NEUROTROPHIC FACTORS AND BRAIN HEALTH IN CHILDREN WITH OVERWEIGHT AND OBESITY: THE ROLE OF CARDIORESPIRATORY FITNESS

Neurotrophic factors, brain health, and fitness

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NEUROTROPHIC FACTORS AND BRAIN HEALTH IN CHILDREN WITH OVERWEIGHT AND OBESITY: THE ROLE OF CARDIORESPIRATORY FITNESS

ABSTRACT

Neurotrophic factors and cardiorespiratory fitness are both considered important in developmental trajectories but their link to brain health remains poorly understood. The aims of the study were to examine whether levels of plasma-derived neurotrophic factors were associated with brain health indicators in children with overweight or obesity; and to test whether these associations were moderated by cardiorespiratory fitness. 100 children (41% girls) were included in this analysis. Plasma levels of brain-derived neurotrophic factor, insulin-like growth factor-1, vascular endothelial growth factor A, and epidermal growth factor were determined by XMap technology. Academic performance and executive function were assessed using validated neuropsychological tests. Hippocampal volume was measured using magnetic resonance imaging. Cardiorespiratory fitness was assessed using the 20-m Shuttle Run Test. Insulin-like growth factor-1 was positively associated with cognitive flexibility. Stratified analyses by fitness categories (i.e., unfit vs. fit) showed that brain-derived neurotrophic factor was positively associated with right posterior hippocampal volume in fit children, and epidermal growth factor was negatively associated with right hippocampal, and right anterior hippocampal volumes in their unfit peers, with a moderating role of cardiorespiratory fitness in these associations. However, all these significant associations disappeared after correction for multiple comparisons. The association between neurotrophic factors and brain health indicators in children with

overweight/obesity was neither strong nor consistent. These results could help enhance our understanding of determinants of brain health in children with overweight/obesity.

<u>Highlights</u>

- This study provides novel findings on the associations between neurotrophic factors and a wide range of brain health indicators in children.
- This study additionally explored the role of cardiorespiratory fitness in these associations.
- The association between neurotrophic factors and brain health in children with overweight/obesity was neither strong nor consistent.
- Cardiorespiratory fitness moderated the associations of brain-derived neurotrophic factor and epidermal growth factor with right hippocampal volume.

Keywords: growth factors, academic performance, cognition, hippocampus, obesity, childhood.

Abbreviations

BDNF, brain-derived neurotrophic factor

IGF-1, insulin-like growth factor-1

VEGFA, vascular endothelial growth factor A

EGF, epidermal growth factor

INTRODUCTION

Childhood obesity, an ongoing worldwide concern, is a major risk factor for the development of multiple diseases.[1] Prior research has suggested that excess body weight in childhood is linked to poorer academic and cognitive performance.[2] Likewise, childhood obesity has been associated with several brain-related alterations such as decreased regional gray matter volume and altered white matter microstructure.[3] In addition, both animal and human studies indicate that neurotrophic factors could be key molecules behind these relationships.[4] Particularly, neurotrophic factors have been related to certain brain processes,[5] which could be negatively influenced by overweight and obesity.[4] Indeed, children with overweight/obesity have shown lower levels of several neurotrophic factors than normal-weight peers.[6]

Neurotrophic factors are protein members of the nerve growth factor family, which are mainly expressed in the central nervous system in humans.[7] Animal and human research[8] has suggested that some neurotrophic factors such as brain-derived neurotrophic factor (BDNF) may be implicated in several brain processes including neuronal proliferation, differentiation and survival, neural morphology and function, synaptic changes (i.e., long-term potentiation in the hippocampus), and neuroplasticity.[5] Other neurotrophic factors such as insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor A (VEGFA), or epidermal growth factor (EGF) may be also important for brain.[9–11] For example, IGF-1 and VEGFA may support brain function by promoting neurogenesis and angiogenesis, as well as blood vessel remodelling, survival and growth,[9] while EGF has been mainly shown to influence the growth and survival of midbrain dopaminergic neurons[11] and to prevent amyloid-beta-induced damage to the brain, which in turn, may influence cognition.[10] Thus, apart from brain structure and

function outcomes, these neurotrophic factors may positively affect a range of other cognitive indicators, such as executive function and academic performance.

Prior research examining the relationship between circulating neurotrophic factors and brain health indicators in humans has been almost exclusively conducted in elderly populations, as well as in psychiatric patients, showing controversial results probably due to differences in methodological issues.[12–14] However, in normal developing children and adolescents, there is a scarcity of literature examining the association between neurotrophic factors and brain health indicators, which has focused only on BDNF and showing conflicting results. In preschool children, greater serum BDNF levels were associated with lower intelligence scores, but were unrelated to attentional capacity.[15] In contrast, serum BDNF was unrelated to subcortical brain volumes (i.e., amygdala and hippocampus) or cortical thickness in children and adolescents.[16] Unfortunately, none of the studies examined children with overweight/obesity, who have shown higher levels of neuroinflammation and poorer cognitive function.[2] In addition, there is no study regarding the association between neurotrophic factors and academic performance, either integrating a wide range of brain health indicators at micro and macro levels (i.e., cellular, brain, cognitive and academic indicators).

Likewise, the above-mentioned studies did not examine these associations in the context of other factors, such as cardiorespiratory fitness, known to influence neurotrophic factors and brain health. In this context, a recent meta-analysis has shown a positive association of cardiorespiratory fitness with academic performance, and particularly with higher-level academic abilities such as language/reading skills and mathematics skills, that are inherently reliant on memory functions.[17] In addition, cardiorespiratory fitness may specifically influence the hippocampus,[18,19] a brain region

critically involved in memory formation and spatial learning. In this sense, human studies suggest that cardiorespiratory fitness may play an important moderating role on hippocampal volumes across the lifespan.[19] Moreover, recent evidence in adolescents and adults showed that cardiorespiratory fitness may also positively influence circulating neurotrophic factors, although this relationship needs to be confirmed.[20]

Collectively, a better understanding of the links between neurotrophic factors, cardiorespiratory fitness, and brain health indicators could help shape our knowledge about the molecular mechanisms of optimizing and enhancing brain health during critical periods of brain development. In addition, given the aforementioned link of obesity with neurotrophic factors and brain health, more research is warranted in the particularly vulnerable population of children with overweight/obesity. Thus, our study aims to analyse the associations between plasma-derived neurotrophic factors (i.e., BDNF, IGF-1, VEGFA, and EGF) and brain health indicators (i.e., academic performance, executive function, and hippocampal volume) in children with overweight/obesity. In addition, given that cardiorespiratory fitness has been positively associated with neurotrophic factors[6,20] and brain outcomes,[17,19] we speculated that circulating levels of neurotrophic factors might be moderated by cardiorespiratory fitness levels to influence brain health indicators. To our knowledge, no previous studies have analysed the interaction effect of cardiorespiratory fitness on the study association. Therefore, a secondary aim was to investigate whether the association between plasma-derived neurotrophic factors and brain health indicators was moderated by cardiorespiratory fitness.

MATERIAL AND METHODS

Participants

The participants of the present cross-sectional study were part of the ActiveBrains project (http://profith.ugr.es/activebrains). Additional information about the methodology of the project can be found elsewhere.[21] The results presented in this cross-sectional analysis belong to the baseline data obtained between November 2014 and February 2016. From a total of 110 participants with overweight/obesity belonging to the ActiveBrains project, 100 participants (7.9-11.9 years, 41 girls) with at least valid data for one neurotrophic factor and one brain health indicator were included in the present study (84 participants had valid data for all variables of the study).

Parents or guardians were informed of the nature and characteristics of the study, and all signed an informed written consent. The ActiveBrains project was approved by the Human Research Ethics Committee of the University of Granada and was registered in ClinicalTrials.gov (identifier: NCT02295072).

Neurotrophic factors

After an overnight fast (at least 12 h), blood samples were drawn from the antecubital vein. Blood samples in tubes containing EDTA were spun immediately at 1000g for 10 min. Plasma was isolated and stored at -80°C until analyses in the Center of Biomedical Research (Granada, Spain).[22] The analysis of mature BDNF (μg/L), IGF-1 (ng/mL), VEGFA (pg/L), and EGF (pg/L) in plasma was performed using the Luminex IS 100/200 system (Luminex Corporation, Austin, TX), with the XMap technology and using human monoclonal antibodies (Milliplex Map Kit, Millipore, Billerica, MA). BDNF concentration was measured (Human Neurodegenerative Disease Magnetic Bead Panel 3; EMD Millipore Corporation, Billerica, U.S.A.) with a sensitivity of 0.23 pg/mL, and intra- and inter-assay precision coefficients of variation

(CVs) of <5.4% and <5.3%, respectively. The IGF-1 concentration was analysed (Human IGF-I, II Magnetic Bead Panel, Millipore Corporation, Billerica, U.S.A.) with a sensitivity of 15 pg/mL and intra- and inter-assay CVs of <10% and <15%, respectively. VEGFA and EGF were quantified (Human Angiogenesis / Growth Factor Magnetic Bead Panel 1, EMD Millipore Corporation, Billerica, U.S.A.) with a sensitivity of 8.1 pg/mL for VEGFA, and 1.0 pg/mL for EGF. The intra- and inter-assay precision CVs were 3.5% and 10% for VEGFA, and 3.2% and 6.8% for EGF, respectively.

Academic performance

Academic performance was assessed using the Spanish version of the Woodcock-Johnson III, the "Bateria III Woodcock-Muñoz Test of Achievement", a well-normed measure of academic performance which has shown high reliability and validity.[23] Thirteen tests were individually administered in one session of 100-120 min, and the obtained data were processed using the Compuscore and profile software version 3.1 (Riverside Publishing Company, Itasca, IL, USA). A standard T-score based on an average of 100 and standard deviations of 15 points was obtained for the following broad academic performance indicators: mathematics, reading, writing, and total achievement.

Executive function

The assessment of executive function was conducted individually for each child, and lasted approximately 45-60 min. Three main indicators were assessed: cognitive flexibility, cognitive inhibition, and working memory, and a broad description can be found elsewhere.

Cognitive flexibility and cognitive inhibition were assessed using two paper-pencil based sub-tests of the Delis-Kaplan Executive Function System (D–KEFS).[24] For cognitive flexibility, we used the Design Fluency Test (DFT),[24] in which participants

were instructed to connect dots using only four straight lines to design as many novel shapes as possible in periods of 60 seconds. The total number of correctly drawn designs across three different conditions was registered and used in the analysis.

For cognitive inhibition, we used a modified version of the Stroop test[25] called the Stroop Colour Word Test, which includes measurements of 1) fundamental linguistic skills (i.e., namely speed of naming), and 2) inhibition, where colour-words are printed in a colour that differs from their meaning, and the task consists of naming the colour of the word and avoiding reading. An interference score was obtained by subtracting completion times (2-1). Because the Stroop interference scores are inversely related to cognitive inhibition, it was multiplied by -1, so that positive scores are indicative of better performance.

Working memory was measured using a modified version of the Delayed Non-Match-to-Sample (DNMS) computerized task, that was previously developed to differentiate between manipulation (high memory load) and maintenance (low memory load) cognitive processes.[26] Each trial was presented on a computer screen using E-Prime and consisted of two phases: sample and choice. Sixteen practice trials plus 140 experimental trials were randomly presented. Participants had to remember 4 sequential Pokémon cartoons (i.e., sample phase), and subsequently, select the cartoon that had not previously appeared (i.e., choice phase) between two different targets. In the high memory load condition, in which the 4 stimuli presented before the choice phase were all different, reaction time and response accuracy were registered. A ratio of working memory was calculated as the quotient between reaction time and response accuracy, and for analytic purposes, this ratio was multiplied by -1, so that a higher ratio indicates better working memory performance.

Hippocampal volume

All images were collected on a 3.0 Tesla Siemens Magnetom Tim Trio scanner (Siemens Medical Solutions, Erlangen, Germany) with a 32-channel head coil. High-resolution T1-weighted images were acquired using a 3D MPRAGE (magnetization-prepared rapid gradient-echo) protocol.[18] Acquisition parameters were: repetition time (TR) = 2300 ms, echo time (TE) = 3.1 ms, inversion time (TI) = 900 ms, flip angle = 9º, field of view (FOV) = 256 x 256, acquisition matrix = 320 x 320, 208 slices, resolution = 0.8 x 0.8 x 0.8 mm, and scan duration of 6 min and 34 s.

Hippocampal volume was calculated using FMRIB's Integrated Registration and Segmentation Tool (FIRST), a semi-automated model-based segmentation tool in FMRIB's Software Library (FSL) version 5.0.7. FIRST uses a Bayesian framework from shape and appearance models obtained from manually segmented images from the Center for Morphometric Analysis, Massachusetts General Hospital, Boston, MA, USA. Briefly, FIRST runs a two-stage affine registration to a standard space template (i.e., Montreal Neurological Institute –MNI– space) using 12 degrees of freedom and uses a subcortical mask to exclude voxels outside subcortical regions. Second, the hippocampus was segmented for both hemispheres. Manual volumetric region labels are parameterized as surface meshes and modeled as a point distribution model. In addition, the hippocampus segmentation from FIRST was then split based on the center of gravity into anterior and posterior sub-regions for each hemisphere separately. This resulted in separate anterior and posterior hippocampal segmentations for each participant, for each hemisphere.[27] The final segmentations were visually inspected for quality. The volume of each region was obtained from FIRST in mm^3.

Cardiorespiratory fitness

Cardiorespiratory fitness was assessed using the 20-m Shuttle Run Test. The final number of completed laps was used in the analyses. For analytic purposes, and to

achieve two homogeneous and comparable groups, participants were classified according to the sex-specific 50th percentile in the present sample (i.e., unfit vs. fit).

Covariates

Sex, peak height velocity (PHV), parental education level, and body mass index (BMI) were included as covariates.

PHV was obtained from weight, height and seated height using Moore's equations.[28] Years from PHV were calculated from the chronological age, and the difference in years was used in the analysis as a value of maturation.

Parental education level was used as a proxy of socioeconomic status. Parent responses were combined as: neither of the parents had a university degree, one of the parents had a university degree, and both parents had a university degree.

Body weight was measured to the nearest 0.1 kg using an electronic scale (SECA 861, Hamburg, Germany) lightly dressed and without shoes. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (SECA 225, Hamburg, Germany). Measures were assessed in duplicate and average measures were used for data analysis. BMI was calculated as weight/height square (kg/m^2), and BMI categories were defined as overweight or obesity according to age- and sex-specific BMI cut-off points.[29]

Total brain volume (cm^3) was calculated from the non-normalized segmented images by adding the volumes of gray and white matter.

Statistical analysis

Descriptive characteristics are shown as mean and standard deviations (SD) or percentages. Prior to all analyses, variables were checked for normality using both graphical (normal probability plots) and statistical (Kolmogorov-Smirnov test) procedures. Due to its skewed distribution, plasma-derived neurotrophic factors,

cognitive inhibition, and working memory were normalized using Blom's formula before analysis.[30]

Multiple linear regression was used to examine the association of plasma-derived neurotrophic factors (i.e., BDNF, IGF-1, VEGFA, and EGF) with brain health indicators (i.e., academic performance, executive function, and hippocampal volume). Analyses were adjusted for sex, PHV, parental education level, and BMI. In addition, when analyzing the associations between plasma-derived neurotrophic factors and hippocampal volume, analyses were further adjusted by total brain volume. Each neurotrophic factor was entered as the predictor variable and each brain health indicator was entered as dependent variable in separate models.

The interaction term was calculated to examine whether the associations of plasma-derived neurotrophic factors with brain health indicators were moderated by the categories of cardiorespiratory fitness (i.e., unfit vs. fit). Additionally, we conducted linear regression models to study the association between plasma-derived neurotrophic factors and brain health indicators by cardiorespiratory fitness categories (i.e., unfit vs. fit).

We conducted false discovery rate (FDR) correction (Benjamini and Hochberg correction on multiple testing) for assessing multiple comparisons between plasma-derived neurotrophic factors and brain health indicators. Briefly, this method uses ranked p-values to determine the cut-off, at which point the Type-I error rate is below 0.05.[31] All analyses were performed using SPSS software (version 24.0, IBM Corporation), and the level of significance was set at $p < 0.050$.

RESULTS

Table 1 presents the descriptive characteristics of the study sample by sex. Participants were 10.0 ± 1.2 years old and had an average BMI of 26.6 kg/m², 26% with overweight and 74% with obesity.

The results of the linear regression analysis showing the association between plasma-derived neurotrophic factors and brain health indicators are presented in **Table 2**. In short, plasma IGF-1 was significantly associated with cognitive flexibility ($R^2=0.316$, $\beta=0.278$; $p=0.002$). However, this significant association disappeared after multiple comparisons correction. There were no other statistically significant associations of any neurotrophic factor with academic performance, executive function, and hippocampal volumes (all $p>0.05$).

The interaction terms of cardiorespiratory fitness with neurotrophic factors in relation to brain health indicators and stratified analyses by cardiorespiratory fitness categories (unfit vs. fit) examining the association between plasma-derived neurotrophic factors and brain health indicators are shown in **Table 3**. Analyses showed no significant interactions of cardiorespiratory fitness with neurotrophic factors in relation to academic performance and executive function indicators (all $p>0.05$). However, we found significant interactions of cardiorespiratory fitness with plasma BDNF and EGF in relation to several hippocampal volume variables (i.e., hippocampus, right hippocampus, right anterior hippocampus, and right posterior hippocampus; interaction p-values<0.05). Specifically, among fit participants, high levels of these neurotrophic factors were associated with larger hippocampal volumes. However, for unfit participants, higher levels of these neurotrophic factors were related to lower hippocampal volumes.

Our stratified results showed that plasma IGF-1 was positively associated with cognitive flexibility in unfit children with overweight/obesity (R^2=0.309, β=0.449; p=0.003). In addition, plasma BDNF concentration was positively associated with right posterior hippocampal volume (R^2=0.407, β=0.272; p=0.039) in fit children with overweight/obesity. Likewise, plasma EGF concentration was negatively associated with right hippocampal (R^2=0.447, β=-0.266; p=0.029) and right anterior hippocampal (R^2=0.474, β=-0.298; p=0.013) volumes in unfit children with overweight/obesity. **Figure 1** graphically showed these associations of plasma BDNF and EGF levels with right hippocampal volumes by cardiorespiratory fitness categories. However, after correcting for multiple comparisons, all the significant associations found in this study disappeared. There were no statistically significant associations between plasma VEGFA and any brain health indicator.

DISCUSSION

This study investigated the association between several plasma-derived neurotrophic factors (i.e., BDNF, IGF-1, VEGFA and EGF) and a wide range of brain health indicators (i.e., academic performance, executive function and hippocampal volume), as well as the moderating role of cardiorespiratory fitness on these associations. We hypothesized that greater concentrations of plasma neurotrophic factors would be associated with better brain health indicators. Consistent with this hypothesis, we only found that higher levels of plasma IGF-1 were associated with better cognitive flexibility. With respect to our second hypothesis, we found that cardiorespiratory fitness moderated the association of plasma BDNF and EGF with hippocampal volume. Specifically, higher plasma BDNF levels were associated with larger right hippocampal volumes in fit children. In addition, higher levels of plasma EGF were associated with smaller right hippocampal volumes in unfit children. However, all these significant associations disappeared after multiple comparisons correction. Our results expand the existing knowledge about the complex interaction between neurotrophic factors, brain health indicators, and cardiorespiratory fitness in children.

Regarding academic performance and executive function, our results only indicated a positive association between plasma IGF-1 and cognitive flexibility in children with overweight and obesity, which showed to be stronger in unfit children and disappeared when correcting for multiple comparisons. To our knowledge this is the first study aimed to examine the association of peripheral neurotrophic factors with academic performance or executive function in youth, which hampers comparisons to other studies. Studies in psychiatric patients and older adults[14,32] are partially in line with this finding. For example, in Parkinson's disease patients, Picillo et al.[14] showed that

low levels of serum IGF-1 were associated with poor executive function, including cognitive flexibility. Similarly, Bellar et al.[32] reported a positive association between serum IGF-1 and cognitive flexibility in healthy older adults. Thus, we speculate that it is likely that, during childhood, in a developing brain, IGF-1 exerts neurotrophic effects in specific parts of the brain that support cognitive flexibility function (but not cognitive inhibition and working memory). More evidence in children is required to confirm or refute this hypothesis.

Inconsistent with our hypotheses, we found that plasma BDNF, VEGFA and EGF were not associated with academic performance or executive function. Although evidence regarding VEGFA and EGF in youths is not available, our results using plasma concentration of neurotrophic factors partially concur with Yeom et al.,[15] who apart from reporting an inverse association of serum BDNF with intelligence, also found a null association between plasma BDNF with intelligence and attention capacity in preschool children. Conversely, in athletic young adults, Belviranli et al.[33] found a positive correlation of plasma BDNF levels with cognitive functioning and verbal fluency. A possible explanation for the lack of associations in children with overweight/obesity found in the present study could be that physiological normal levels of neurotrophic factors may be helpful for cognition, but a reduced level due to their overweight/obesity condition might induce inhibitory or excitatory neurotransmission in the brain altering the studied associations.[34] Since little is known regarding these associations in children, more theoretical and practical research is needed to understand how neurotrophic factors are related to complex higher-order cognition, as well as their relationship with academic performance, in the developing brain.

Our study revealed no association of plasma-derived neurotrophic factors with hippocampal volumes in children with overweight/obesity in contrast to findings in

patient and adult populations, which suggested that BDNF could be related to brain structure, specifically to hippocampal regions.[8,32] However, additional analysis by cardiorespiratory fitness categories (unfit vs. fit) indicated a positive association between plasma BDNF levels and right posterior hippocampal volume in fit children with overweight/obesity, and negative associations of plasma EGF with right hippocampal and right anterior hippocampal volumes in their unfit peers. Although these associations disappeared after multiple comparisons correction, our findings complement prior knowledge by suggesting that cardiorespiratory fitness may play a key role in the association between neurotrophic factors and hippocampal structure throughout the lifespan.

Prior literature investigating the association between circulating neurotrophic factors and brain structure, and specifically hippocampal volume, in youths remains scant. De Araujo et al.[16] reported a null association of peripheral serum BDNF concentrations with subcortical volumes (i.e., left and right amygdala and hippocampus) and cortical thickness in children and adolescents. Likewise, in another study carried out in children and adolescents with bipolar disorder, peripheral serum BDNF levels were not correlated with either left, right or total hippocampal volumes.[35] Similarly, Mansur et al.[12] reported a lack of association between serum BDNF and hippocampal volume in children. Nevertheless, it is important to point out that none of the reviewed studies measured plasma BDNF levels nor considered cardiorespiratory fitness in their analyses. The mechanisms underlying the moderating role of cardiorespiratory fitness in the association between plasma BDNF and EGF with right hippocampal volumes in the present study remains to be determined. However, we speculate that aerobic exercise (i.e., planned and structured physical activity), which has been directly related to cardiorespiratory fitness, may increase hippocampal volume,[27] and neurotrophic factors

in the hippocampus.[9] These molecules may cross the blood-brain barrier, showing a positive association with this specific brain region in fit children. Moreover, since these neurotrophic factors are also stored and released by platelets, it is likely that any attribute closely linked to cardiorespiratory fitness (e.g., physical activity), which affects platelet functioning, might influence BDNF and EGF levels. Indeed, in a previous study with the present sample, we found that physical activity was positively associated with BDNF, but not with IGF-1 and VEGFA.[22] Likewise, cardiorespiratory fitness was positively associated with hippocampal volume in our[18] and other samples in children.[19] Taken together, further studies should consider cardiorespiratory fitness when analysing the association between neurotrophic factors and brain structure.

Beyond these observations, the relative and divergent contribution of BDNF and EGF on right hippocampal volumes cannot be elucidated in the present study; however, we suggest some possible explanations behind these associations. On the one hand, neurotrophic factors may influence hippocampal volume by influencing cell proliferation and survival since animal studies have shown that these molecules are closely linked to hippocampal neurogenesis.[9] On the other hand, the fact that we only found associations with right hippocampal volume could be due to differences between left and right hippocampus regarding its structure and neurotrophic factor expression.[36,37] For example, prior evidence found that children presented a larger right hippocampus, compared to the left, showing an asymmetric hippocampal structural development,[36] which is also shown in the present study. Animal models have also shown that BDNF was asymmetrically expressed in the hippocampus,[37] and specifically reported higher BDNF gene expression in the right hippocampus compared to the left.[38] Thus, it is reasonable to believe that the influence of neurotrophic factors on hippocampal volume may show hippocampal lateralization. For a better understanding of the association

between neurotrophic factors and brain volumes, future studies should extend this work beyond the hippocampus to other brain regions involved in academic performance and executive function.

Our results should be interpreted with caution due to differences in methodological issues (e.g., the study design, analysis techniques), the multifactorial nature of academic performance and executive function, as well as to the specific characteristics of our sample (i.e., children with overweight or obesity). In this context, the fact that this population may present difficulties to perform physical activity and that has shown lower levels of neurotrophic factors compared to normal-weight children,[6,22] could also have influenced the results such that there could be an attenuated or exaggerated physiological response to physical activity. In addition, it is likely that our study was underpowered to determine statistically significant results, which also hinders direct comparisons across studies. Importantly, different results could be due to the matrix in which neurotrophic factors are measured (serum vs. plasma). Indeed, in the case of BDNF, some studies have indicated that plasma BDNF is a reliable indicator of brain BDNF levels,[39] while serum BDNF reflects the concentration of BDNF stored by platelets during illness or treatment periods.[13] Accordingly, we measured neurotrophic factors in plasma. Secondly, the kit used in other studies (e.g. ELISA) may not allow to differentiate between mature BDNF or pro-BDNF, which exert different and opposed functions.[40] Lastly, genetic features may influence the associations between neurotrophic factors and brain health indicators. Therefore, future studies should include genetic variants, as well as neurotrophic factors measured in both serum and plasma, and different brain health indicators, to gain a better understanding of the associations.

Overall, taking our results into account, we speculate that although neurotrophic factors may play a key role in brain health in youths,[33] the positive influence of these

molecules on brain health may only become evident during adulthood and old age. Thus, healthy lifestyle promotion programs designed to improve brain health in the long-term should take into account cardiorespiratory fitness and neurotrophic factor levels. In this sense, early identification of low levels of cardiorespiratory fitness would be important in order to provide personalized assistance aimed to enhance brain health indicators.

Limitations and strengths

The current study has some limitations that must be mentioned. First, the cross-sectional design of our analyses prevents us from inferring causal relationships. In addition, our study only investigated children with overweight and obesity, which limits the generalizability of our findings. Likewise, our analyses need replication with a larger sample size in order to confirm and expand upon the study associations. Lastly, inflammatory biomarkers closely linked to overweight and obesity were not considered in this study, but they could influence the expression of neurotrophic factors and brain health indicators. On the other hand, the main strength of this study was its novelty, being the first one to examine the association of several plasma-derived neurotrophic factors with academic performance, executive function and hippocampal volume, as well as the role of cardiorespiratory fitness on these associations in children with overweight/obesity. Additional strengths comprise the use of the most advanced techniques to quantify neurotrophic factors (i.e., Luminex 200), and the inclusion of a wide range of brain health indicators assessed through validated tools.

Conclusions

The association between plasma-derived neurotrophic factors and brain health indicators in children with overweight/obesity was neither robust nor consistent. Our findings suggest that only plasma IGF-1 was positively associated with cognitive

flexibility, showing null associations of plasma BDNF, VEGFA and EGF with academic performance and executive function. In addition, this study revealed that plasma BDNF and EGF (but not IGF-1 and VEGFA) were associated with right hippocampal volumes only in sub-groups of fitness. However, all these significant associations disappeared after correction for multiple comparisons. Cardiorespiratory fitness only showed a moderating role in the associations of plasma BDNF and EGF with right hippocampal volume. These results contribute to increasing the knowledge of factors influencing cognition and brain structure in children with overweight/obesity, which could help enhance our understanding of determinants of brain health.

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Competing interests. The authors declare that they have no competing interests.

Data accessibility. We did not obtain children's parents consent to widely share the data nor was it included in the IRB protocol.

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Table 1. Descriptive characteristics of the study sample.

	n	All	Boys	Girls
		100	59	41
Physical characteristics	100			
Age (years)		10.0 ± 1.2	10.2 ± 1.2	9.9 ± 1.1
Peak height velocity (years)		-2.3 ± 1.0	-2.7 ± 0.8	-1.7 ± 1.0
Weight (kg)		55.4 ± 10.7	56.1 ± 10.3	54.3 ± 11.2
Height (cm)		143.8 ± 8.4	144.3 ± 7.8	142.9 ± 9.3
Body mass index (kg/m^2)		26.6 ± 3.5	26.8 ± 3.6	26.3 ± 3.4
Body mass index category (%)				
Overweight		26	26	27
Obesity grade (I/II/III)		46/19/9	49/15/10	42/24/7
Cardiorespiratory fitness (laps)	100	16.0 ± 7.7	17.1 ± 8.0	14.4 ± 7.1
Parental education university level (Neither/ One/ Both parents) (%)	100	64/19/17	71/15/14	54/24/22
Neurotrophic factors [a]				
Brain-derived neurotrophic factor (μg/L)	99	4.7 ± 5.0	4.4 ± 4.5	5.2 ± 5.6
Insulin-like growth factor 1 (ng/mL)	100	95.8 ± 38.3	86.1 ± 28.6	109.8 ± 45.8
Vascular endothelial growth factor A (pg/L)	98	54.6 ± 53.7	51.8 ± 46.2	58.6 ± 63.5
Epidermal growth factor (pg/L)	92	8.3 ± 18.7	8.9 ± 19.0	7.4 ± 18.4
Academic performance [b]	100			
Mathematics		101.6 ± 10.6	102.0 ± 11.1	101.1 ± 9.9
Reading		108.1 ± 12.9	108.4 ± 11.0	107.6 ± 15.4
Writing		113.9 ± 12.3	113.0 ± 11.1	115.2 ± 13.8
Total achievement		109.2 ± 11.7	109.1 ± 10.4	109.3 ± 13.4
Executive Function	100			
Cognitive flexibility [c]		19.8 ± 6.5	20.4 ± 6.8	19.0 ± 6.1
Cognitive inhibition [a, d]		-41.0 ± 17.4	-38.9 ± 14.9	-44.0 ± 20.2
Working memory [a, e]		15.2 ± 6.9	15.0 ± 6.8	15.5 ± 7.0
Hippocampal volume (mm^3)	95			
Hippocampus		7019.2 ± 645.1	7185.2 ± 613.5	6780.7 ± 620.9
Left hippocampus		3442.4 ± 354.6	3511.1 ± 363.9	3343.7 ± 320.1
Left anterior hippocampus		1981.4 ± 211.0	2023.6 ± 210.7	1920.8 ± 198.9
Left posterior hippocampus		1461.0 ± 154.2	1487.5 ± 163.3	1423.0 ± 133.0
Right hippocampus		3576.8 ± 372.5	3674.2 ± 365.9	3436.9 ± 339.8
Right anterior hippocampus		2053.0 ± 234.8	2106.4 ± 231.8	1976.3 ± 220.0
Right posterior hippocampus		1522.9 ± 146.8	1567.8 ± 145.7	1458.5 ± 124.1
Total brain volume (cm^3)	95	1195.7 ± 101.5	1238.5 ± 84.1	1134.2 ± 93.0

Table 2. Linear regression analyses examining the associations of plasma-derived neurotrophic factors with brain health indicators in children with overweight/obesity.

	BDNF [a]			IGF-1 [a]			VEGFA [a]			EGF [a]		
	n	β	p	n	β	p	n	β	p	n	β	p
Academic performance	99			100			98			92		
Mathematics		0.009	0.923		0.075	0.462		0.057	0.553		0.031	0.754
Reading		-0.021	0.821		0.028	0.781		-0.127	0.180		-0.150	0.127
Writing		-0.005	0.959		0.148	0.152		-0.106	0.278		-0.028	0.781
Total achievement		0.004	0.968		0.085	0.389		-0.042	0.655		-0.047	0.625
Executive function	99			100			98			92		
Cognitive flexibility		0.034	0.702		0.278	**0.002**		0.000	0.999		0.028	0.759
Cognitive inhibition [a, b]		-0.005	0.954		0.060	0.541		-0.042	0.647		-0.029	0.764
Working memory [a, b]		-0.032	0.759		-0.132	0.230		0.033	0.752		-0.038	0.725
Hippocampal volume [c]	95			96			94			88		
Hippocampus		0.009	0.908		-0.038	0.653		0.083	0.297		-0.005	0.950
Left hippocampus		0.011	0.905		-0.037	0.695		0.135	0.129		0.064	0.490
Left anterior hippocampus		-0.024	0.796		-0.040	0.676		0.102	0.251		0.032	0.731
Left posterior hippocampus		0.057	0.540		-0.032	0.748		0.172	0.061		0.103	0.285
Right hippocampus		0.006	0.946		-0.031	0.732		0.015	0.853		-0.069	0.440
Right anterior hippocampus		-0.033	0.974		-0.055	0.542		0.011	0.892		-0.103	0.254
Right posterior hippocampus		0.020	0.817		0.012	0.898		0.026	0.762		-0.016	0.857

Analyses were adjusted by sex, peak height velocity, parental education level, and body mass index. [c] Further adjusted by total brain volume. β: standardized regression coefficients. Statistically significant associations that are shown in bold disappeared when p values were adjusted for multiple comparisons using the Benjamini-Hochberg method. BDNF: Brain-derived neurotrophic factor; IGF-1: Insulin-like growth factor 1; VEGFA: Vascular endothelial growth factor A; EGF: Epidermal growth factor. [a] Blom's normalized values were used in the analysis. [b] The original score was multiplied by -1 to invert the variable, so that a higher score indicates a higher executive function.

Table 3. Associations between plasma-derived neurotrophic factors and brain health indicators by cardiorespiratory fitness categories in children with overweight/obesity.

		BDNF [a]					IGF-1 [a]					VEGFA [a]					EGF [a]				
		Unfit		Fit			Unfit		Fit			Unfit		Fit			Unfit		Fit		
		β	p	β	p	p_i	β	p	β	p	p_i	β	p	β	p	p_i	β	p	β	p	p_i
Academic performance	n	46		53			47		53			47		51			45		47		
Mathematics		-0.040	0.788	-0.061	0.620	0.953	0.042	0.802	0.030	0.815	0.772	-0.117	0.431	-0.223	0.078	0.547	-0-065	0.670	-0.229	0.097	0.338
Reading		0.001	0.995	-0.004	0.977	0.879	0.119	0.476	0.053	0.692	0.673	0.001	0.993	0.099	0.450	0.581	-0.045	0.769	0.159	0.238	0.513
Writing		0.120	0.438	-0.072	0.585	0.295	0.156	0.360	0.099	0.465	0.842	-0.004	0.981	-0.152	0.244	0.560	0.118	0.445	-0.090	0.512	0.217
Total achievement		0.042	0.778	-0.045	0.719	0.722	0.100	0.551	0.069	0.592	0.623	-0.028	0.851	-0.071	0.576	0.872	-0.011	0.942	-0.025	0.848	0.732
Executive function	n	46		53			47		53			47		51			45		47		
Cognitive flexibility		0.151	0.283	-0.010	0.938	0.381	0.449	**0.003**	0.151	0.247	0.193	0.097	0.490	-0.027	0.834	0.601	0.218	0.127	-0.113	0.428	0.093
Cognitive inhibition [a, b]		-0.195	0.210	0.059	0.693	0.246	0.090	0.607	0.103	0.508	0.846	-0.259	0.088	0.063	0.680	0.141	-0.056	0.724	0.017	0.918	0.966
Working memory [a, b]		-0.225	0.140	0.165	0.258	0.081	-0.171	0.317	-0.121	0.423	0.640	-0.048	0.751	0.104	0.478	0.475	-0.100	0.522	0.008	0.961	0.524
Hippocampal volume	n	44		51			45		51			45		49			43		45		
Hippocampus		-0.125	0.254	0.157	0.236	0.091	0.051	0.672	-0.133	0.290	0.407	0.039	0.719	0.175	0.155	0.507	-0.177	0.103	0.244	0.092	**0.034**
Left hippocampus		-0.045	0.687	-0.004	0.977	0.773	0.103	0.390	-0.130	0.363	0.209	0.157	0.145	0.124	0.381	0.730	-0.062	0.572	0.154	0.359	0.332
Left anterior hippocampus		-0.070	0.551	-0.037	0.809	0.779	0.113	0.362	-0.161	0.271	0.198	0.127	0.254	0.100	0.490	0.773	-0.051	0.654	0.073	0.673	0.620
Left posterior hippocampus		-0.006	0.959	0.038	0.801	0.783	0.082	0.517	-0.084	0.550	0.272	0.189	0.092	0.148	0.288	0.697	-0.074	0.519	0.242	0.136	0.135
Right hippocampus		-0.185	0.138	0.261	0.054	**0.012**	-0.006	0.966	-0.092	0.483	0.924	-0.078	0.524	0.167	0.184	0.146	-0.266	**0.029**	0.231	0.127	**0.015**
Right anterior hippocampus		-0.197	0.114	0.238	0.089	**0.014**	-0.069	0.610	-0.084	0.534	0.777	-0.077	0.531	0.143	0.261	0.162	-0.298	**0.013**	0.173	0.270	**0.023**
Right posterior hippocampus		-0.154	0.234	0.272	**0.039**	**0.021**	0.096	0.497	-0.093	0.469	0.473	-0.076	0.551	0.198	0.112	0.159	-0.197	0.126	0.238	0.056	**0.022**

Analyses were adjusted by sex, peak height velocity, parental education level, and body mass index. Further adjusted by total brain volume. β: standardized regression coefficients. p_i: p-value for interaction of cardiorespiratory fitness categories on the association between neurotrophic factors and brain health indicators. Statistically significant associations that are shown in bold disappeared when p values were adjusted for multiple comparisons using the Benjamini-Hochberg method. BDNF: Brain-derived neurotrophic factor; IGF-1: Insulin-like growth factor 1; VEGFA: Vascular endothelial growth factor A; EGF: Epidermal growth factor. Unfit indicates participants with cardiorespiratory fitness < sex-specific 50th percentile. Fit indicates participants with cardiorespiratory fitness ≥ sex-specific 50th percentile. [a] Blom's normalized values were used in the analysis.

Figure 1. Associations of plasma BDNF and EGF with right hippocampal volume by cardiorespiratory fitness categories in children with overweight/obesity.

β: standardized regression coefficients. p interaction: p-value for interaction of cardiorespiratory fitness categories in the association of BDNF and EGF with right hippocampal volume. Analyses were adjusted by sex, peak height velocity, parental education level, body mass index and total brain volume. BDNF: Brain-derived neurotrophic factor; EGF: Epidermal growth factor. Unfit indicates participants with cardiorespiratory fitness < sex-specific 50th percentile. Fit indicates participants with cardiorespiratory fitness ≥ sex-specific 50th percentile. [a] Blom's normalized values were used in the analysis. *p<0.05.