis of six cognitive domains and the most frequently reported neuropsychological tests within domains. *J Int Neuropsychol Soc* 20:278–291. <https://doi.org/10.1017/S1355617713001483>
- Pan W, Banks WA, Fasold MB, et al (1998) Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology* 37:1553–1561. [https://doi.org/10.1016/S0028-3908\(98\)00141-5](https://doi.org/10.1016/S0028-3908(98)00141-5)
- Paula JJ de, Costa MV, Andrade G de F de, et al (2016) Validity and reliability of a “simplified” version of the Taylor Complex Figure Test for the assessment of older adults with low formal education. *Dement Neuropsychol* 10:52–57. <https://doi.org/10.1590/s1980-57642016dn10100010>
- Rasmussen P, Brassard P, Adser H, et al (2009) Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Exp Physiol* 94:1062–1069. <https://doi.org/10.1113/expphysiol.2009.048512>
- Rozanska O, Uruska A, Zozulinska-Ziolkiewicz D (2020) Brain-derived neurotrophic factor and diabetes. *Int J Mol Sci* 21:. <https://doi.org/10.3390/ijms21030841>
- Sadanand S, Balachandar R, Bharath S (2016) Memory and executive functions in persons with type 2 diabetes: a meta-analysis. *Diabetes Metab Res Rev* 32:132–142. <https://doi.org/10.1002/dmrr.2664>
- Sanders LMJ, Hortobágyi T, Gemert S la B van, et al (2019) Dose-response relationship between exercise and cognitive function in older adults with and without cognitive impairment: A systematic review and meta-analysis. *PLoS One* 14:1–24. <https://doi.org/10.1371/journal.pone.0210036>
- Sartorius A, Hellweg R, Litzke J, et al (2009) Correlations and discrepancies between serum and brain tissue levels of neurotrophins after electroconvulsive treatment in rats. *Pharmacopsychiatry* 42:270–276. <https://doi.org/10.1055/s-0029-1224162>
- Silveira-Rodrigues JG, Pires W, Ferreira Gomes P, et al (2021) Combined exercise training improves specific domains of cognitive functions and metabolic markers in middle-aged and older adults with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 173:108700. <https://doi.org/10.1016/j.diabres.2021.108700>

Sinclair AJ, Girling AJ, Bayer AJ (2000) Cognitive dysfunction in older subjects with diabetes mellitus: impact on diabetes self-management and use of care services. All Wales Research into Elderly (AWARE) Study. *Diabetes Res Clin Pract* 50:203–12

Squire LR (2004) Memory systems of the brain: A brief history and current perspective. *Neurobiol Learn Mem* 82:171–177. <https://doi.org/10.1016/j.nlm.2004.06.005>

Sun Z, Yu J, Liu Y-L, et al (2018) Reduced Serum Levels of Brain-Derived Neurotrophic Factor Are Related to Mild Cognitive Impairment in Chinese Patients with Type 2 Diabetes Mellitus. *Ann Nutr Metab* 73:271–281. <https://doi.org/10.1159/000493275>

Swift DL, Johannsen NM, Myers VH, et al (2012) The effect of exercise training modality on serum brain derived neurotrophic factor levels in individuals with type 2 diabetes. *PLoS One* 7:1–7. <https://doi.org/10.1371/journal.pone.0042785>

Szuhany KL, Bugatti M, Otto MW (2015) A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. *J Psychiatr Res* 60:56–64. <https://doi.org/10.1016/j.jpsychires.2014.10.003>

Tang L, Kang Y, Yin B, Fan X-S (2017) Effects of weight-bearing ladder and aerobic treadmill exercise on learning and memory ability of diabetic rats and its mechanism. *Chinese J Appl Physiol* 33:436–440. <https://doi.org/10.12047/j.cjap.5570.2017.105>

Vedhara K, Wadsworth E, Norman P, et al (2004) Habitual prospective memory in elderly patients with Type 2 diabetes: implications for medication adherence. *Psychol Health Med* 9:17–27. <https://doi.org/10.1080/13548500310001637724>

Voss MW, Erickson KI, Shaurya R, et al (2013) Neurobiological markers of exercise-related brain plasticity in older adults. *Brain Behav Immun* 28:90–99. <https://doi.org/10.1016/j.bbi.2012.10.021>

Walsh JJ, Tschakovsky ME (2018) Exercise and circulating BDNF: Mechanisms of release and implications for the design of exercise interventions. *Appl Physiol Nutr Metab* 43:1095–1104. <https://doi.org/10.1139/apnm-2018-0192>

Zhen YF, Zhang J, Liu XY, et al (2013) Low BDNF is associated with cognitive deficits in patients with type 2 diabetes. *Psychopharmacology (Berl)* 227:93–100. <https://doi.org/10.1007/s00213-012-2942-3>

## Figures

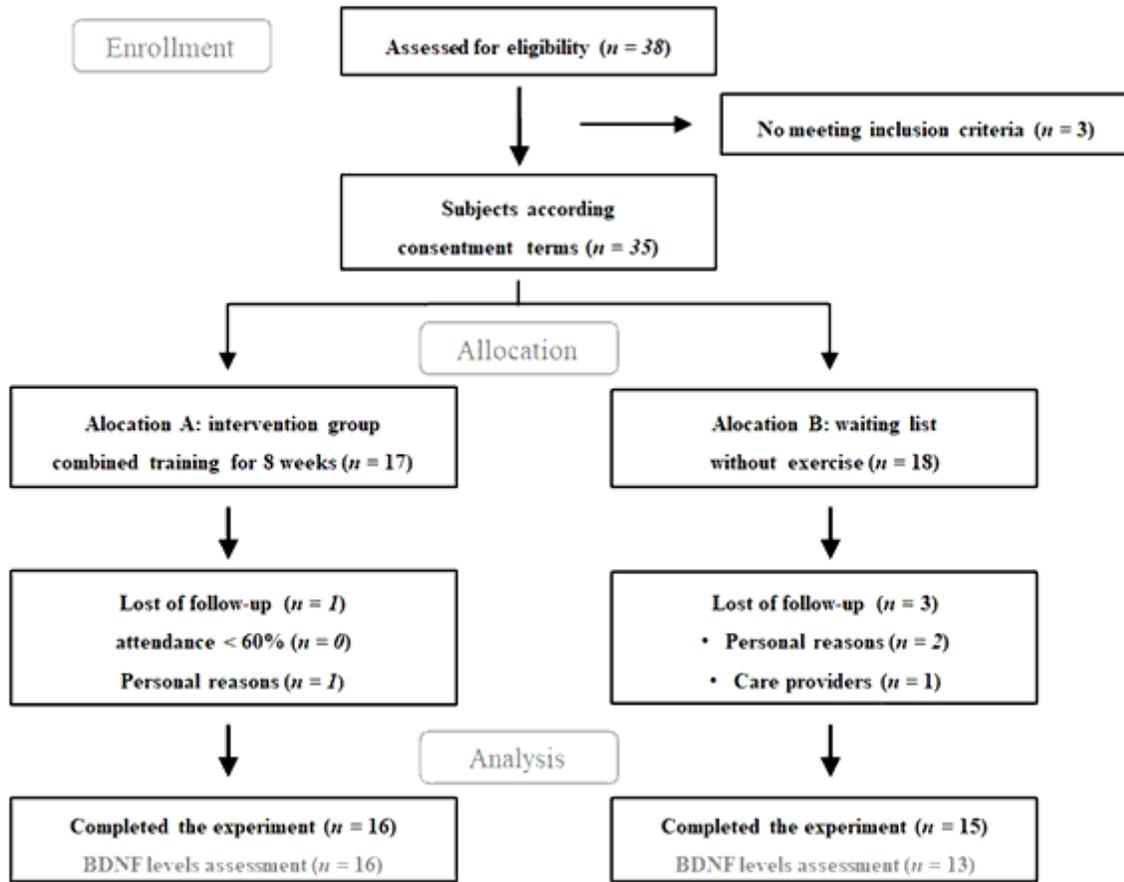


Figure 1

CONSORT Flow chart of the study.

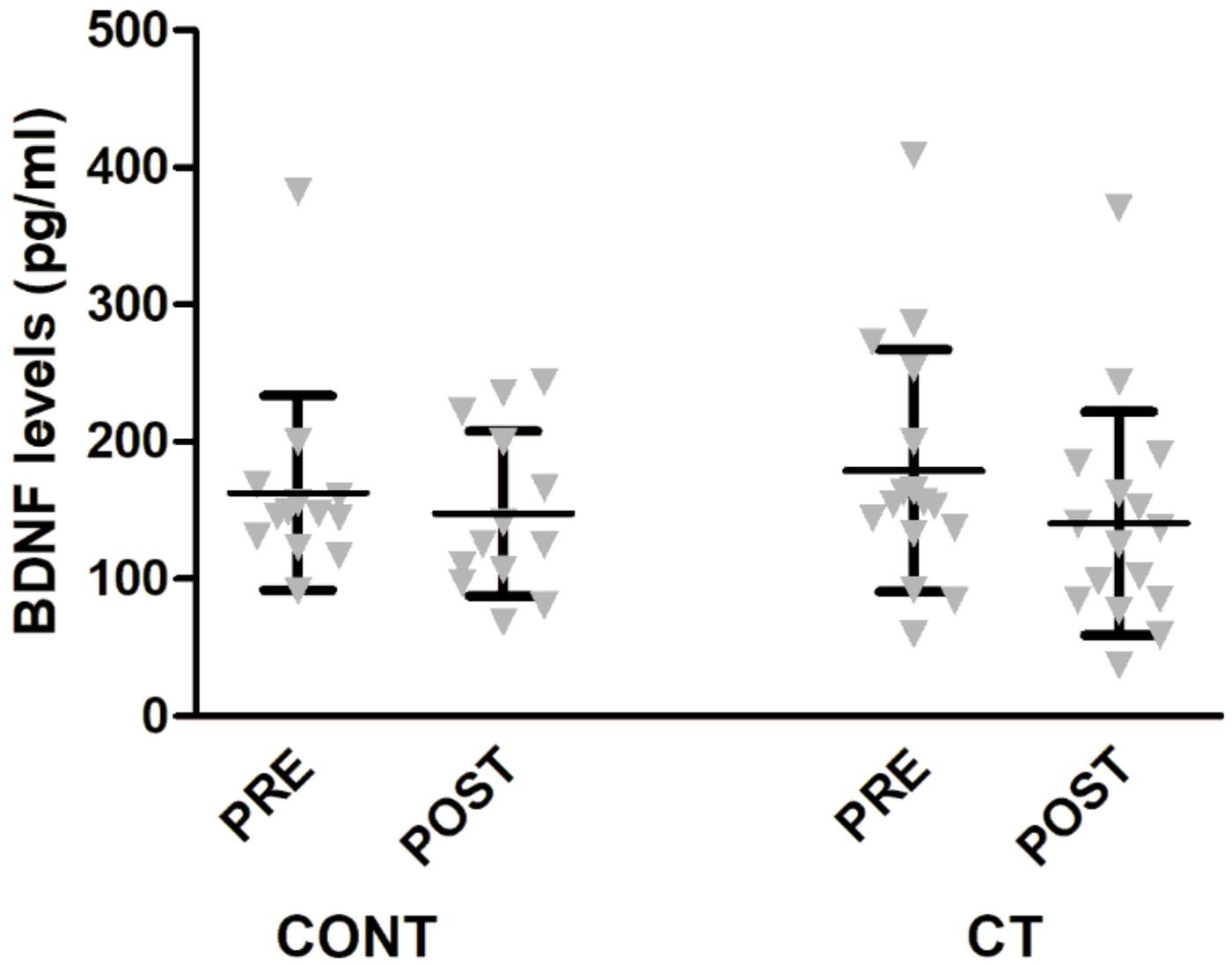


Figure 2

Pre- and post-intervention plasmatic BDNF levels (Combined training group, CT, n=16 and Control group, CONT, n=13). Values are mean  $\pm$  SD.

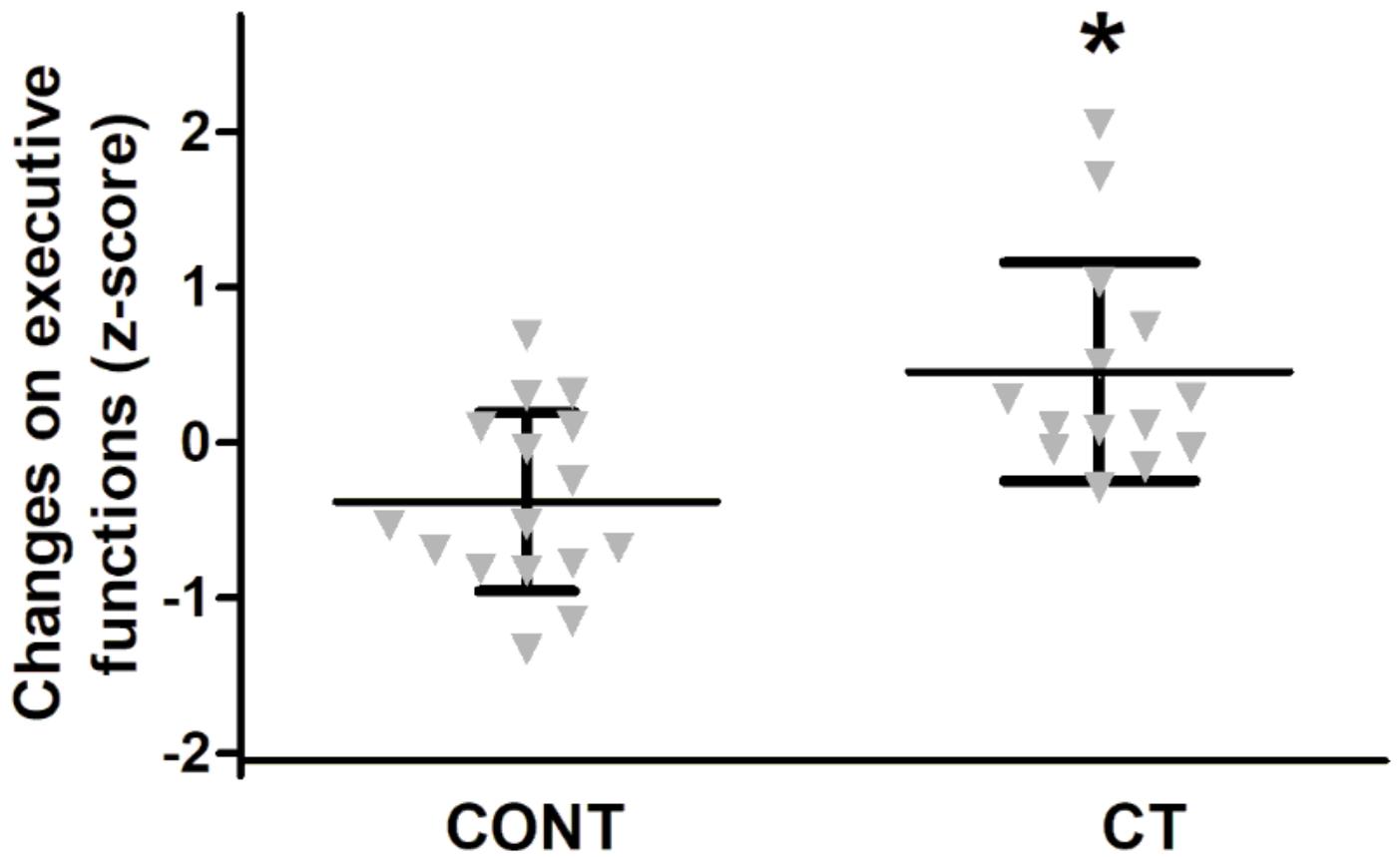


Figure 3

Changes (Post minus Pre) in composite score of executive functions (Combined training group, CT, n=16 and Control group, CONT, n=14). Values are mean  $\pm$  SD. \* indicates a significant difference ( $p < 0.05$ ) in unpaired Student's T-test.

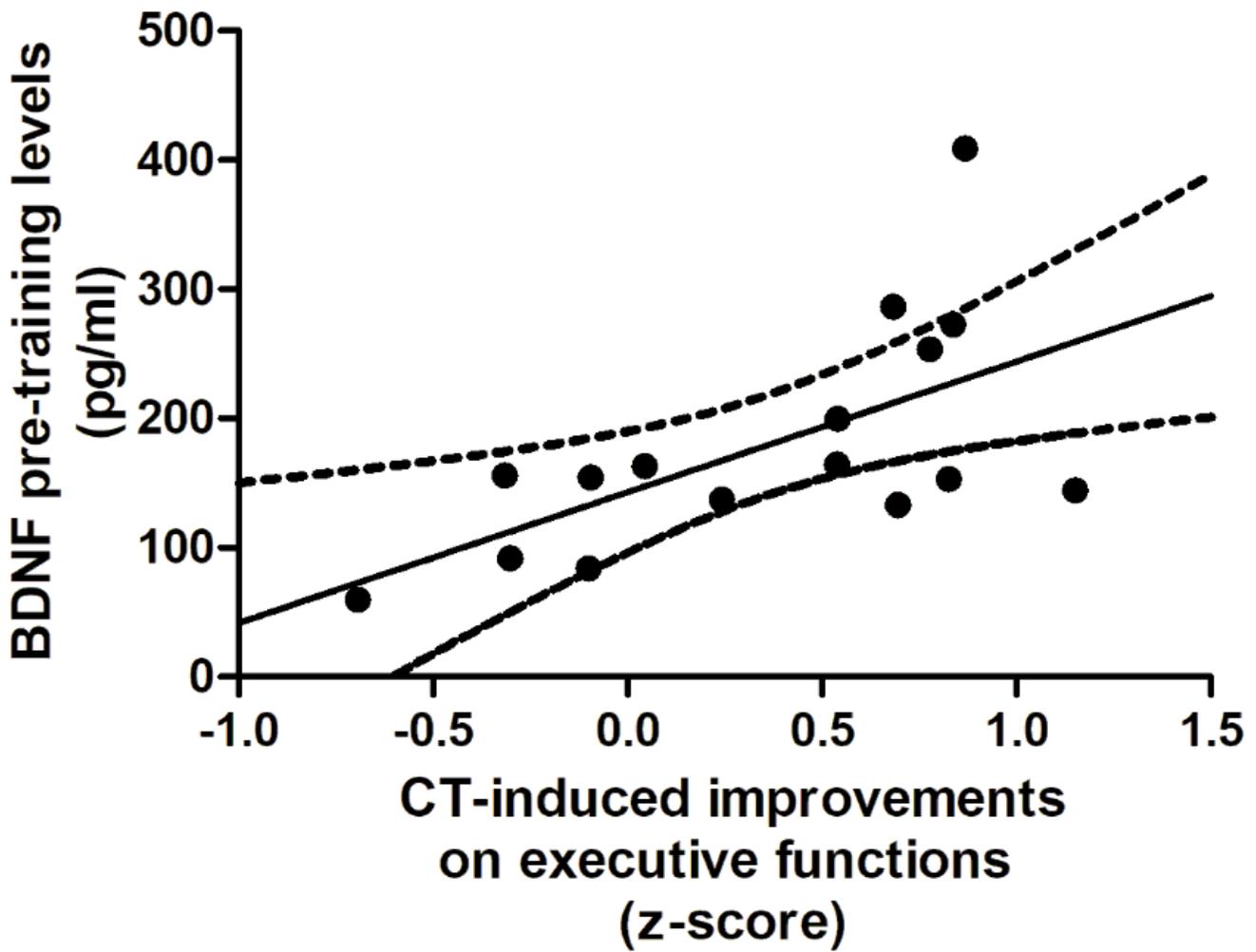


Figure 4

Relationship between pre-training plasma BDNF levels and composite z-score of CT-induced improvements on executive functions. Note: dotted line represents the 95% confidence interval. Regression equation: CT-induced improvements on executive functions =  $0.444 - (0.00461 * \text{pre-CT resting BDNF levels})$ .

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [OpenData.rar](#)