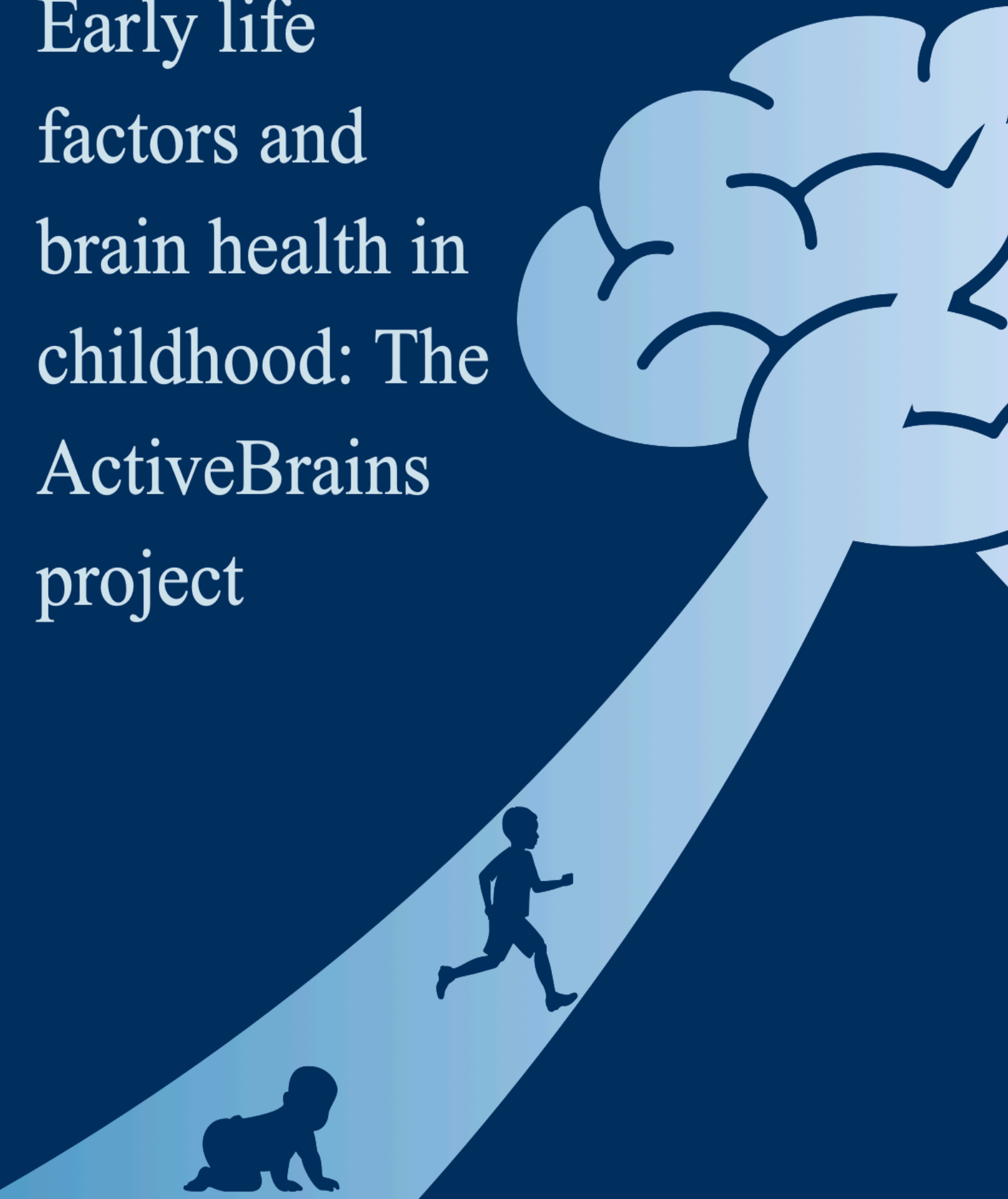
Early life factors and brain health in childhood: The ActiveBrains project



Patricio Solis Urra

International Doctoral Thesis

UNIVERSIDAD DE GRANADA

Early life factors and brain health in childhood:
The ActiveBrains project

Patricio Solis-Urra

International Doctoral Thesis / Tesis Doctoral Internacional

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Factores de la vida temprana y salud cerebral en la infancia: Proyecto ActiveBrains

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Early life factors and brain health in childhood: The ActiveBrains project

Factores de la vida temprana y salud cerebral en la infancia: Proyecto ActiveBrains

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Granada, Diciembre 2020

Lo importante es no dejar de hacerse preguntas;
Entonces, ¿Para que sirve la utopía?

A quienes me dan la libertad de resolver
mis curiosidades y caminan conmigo cada día.
En especial, a mis guapas que me acompañan en casa.

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1. Research project and funding

Research project and funding

The present International Doctoral Thesis was carried out under the framework of the ActiveBrains study (http://profith.ugr.es/activebrains?lang=en), which was funded by the following organizations:

- Spanish Ministry of Economy and Competitiveness and Fondo Europeo de Desarrollo Regional (FEDER) (DEP-2013-47540, DEP2016-79512-R, DEP2017-91544-EXP, BES-2014-068829, FJCI-2014- 19563, IJCI-2017-33642, and RYC-2011-09011).
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2. Abstract/Resumen

Abstract

Background Early life environment has shown to have an impact on future physical health outcomes, which is known as early life programming, and it may also influence brain health later in life.

Purpose The present doctoral thesis aimed to study the association of early life factors, including indicators of the prenatal period (i.e., birth weight and birth length) and breastfeeding practices, with brain health in overweight/obese children, and its implication in academic performance. Specifically, this doctoral thesis investigated the association of early life factors with gray matter volumes (**Study 1)**, white matter microstructure (**Study 2),** structural brain networks (**Study 3),** and resting state functional connectivity of hippocampus (**Study 4**).

Method To address the aims, we used baseline data of the ActiveBrains project corresponding to 110 children with overweight/obesity. Birth weight and birth length were assessed by birth records and breastfeeding practices reported by parents. Magnetic resonance imaging was used to asses brain health. In **study 1**, gray matter volume was determined by whole brain voxel-based morphometry; in **Study 2,** white matter microstructure was derived by tractography; in **Study** 3, structural brain networks were determined by structural covariance analysis; and in **Study 4,** hippocampal connectivity was derived by resting state functional connectivity (rsFC) of hippocampus.

Results and main findings In **Study 1,** higher birth weight and birth length, and any breastfeeding duration were associated with greater gray matter volume in different brain regions including the middle frontal gyrus, rectal gyrus, thalamus, putamen, middle temporal gyrus, lingual gyrus, middle occipital gyrus, calcarine cortex cerebellum, inferior frontal gyrus and rolandic operculum. In **study 2**, none early life indicator was associated with WM microstructure tracts after controlling for multiple comparison. In **study 3,** birth

weight and birth length were associated with 5 overlapped structural networks covering mainly cerebellum, posterior cingulate gyrus and anterior precuneus cortex; and subcortical structures as hippocampus, parahippocampal gyrus, caudate, putamen, pallidum, accumbens and amygdala. In **study 4**, birth weight was positively associated with rsFC of hippocampus with precentral, postcentral gyri and cerebellum. Birth length was not related to hippocampal connectivity. Exclusive breastfeeding was negatively associated with rsFC of hippocampus with angular gyrus and primary motor cortex; and any breastfeeding was positively associated with rsFC of hippocampus with middle temporal gyrus. Finally, across studies, none of these brain regions related to early life factors were post-hoc associated with academic performance.

Overall conclusion Early life factors are associated with brain structure and function in overweight/obese children, although its implications on academic performance are not observed in this thesis. The results of the present doctoral thesis expand the understanding of the early life programming on brain health in children with overweight/obesity.

Resumen

Antecedentes El ambiente en el que se desarrollan las primeras etapas de la vida, ha demostrado estar relacionado con parámetros de salud física en el futuro, lo que se conoce como programación temprana, podría también influir en la salud del cerebro más adelante en la vida.

Objetivos La presente tesis doctoral internacional tiene como objetivo estudiar la asociación de los factores de la vida temprana, incluyendo indicadores del periodo prenatal (como el peso y la longitud al nacer) y la lactancia materna con la salud cerebral en niños con sobrepeso/obesidad. Específicamente, esta tesis doctoral investiga la asociación de los factores de la vida temprana con el volumen de materia gris (**Estudio 1**); con la microestructura de materia blanca (**Estudio 2**); con las redes estructurales cerebrales (**Estudio 3**) y con la conectividad funcional en reposo del hipocampo (**Estudio 4**).

Método Para llevar a cabo estos objetivos hemos usado datos de la evaluación inicial del proyecto ActiveBrains, correspondientes a 110 niños con sobrepeso/obesidad. El peso y la longitud al nacer se recogieron de la cartilla de nacimiento, y la lactancia materna fue preguntada a los padres. Se utilizó resonancia magnética por imagen para determinar la salud cerebral. En el estudio 1, volúmenes de materia gris fueron determinados por análisis de morfometría basado en voxel; en el estudio 2, la microestructura de la materia blanca se determinó por tractografía; en el estudio 3, se construyeron redes estructurales a través de análisis de covarianza estructural; y en el estudio 4, la se analizó la conectividad funcional del hipocampo a través de análisis de conectividad funcional en reposo.

Resultados y hallazgos principales Un mayor peso y longitud al nacer y la duración de la lactancia completa fueron asociados con mayor volumen de materia gris en

diferentes regiones del cerebro, incluyendo el giro frontal medio, giro recto, tálamo, putamen, giro temporal medio, giro lingual, giro occipital medio, corteza calcarina, cerebelo, giro frontal inferior y el opérculo de rolando **(Estudio 1)**. Ningún indicador se asoció con la microestructura de la materia blanca. **(Estudio 2)**. El peso y la longitud al nacer fuero asociados de manera conjunta con 5 redes estructurales que cubren principalmente cerebelo, giro cingulado posterior, cortex precuneo anterior, y estructuras subcorticales como hipocampo, giro para hipocampal, caudado, putamen, globo pálido, nucleo accumbens y amígdala **(Estudio 3)**. Finalmente, el peso al nacer fue asociado positivamente con la conectividad funcional en reposo entre el hipocampo y el giro precentral, giro postcentral y el cerebelo. La lactancia materna se asoció negativamente con la conectividad funcional en reposo entre el hipocampo y el giro angular y la corteza motora (lactancia exclusiva) y positivamente con el giro temporal medio (lactancia completa) **(Estudio 4)**. A lo largo de los estudios, los indicadores cerebrales no fueron asociados con el rendimiento académico.

Conclusión general Los factores de la vida temprana fueron asociados con la estructura y función cerebral en niños con sobrepeso/obesidad, pero no se encontraron consecuencias sobre su rendimiento académico. Los resultados de la presente tesis doctoral amplían el entendimiento de la influencia de la primera etapa de la vida sobre la salud cerebral en niños con sobrepeso/obesidad.

3. General introduction

Childhood obesity is one of the most public health concerns [1]. Worldwide prevalence of childhood obesity is raising during the las decades. Estimations of world health organization indicated that around 41 millions of newborn and children were overweight or obese in 2016, and this situation is still raising [1]. For example, it's expected that 91 millions of children and adolescent will have obesity in 2025 [2]. This situation is critical as it has been demonstrated that obesity at childhood have a high likelihood of maintain their health status at adulthood, increasing the risk of develop obesity-related disorders [3]. Childhood obesity have serious consequences in a broad spectrum of adverse health outcomes, including hypertension, dyslipidemia, insulin resistance, fat liver disease among others [1,3]. In the last years, the research on consequences of obesity on brain health is increasing.

The brain health is a broad term related to the optimal functioning of behavioral and biological measures of the brain and the subjective experiences arising from brain function, including outcomes related to biological markers of the brain (e.g., structural brain morphology, brain function) or the subjective behavioral manifestations of brain [4]. Childhood is a period of sensitive changes in brain structure and function, since the brain is still in develop and wiring its connections [5]. In addition, childhood is a critical period for obesity-related brain disorders such as attention deficit, and depression [6,7]. Childhood obesity has been also associated with evident structural and functional brain abnormalities [8]. For example, brain-related alterations in obesity include reduced hippocampal, frontal and thalamus volume as well as disrupted structural connectivity in motor and in reward system, among others [8,9].

Fetal environment is the one of the most important period for brain development [10]. The rapid growth of the brain during the early life stage indicates its crucial vulnerability to environmental factors, being one of the most affected organs [10-12]. Simple environmental

indicators during early stage are anthropometric neonatal data (i.e., birth weight and birth length) and breastfeeding practices, that have been related with several indicators of brain, cognition and neurological disorders [10]. Importantly, both prenatal as postnatal stages are critical periods for brain growth. Int the first weeks after birth, the brain volume has about 35% of adult volume reaching the size of 80% of adults size at the second year of life [12]. The different tissues of brain have distinct maturation trajectories, while the gray matter volume growth a 108-149% at 1 year of life, and 14-19% between the first and second year of life, WM volume increase around 11 and 19%, respectively [12]. Thus, while after age 2 the gray matter shows marginal absolute increments with important decreasing in adolescence, WM increases gradually until adulthood. Interestingly, unlike to WM volume development, WM microstructure (e.g., tracts) show similar patterns of maturation than gray matter regions, with exponential rates until 1 age, and with gradually increases onwards; however, while gray matter of brain represents principally cell bodies of the neurons, WM microstructure represents the integrity of axonal bundles of the neurons [5,13]. Parallelly, previous studies found that development of brain functional networks begins prenatally [12], and specific patterns of maturation depends of the different networks. Thus, a broader analysis of the association of different early life factors and different measure of brain health could provide a better understanding of the influence of early life factors on brain development, with consequences at childhood.

Birth weight and birth length, represent the prenatal stage, specifically the nutrition and environment during the early life stage, and these indicators have been related to gray matter volumes, white matter (WM) microstructure and connectivity between brain regions, at birth[1,14,15], and even at childhood age [16,17]. Multiple factors are related to the early environment and its consequences in brain health [18,19]. For example, the stress during early life disrupt neuroendocrine system related to growth, metabolic processes and

glucose-insulin homeostasis leading to disruption in neurodevelopment, as well as cardiometabolic and obesity disfunctions [18]. This is mainly due to stressing fetal environment is related to endocrine-disrupting chemicals, leading children more vulnerable to environmental stressors and in consequences to poorer neurobehavioral outcomes. On the other hand, mechanisms related to synaptic system, atrophy, and glucocorticoids system are suggested to determine how early life stress leads to effect in neurodevelopment [20].

Most evidence indicated that preterm birth had specific disruption on gray matter, WM and connectivity during the first stage of life and with long-lasting consequences [14,15]. For example, birth weight predicts gray matter volume (e.g., hippocampus, cerebellum) [14,15], as well as WM microstructure in school-aged children [15]. Likewise, premature born has demonstrated long-lasting effect on structural brain networks. For example, several disruptions in the structural brain network during adolescence and childhood have been explained by premature born and gestational age in regions principally involved in language and executive functions [16,17]. However, less is known about the extent to which early life including the wide birth weight spectrum may predict brain structural and functional connectivity [21,22].

Breastfeeding practices are important factors related to brain development [23]. Indeed, breastfeeding is the most natural nutritional strategy during the first months of life. Breast milk provide key nutrients contain key components related to proliferation, myelinization and maturation of brain cells (ie., docosahexaenoic, arachidonic acid, taurine) [24,25]. During postnatal period, both exclusive and any breastfeeding are crucial to gray matter and WM development with long-lasting consequences [26,27]. For example, exclusive breastfeeding was associated to gray matter volume at term age [26] and cortical

thickness in adolescence, and in turn, was coupled with intelligence, mathematics and working memory benefits [26,28]. In addition, breastfed children showed better WM development in associative regions [27]. In contrast, the role of breastfeeding on structural and functional connectivity is poorly understood, and there are no previous studies in the context of childhood obesity.

Early life factors are also key predictors of obesity during childhood, and in turn, obesity may have consequences in several brain outcomes such as gray matter volumes [29,30], WM microstructure [30] and brain connectivity [31]. For example, obese population has shown smaller gray matter volumes in basal ganglia, hippocampus, thalamus [30], prefrontal cortex, cerebellum and left temporal pole [29]. In addition, there is extensive evidence showing children with overweight/obesity have lower academic performance compared with normal weight peers [32,33]. Thus, it is of great relevance to understand the influence of early life factors on brain structure and function in a population of children with overweight/obesity, and turn, its academic implications [34]. Furthermore, a key unexplored variable in this association is cardiorespiratory fitness (CRF), also known as aerobic capacity. Early life factors also predicted later CRF [8,35], and in turn, CRF was associated with lower risk of obesity, and greater gray matter volumes [36], WM microstructure [37,38], and functional connectivity at childhood [39]. Thus, it is needed to take in account this important health-related variable in the association of early life factors with brain outcomes.

In summary, previous studies related to early life environment investigated mainly the effect of being born preterm on brain health outcomes, but less studies shows the effects of a low weight at birth, which is an indicator of poor intrauterine nutrition. Further, examining different dimensions of brain structure, and also of brain function will provide a more comprehensive understanding of the influence of early life factors on brain health

later in life [12]. The present doctoral thesis provides a complete picture on how early life factors may influence brain structure (i.e., gray matter volumes, WM integrity and structural brain networks) and function (i.e., functional connectivity of hippocampus), and its potential implications on academic performance

4. Aims

Overall aim

The overall aim of the present international doctoral thesis was to study the association of early life factors, including anthropometric neonatal data (i.e., birth weight and birth length) and breastfeeding practices (i.e., exclusive and any breastfeeding), with brain health outcomes in children with overweight/obesity.

Specific aims

The specific aims of the present doctoral thesis were:

1. To investigate the associations of early life factors such as anthropometric neonatal data (i.e., birth weight and birth length), and breastfeeding practices (i.e., exclusive and any breastfeeding) with gray matter volume, and ii) to test whether these early-life factor-related regional gray matter volume are associated with academic performance in overweight/obese children **(Study 1).**
2. To investigate the associations of early life factors such as anthropometric neonatal data (i.e., birth weight and birth length) and breastfeeding practices (i.e., exclusive and any breastfeeding) with WM microstructure, and ii) to test whether WM tract related to early life factors are associated with academic performance in children with overweight/obesity **(Study 2).**
3. To investigate the association of early life factors such as anthropometric neonatal data (i.e., birth weight and birth length) and breastfeeding practices (i.e., exclusive and any breastfeeding) with structural brain network, and (ii) to test whether structural brain network related to early life factors are associated with academic performance in children with overweight/obesity **(Study 3).**
4. To investigated the associations of early life factors such as anthropometric neonatal data (i.e., birth weight and birth length) and breastfeeding practices (i.e., exclusive and any breastfeeding) with hippocampal resting state functional

connectivity (rsFC), and ii) to test whether connectivity related to early life factors are associated with academic performance in children with overweight/obesity **(Study 4).**

5. Methods, results and discussion

The present International Doctoral Thesis contains a total of 4 studies. All studies contain baseline data from children with overweight/obesity including in the ActiveBrains project. **Table 1** shows an overview of the design, participants and variables included in each study.

Table 1. Study methodology overview

	Specific aim	Participants	Analytic approach	Predictors	Br
Study 1	To investigate the associations between early life factors and gray matter volume, and ii) to test whether these early-life factor-related differences in regional gray matter volume are associated with variability in academic performance.	96 children with overweight/obesity (10.0 ± 1.1, 37.5 % girls)	Voxel based morphometry	Birth weight, birth length, and exclusive and any breastfeeding	C
Study 2	To investigate the associations of early life factors with white matter microstructure, and ii) to test whether white matter tract related to early life factors are associated with academic performance.	98 children with overweight/obesity (10.03 ± 1.16, 38.7 girls)	Tractography		W mi
Study 3	To investigate the association of early life factors with structural brain network and (ii) to test whether structural brain network associated to early life factors are associated in academic performance.	96 children with overweight/obesity (10.01 ± 1.14, 37.5 girls)	Structural covariance		C
Study 4	To investigate the associations of early life factors with hippocampal resting state functional connectivity, and ii) we tested whether connectivity related to early life factors are associated with academic performance	96 children with overweight/obesity (10.01 ± 1.14, 37.5 girls)	Resting state functional connectivity		H c

5.1. Study 1: Early life factors, gray matter brain volume and academic performance in children with overweight/obesity: The ActiveBrains project

Introduction

Perinatal nutrition and development are crucial factors for long-term health outcomes [40]. Specifically, birth weight, birth length and infant feeding, which are key indicators of prenatal and perinatal nutrition [41,42], are predictors of long-term physical [43,44] and mental outcomes [45-47]. In addition, early life factors may influence brain and academic outcomes later in life, especially during childhood [47-49].

Brain size at birth is only one quarter to one-third of its adult volume and fetal development is one of the most important periods of maturation [50]. Indeed, the period between conception though 2 years of age has been recognized as a critical stage of development, in which brain may be altered from various exposures, resulting in long-term consequences to both its morphology and function [23]. Specifically, the first year of postnatal life is associated with an increase of 108-149% of cortical gray matter volume, and of 14-19% in the second year, with wide variability between regions [12,51]. Importantly, intrauterine growth restriction from inadequate prenatal and perinatal fetal environments can profoundly affect post-natal neurodevelopment later in life [15,23].

Previous studies on prenatal environmental factors (i.e., birth weight or birth length) in childhood have mainly focused on preterm or low birth weight infants in relation to brain development. For example, school-aged children born with low birth weight have overall and regional (e.g., hippocampus, cerebellum) reductions in gray matter volume [14,15] that, in turn, may relate to lower academic performance [52]. Importantly, the association between these early life factors and brain development could differ according to gestational age, as these infants are exposed to different medical complications during perinatal period [22]. While low birth weight infants (i.e., <2500 g or very low birth <1500 g) have shown lower brain health [45,47], little is known about the extent to which early life across the birth weight

spectrum may predict brain structure [21,22]. Thompson et al. found that variation in birth weight predicts brain development in all groups of gestational age [21]. Furthermore, Murray et al., found a differential effects of intrauterine growth restriction on neurodevelopment as a function of gestational age [53]. However, there are not studies that have investigated the association of birth length and gray matter volume. As such, more studies are needed to examine whether birth weight and birth length separately predict brain development in children across the birth weight spectrum, since both factors have been differentially related to brain health outcomes [54]. This is important for better understanding individual variability of brain development in childhood.

Postnatal environmental factors, such as breastfeeding, are potential nutritional opportunities for influencing healthy brain development [23]. Breast milk provides nutrients (e.g. docosahexaenoic acid) that are rapidly incorporated into the central nervous system during the first months of life and, in turn, could help stimulate gray matter development [55]. Additionally, taurine is the major free amino acid in breast milk, which has a crucial role in the optimal development, proliferation and maturation of brain cells promoting healthy neurodevelopment [25]. To date, few studies have examined the long-term effect of breastfeeding on child's gray matter volume, and in turn on their academic abilities [49,56]. Ou et al. showed that children who were breastfed had greater gray matter volume in temporal and parietal regions compared with those who were fed with cow formula [57]. Belfort et al. found that those who predominantly were breastfed at the 28 day of life had greater deep gray matter volume in the first week of life, but this association disappeared at 7 years, and was only associated with a higher intelligence quotient and better performance in mathematics, working memory and motor function at this age [49]. Thus, whether breastfeeding has long lasting effects on gray matter and neurocognitive outcomes remain unclear.

Collectively, a better understanding of the association between early life factors and regional gray matter volume in school age children is needed, and specifically this association has not been studied in children with overweight/obesity. This is particularly important in an overweight/obese population, since suboptimal early life factors have been associated with a higher body mass index (BMI) [58] lower CRF [59,60] and lower gray matter volume during childhood [48]. In turn, children with overweight/obesity have worse academic abilities compared to normal-weight children [61-63]. In addition, despite the marked relevance of CRF on gray matter volume in normal weight children [64] and in overweight/obese children [36], previous studies have not considered CRF or BMI when examining the influence of early life factors on brain development. Therefore, the aim of the present study was twofold, i) to investigate the associations between early life factors (i.e., birth weight, birth length, and exclusive and any breastfeeding) and gray matter volume adjusting for several covariates including CRF and BMI, and ii) to test whether these early life factors-related differences in regional gray matter volume are associated with variability in academic performance in overweight/obese children.

Material and method

Participants

We included 96 overweight/obese children aged 8–11 years from the ActiveBrains project (www.profith.ugr.es/activebrains) with valid measures of early life factors, brain and academic performance variables. Participants met the following inclusion/exclusion criteria to be in the study: 1) 8 to 11.9 years-old; 2) classified as overweight or obese at baseline according to sex and age specific World Obesity Federation cut- off points [65-67]; 3) an absence of physical disabilities or neuro- logical disorders that prohibited them from exercise; 4) in the case of girls, not to have started menstruation at the moment of baseline assessments; 5) reporting no use of medications that influence central nervous system

function; 6) right-handed (i.e., measured by the Edin- burgh inventory) since right-handed individuals substantially differ in brain hemisphere structure (i.e., dominant and non-dominant hemi- sphere) from left-handed ones; and 7) an absence of attention-deficit hyperactivity disorder (ADHD) above the 85th percentile measured by the ADHD rating scale [68] The present cross-sectional analysis was carried out using baseline data prior to randomization for an exercise intervention. Measurements were carried out from November 2014 to February 2016. Parents or legal guardians were informed of the purpose of the study and written informed parental consents were obtained. The ActiveBrains project was approved by the Ethics Committee on Human Research (CEIH) of the University of Granada and was registered in ClinicalTrials.gov (identifier: NCT02295072).

Early life factors

Birth weight (kg), birth length (cm) and gestational age (weeks) were collected from birth records (parents' record with the perinatal information of each child). The duration of exclusive and any breastfeeding in months was reported by parents. Parents were asked how long (months) the child received only breast milk (neither formula nor other liquid or solid). This answer was classified as exclusive breastfeeding. In addition, parents were also asked how long (months) the child received any breast milk (combined with other liquid, formula, or solid). This answer was classified as any breastfeeding.

Magnetic Resonance Imaging (MRI) acquisition and processing

MRI assessment was performed with a 3.0 T Magnetom Tim Trio system (Siemens Medical Solutions, Erlangen, Germany) equipped with a 32-channel head coil. Three-dimensional, high-resolution, T1-weighted images were collected using a magnetization-prepared rapid gradient-echo (MPRAGE) sequence. The sequence parameters were as

follows: 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.

The processing protocol was detailed in a previously published paper [36]. In brief, imaging pre-processing steps included quality control, alignment and segmentation into gray matter tissue, WM tissue and cerebrospinal fluid. First, each individual image was checked for acquisition artifacts and alignment along the horizontal anterior commissure and posterior commissure plane. Then, gray matter images were spatially normalized to Montreal Neurological Institute (MNI) space and used to create a template using Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL). Subsequently, images were normalized to the DARTEL template via non-linear transformation and modulated with Jacobian determinants. Finally, the images were smoothed by convolving them with an isotropic Gaussian kernel of 8 mm full width at half maximum (FWHM).

Academic performance

The Bateria III Woodcock-Muñoz Tests of Achievement was used to assess academic performance (i.e., Spanish version of the Woodcock- Johnson III) [69] A trained evaluator individually administered the tests to each child, explained the instructions and assessed the child during the session. The full administration time was between 100 and 120 min. The Bateria III Woodcock-Muñoz is a standardized test of achievement comprising several sub-tests. In this study we included four indicators: reading, writing, mathematics, and a total achievement standard score.

Early life factors and gray matter

Covariates

Sex, gestational age, peak height velocity (PHV), parental education, BMI and CRF were used as covariates. Pubertal maturity status was determined by PHV and was obtained from the [70] equation for boys and girls; PHV offset was calculated by subtracting the age of PHV from the chronological age. The difference in years was defined as a value of maturity offset. Both father and mother's educational level were considered indicators of socioeconomic status. Thus, parents were asked to report their maximum completed level of education and answers were categorized as: none of the parents had university degree, one of the parents had a university degree or both parents had a university degree [71]. BMI was computed as weight in kilograms divided by height in meters squared (kg/m2). CRF was assessed through the 20-m shuttle-run test and maximal oxygen consumption (VO2max, mL/kg/min) was calculated using the Leger equation [72].

Statistical analysis

Participant's characteristics are shown as mean and standard deviation (SD) for continuous variables, and percentages for categorical variables. Whole-brain voxel-wise multiple regression models were performed in SPM12 for the analyses of imaging data. The associations between each early life factor (i.e., birth weight, birth length, exclusive and any breastfeeding) and gray matter volume were tested in separate linear regressions. The model included adjustment for sex, gestational age, PHV offset, parental education level, BMI and CRF. We extracted eigenvalues of the peak coordinates of each significant cluster that showed association with each early life factor and we performed separate regression models in SPSS including eigenvalues as outcomes and each early life factor as a predictor, adjusted for covariates mentioned above. Additionally, we performed linear regression in SPSS (version 21 for Macintosh; P set at < 0.05) to test the association

between each significant gray matter region using those eigenvalues as predictors and academic performance indicators as outcomes adjusting for sex, PHV offset, parental education level, BMI and CRF. The spatial extent threshold was determined using AlphaSim as implemented in Resting-State fMRI Analysis Toolkit toolbox (RESTplus). Input parameters include a brain mask of 128190 voxels and a cluster connection radius of 5 mm considering the smoothness of data after model estimation. The voxel-level alpha significance (threshold, $p < 0.001$ uncorrected) along with the appropriate cluster size for controlling for multiple comparisons in each analysis was indicated in the results. Finally, resulting cluster extents were adjusted to account for the non-isotropic smoothness of structural images, in accordance with Hayasaka et al [73]. The clusters are reported after applying these corrections. Additionally, sensitivity analyses were performed excluding preterm children.

Results

Table 1 shows descriptive characteristics of the overall sample and separated by boys and girls. Table 2 presents the associations for each early life factor showing a positive association with regional gray matter volume, independently of CRF and BMI. Consistent with our predictions, higher birth weight was associated with greater gray matter volume ($p < 0.001$, $k = 45$) in seven clusters, with t values ranging from 3.46 to 5.62, a cluster size between 82 and 4445 and a standardized coefficient (β) between 0.361 and 0.539, specifically, in frontal regions (i.e., middle frontal gyrus and rectal gyrus), temporal regions (middle temporal gyrus), thalamus, and cerebellum (i.e. cerebellum III, and cerebellum crus II bilaterally) (**Table 2, Fig. 1**).

Similarly, higher birth length was associated with greater gray matter volume ($p < 0.001$, $k = 38$) in seven clusters, with t ranging from 3.78 to 5.44, cluster size between 97

and 4478 and a standardized coefficient (β) between 0.378 and 0.537, specifically, in putamen, temporal regions (middle temporal gyrus), occipital regions (lingual, middle occipital gyrus), calcarine cortex and cerebellum crus II bilaterally (**Table 2, Fig. 2**).

We also found that duration of any breastfeeding was associated with greater gray matter volume ($p < 0.001$, k 1⁄4 39) in 3 clusters, with t ranging from 4.01 to 4.32, cluster size between 64 and 171 and standardized coefficient (β) between 0.359 and 0.408, specifically, in frontal regions (inferior frontal gyrus pars orbital bilaterally) and rolandic operculum (**Table 2, Fig. 3**). No significant associations were found be- tween duration of exclusive breastfeeding and gray matter volume.

In sensitivity analysis after excluding preterm children, the associations were maintained for birth weight in four regions (i.e., middle frontal gyrus, middle temporal gyrus, cerebellum crus II left and cere- bellum crus II right), for birth length in five regions (i.e., putamen, lingual, calcarine cortex, cerebellum crus II left and cerebellum crus II right) and for duration of any breastfeeding in two regions (i.e., inferior frontal gyrus pars orbital and rolandic operculum) (data not shown). These associations were also independent of BMI and CRF.

Table 1. Characteristics of study sample.

	All		*Boys*		*Girls*	
	n		*n*		*n*	
Physical characteristics	96		60		36	
Age (years)		10.0 ± 1.1		10.2 ± 1.1		9.8 ± 1.1
Weight (kg)		55.7 ± 11.2		56.7 ± 10.7		54.0 ± 11.8
Height (cm)		143.8 ± 8.3		144.7 ± 7.4		142.3 ± 9.6
Peak height velocity offset (year)		-2.3 ± 1.0		-2.7 ± 0.8		-1.2 ± 0.8
Body mass index (kg/m^2)		26.7 ± 3.7		26.9 ± 3.8		26.4 ± 3.5
Cardiorespiratory fitness (mL/kg/min)*		40.9 ± 2.8		40.8 ± 2.8		40.9 ± 2.8
Body mass index category (%)	96		60		36	
Overweight		25.0		23.3		27.8
Obesity type I		42.7		45.0		38.9
Obesity type II/III		32.3		31.7		33.3
Parental education university level (%)	96		60		36	
None of the parents		66.7		71.7		58.3
One of the two parents		17.7		16.7		19.4
Both parents		15.6		11.5		22.2
Gestational age (%)	96		60		36	
< 37 weeks		17.7		16.7		19.4
37 – 40 weeks		64.6		68.3		58.3
> 40 weeks		17.7		15.0		22.2
Neonatal characteristics						
Birth weight (g)	94	3,343.7 ± 0.5	59	3,359.0 ± 0.6	35	3,318.0 ± 0.5
Birth length (cm)	85	50.7 ± 2.7	57	50.6 ± 3.0	28	50.9 ± 1.8
Gestational age (weeks)	96	38.6 ± 2.6	60	38.6 ± 2.6	36	38.7 ± 2.6
Exclusive breastfeeding[#] (%)	92		59		33	
Never		31.5		28.2		36.4
< 3 months		17.4		20.3		12.1
3-5 months		23.9		18.6		33.3
≥ 6 months		27.2		32.2		18.2
Any breastfeeding[##](%)	92		59		33	
Never		20.7		20.3		21.2
< 3 months		14.1		13.6		15.2
3-5 months		25.0		22.0		30.3
≥ 6 months		40.2		44.1		33.3
Academic performance (standard score)**	96		60		36	
Mathematics		102.0 ± 10.7		102.4 ± 11.2		101.4 ± 9.8
Reading		108.5 ± 12.9		108.3 ± 11.1		108.9 ± 15.7
Writing		114.0 ± 12.0		112.6 ± 11.9		116.4 ± 11.9
Total achievement		109.5 ± 11.7		109.0 ± 10.7		110.3 ± 13.3

Values are mean ± SD or percentage. *Measured by the 20-m shuttle run test; **Measured by the Bateria III Woodcock-Muñoz Tests of Achievement. [#]Months the child received only breast milk. [##]Months the child received breast milk combined with other liquid, or solid.

Table 2. Brain regions showing positive associations of birth weight* (n=95), birth length (n=85) and any breastfeeding (n=92) with gray matter volume in overweight/obese children.

Brain Regions (mm³)	***x***	***y***	***z***	***t***	***Cluster size***	***Hem***	**B (95% CI)**	***β***
Birth weight								
Middle frontal gyrus	32	42	21	4.32	82	L	0.101 (0.055,0.148)	0.462
Rectal gyrus	2	38	-30	3.92	328	L	0.030 (0.015,0.045)	0.392
Thalamus	16	-21	12	3.46	100	L	0.023 (0.010,0.037)	0.371
Middle temporal gyrus	-45	-16	-9	3.82	180	R	0.040 (0.019,0.060)	0.390
Cerebellum III	-9	-36	-20	3.68	166	R	0.025 (0.012,0.038)	0.361
Cerebellum crus II	24	-84	-46	5.62	4445	L	0.052 (0.034,0.070)	0.539
Cerebellum crus II	-36	-78	-51	4.62	2638	R	0.030 (0.017,0.043)	0.453
Birth length								
Putamen	-21	14	-14	3.93	430	R	0.005 (0.003,0.008)	0.378
Middle temporal gyrus	-50	-27	-8	3.87	375	R	0.017 (0.008,0.026)	0.431
Lingual	22	-74	-8	4.09	97	L	0.013 (0.007,0.020)	0.439
Middle occipital gyrus	40	-76	26	3.78	150	L	0.012 (0.006,0.018)	0.408
Calcarine cortex	-10	-84	-2	3.80	283	R	0.010 (0.005,0.016)	0.425
Cerebellum crus II	33	-74	-51	5.44	4478	L	0.015 (0.010,0.021)	0.537
Cerebellum crus II	-40	-70	-54	4.76	1878	R	0.007 (0.004,0.010)	0.496
Any breastfeeding								
Inferior frontal gyrus pars orbital	16	18	-27	4.01	64	L	0.002 (0.001,0.003)	0.359
Inferior frontal gyrus pars orbital	-16	21	-27	4.26	133	R	0.002 (0.001,0.002)	0.364
Rolandic operculum	56	-2	14	4.32	171	L	0.004 (0.002,0.006)	0.408
Exclusive breastfeeding								
Non-significant association	-	-	-	-	-		-	-

Analyses were adjusted by sex, peak height velocity offset (years), parent education university level (neither/one/both), gestational age (weeks), body mass index (kg/m^2) and cardiorespiratory fitness (mL/kg/min). All contrasts were thresholded using AlphaSim at $P < 0.001$ with k= 45 voxels for birth weight, k= 38 voxels for birth length and k= 39 voxels for any breastfeeding, and surpassed Hayasaka correction. Anatomical coordinates (X, Y, Z) are given in Montreal Neurological Institute (MNI) Atlas space. Hem, hemisphere; R, right, L, left *Values in Kg.

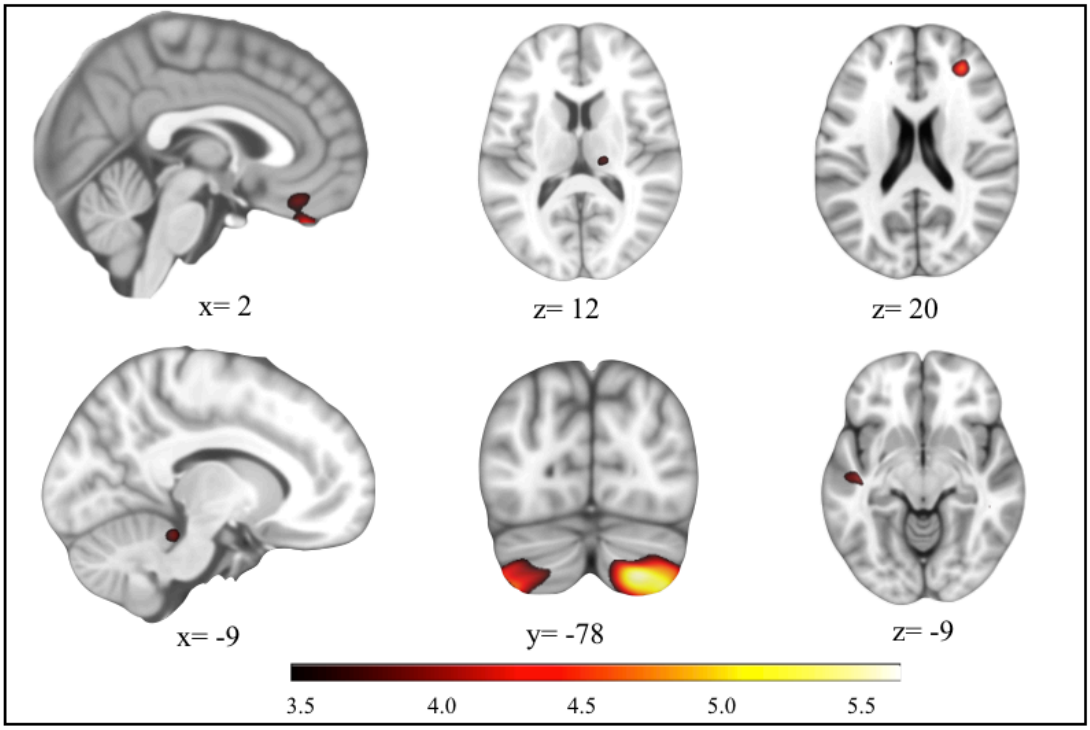

Figure 1. Brain regions showing positive association with birth weight. Images corresponding to model adjusted by sex, peak height velocity offset (years), parental education level (neither/one/both), body mass index (kg/m^2) and cardiorespiratory fitness (mL/kg/min). Maps were thresholded using AlphaSim at $P < 0.001$ with $k = 45$ and surpassed Hayasaka correction. The color bar represents t-values, with lighter yellow color indicating higher significant association. Images are displayed according to neurological convention; therefore the right hemisphere corresponds to the right side in coronal displays. x: indicates coordinate of sagittal view; y: indicates coordinate of coronal view; z indicates coordinate of axial view.

There were no negative significant relationships between early life risk factors and gray matter volume in any brain region ($p > 0.05$). **Table 3** shows the association between brain regions corresponding to each early life factor and academic performance. No significant associations were found (all $p > 0.05$).

Discussion

The main finding indicates that early life factors (i.e., birth weight, birth length and any breastfeeding) were positively associated with gray matter volume in numerous cortical and subcortical brain structures in children with overweight/obesity and these associations

were independent of BMI and CRF. Additionally, regional gray matter volumes of clusters associated with early life factors were not associated with aca- demic performance. These results have important public health implications since they suggest that gray matter volume during childhood could be partially influenced by fetal or early infancy environment.

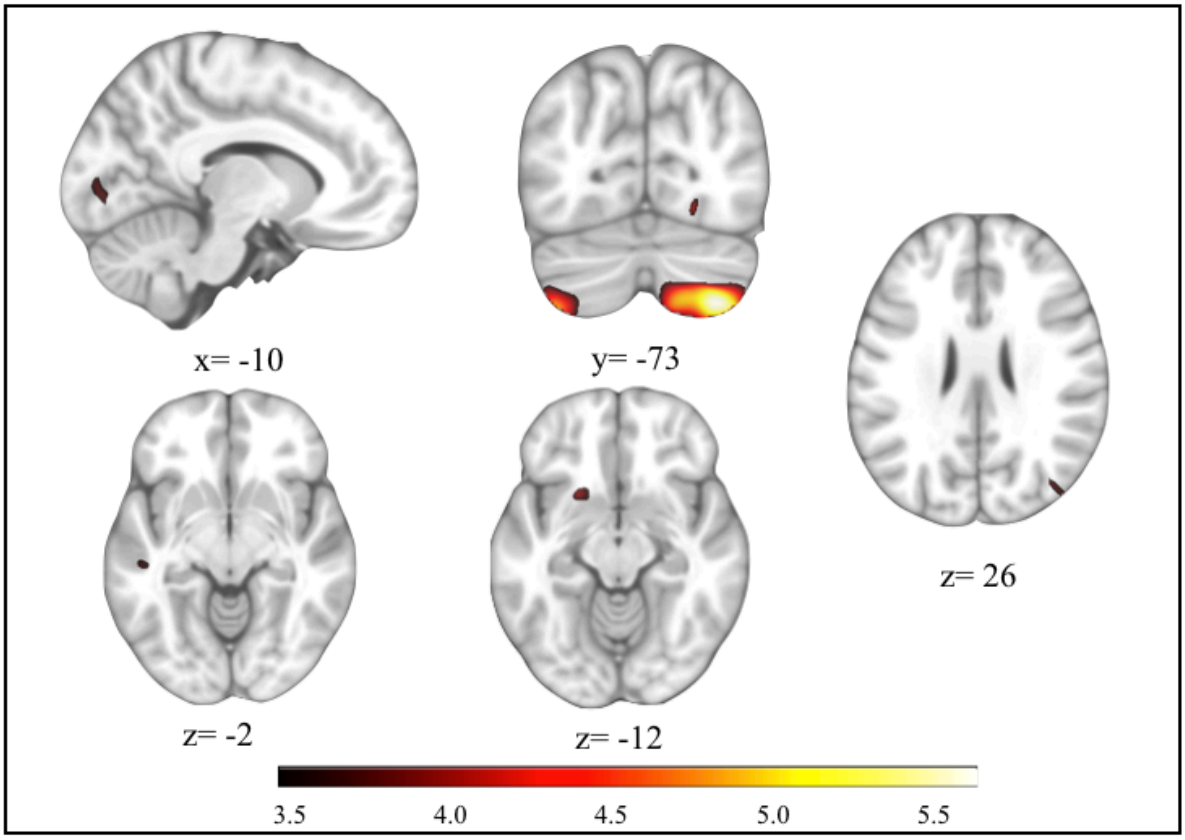

Figure 2. Brain regions showing positive association with birth length. Images corresponding to model adjusted for sex, peak height velocity offset (years), parental education level (neither/one/both), body mass index (kg/m2) and cardiorespiratory fitness (mL/kg/min). Maps were thresholded using AlphaSim at $P < 0.001$ with $k = 38$ and surpassed Hayasaka correction. The color bar represents t-values, with lighter yellow color indicating higher significant association. Images are displayed ac- cording to neurological convention; therefore, the right hemisphere corresponds to the right side in coronal displays. x: indicates coordinate of sagittal view; y: indicates coordinate of coronal view; z: indicates coordinate of axial view.

Several mechanisms might explain the present results. During pregnancy, intrauterine growth restriction profoundly and negatively influences brain development; the number of neurons, dendritic and synaptic head architecture, the concentrations of neurotransmitters and growth factors are affected in a suboptimal intrauterine environment

[23]. Poor fetal environment can lead to fetal circulatory redistribution, which is considered an adaptive fetal response that preserves oxygen supplied to the brain (and other vital organs) in conditions of chronic hypoxia [53]. This does not apparently ensure normal brain development, and even more, cortical gray matter volume could decrease independently of a reduction in overall brain volume [41,42]. Thus, poor fetal nutrition as well as other aspects of the environment reflected in lower weight and length at birth could affect brain development and subsequent gray matter volume in early childhood.

Table 3. Associations of early life factors-related changes in gray matter with academic performance*.

	Mathematics		*Reading*		*Writing*		*Total achievement*	
	β	***p***	***β***	***p***	***β***	***p***	***β***	***p***
Birth weight								
Middle frontal gyrus	0.038	0.705	0.106	0.295	0.100	0.308	0.100	0.297
Rectal gyrus	0.188	0.081	0.064	0.564	-0.134	0.209	0.041	0.698
Thalamus	0.139	0.172	0.182	0.078	-0.038	0.703	0.119	0.226
Middle temporal gyrus	0.014	0.894	0.129	0.217	-0.012	0.908	0.053	0.591
Cerebellum III	0.081	0.470	0.194	0.086	0.069	0.533	0.143	0.186
Cerebellum crus II	0.074	0.482	-0.002	0.983	-0.016	0.877	0.012	0.905
Cerebellum crus II	0.087	0.820	-0.037	0.736	0.075	0.474	0.032	0.755
Birth length								
Putamen L	0.068	0.562	0.124	0.307	-0.088	0.461	0.056	0.626
Middle temporal gyrus	-0.014	0.890	0.001	0.989	-0.057	0.575	-0.032	0.745
Lingual	-0.185	0.072	0.044	0.682	0.016	0.882	-0.035	0.729
Middle occipital gyrus	0.028	0.790	0.121	0.259	-0.053	0.614	0.052	0.609
Calcarine cortex	-0.104	0.300	0.074	0.478	-0.077	0.453	-0.028	0.772
Cerebellum crus II	-0.080	0.446	-0.018	0.867	-0.005	0.960	-0.042	0.684
Cerebellum crus II	-0.048	0.647	-0.172	0.106	-0.023	0.828	-0.115	0.256
Any breastfeeding								
Inferior frontal gyrus pars orbital	0.207	0.059	0.056	0.621	0.043	0.693	0.114	0.286
Inferior frontal gyrus pars orbital	0.220	0.053	0.054	0.644	0.067	0.554	0.124	0.267
Rolandic operculum	0.082	0.423	-0.087	0.400	0.041	0.685	0.009	0.932

Values are standardized regression coefficients (β). Analyses were adjusted by sex, peak height velocity offset (years), parent education university level (neither/one/both), gestational age (weeks), body mass index (kg/m^2) and cardiorespiratory fitness (mL/kg/min). *Measured by the Bateria III Woodcock-Muñoz Tests of achievement.

Early life factors and gray matter

Additionally, the early postnatal period, specifically the first year of life, is crucial to gray matter growth, since during this period gray matter volume may increase more quickly than WM volume, reaching 80% of adult capacity by the second year [12]. From age 2, gray matter volume shows minimal absolute increases in comparison with WM volume that increases much more gradually [12]. Indeed, the importance of docosahexaenoic acid (an abundant ingredient in human milk) as well as taurine and iron for neurogenesis, neuronal migration, and synaptogenesis might be leading to some of these associations [23,74]. Breastfeeding provides the essential components to infant growth and development in each period according to the personal needs of each child [75,76]. Therefore, breastfeeding in the early postnatal period might be a nutritional strategy for sustaining healthy brain development [23,42].

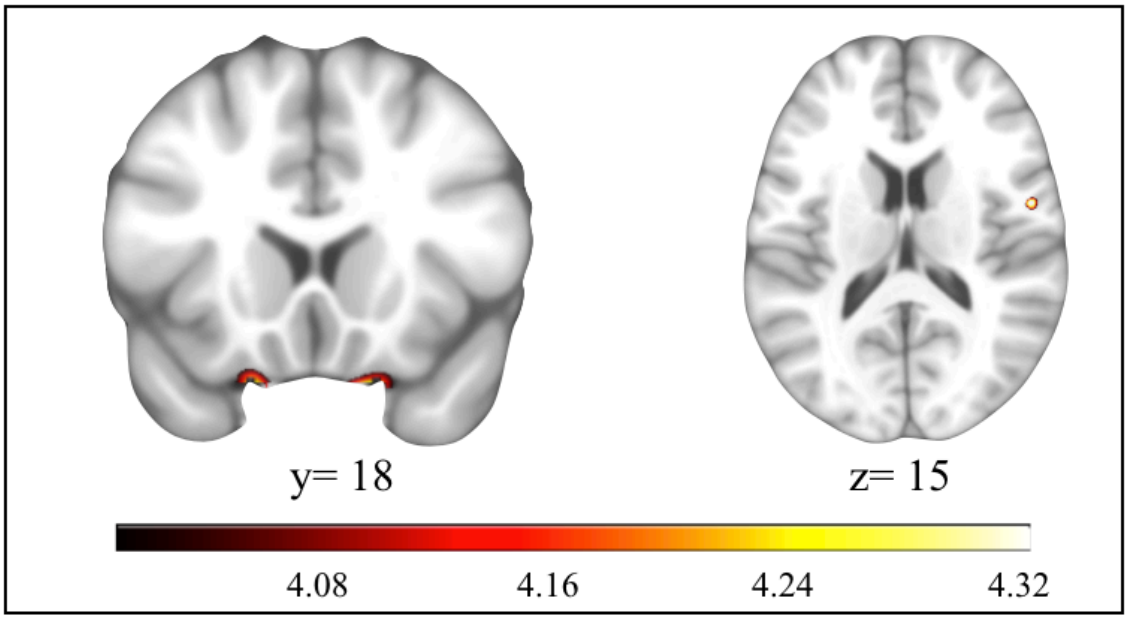

Figure 3. Brain regions showing positive association with any breastfeeding. Images corresponding to model adjusted for sex, peak height velocity offset (years), parental education level (neither/one/both), body mass index (kg/m2) and cardiorespiratory fitness (mL/kg/min). Maps were thresholded using AlphaSim at $P < 0.001$ with $k = 39$ and surpassed Hayasaka correction. The color bar represents t-values, with lighter yellow color indicating higher significant association. Images are displayed according to neurological convention; therefore, the right hemisphere corresponds to the right side in coronal displays. x: indicates coordinate of sagittal view; y: indicates coordinate of coronal view; z: indicates coordinate of axial view.

To the best of our knowledge, there are no previous studies examining the relationship of early life factors with gray matter volume specifically in children with overweight/obesity. In infants, higher birth weight was associated with larger brain volumes and regional cortical gray matter volumes in those individuals scanned between week 41.4–42.4 of post- menstrual age [21]. Additionally, low birth weight was the most predominant predictor of lower brain volume and altered microstructure, in comparison with multiple birth, social risk or postnatal growth factors [21]. Previous systematic reviews in healthy normal-weight children support that low birth weight (i.e., <2500 g or very low birth <1500 g) negatively affects several cortical gray matter areas, cerebellar gray matter, and thalamus volume [14,15,48]. For instance, our results showed that the cerebellum was an important region associated with both body weight and length at birth. These associations remained after sensitivity analysis excluding preterm children (data not shown). Recently, the cerebellum has been related to plasticity in the cerebral cortex; it plays an important role in language, motor and cognitive maturation, and is considered a domain-general region in all motor and cognitive processes [77]. These results suggest that the cerebellum might be particularly sensitive to the intrauterine environment due its major growth and development during the second half of gestation, and sub-optimal conditions in this period could lead to less body size [41,78-80].

Moreover, middle temporal gyrus was consistently associated with both birth length and birth weight. This finding is in line with previous studies showing that temporal brain regions are particularly susceptible to disruption of the gestational environment which may have long-term consequences [81]. This region is involved in both linguistic and nonlinguistic semantic-level processes [82,83], as well as in memory and several other cognitive processes [84,85].

Early life factors and gray matter

Additionally, we found that birth length was associated with greater gray matter volume in occipital regions (e.g., lingual gyrus, middle occipital gyrus and calcarine cortex), which have a principal role in visual processing [86] in line with previous findings indicating that the prenatal environment has long-term consequences in these regions [87,88]. Collectively, although previous studies have focused on preterm or low or very low birth weight infants, our findings across a wide range of birth weight partially concur with previous studies showing that birth size was associated with greater regional gray matter independently of important health-related factors such as CRF or BMI [36,62]

Exclusive and any breastfeeding have been related to gray matter development [49]. In the present study, exclusive breastfeeding was not associated with gray matter volume; however, any breastfeeding was positively associated with regional gray matter in the inferior frontal gyrus and rolandic operculum, two regions mainly related to language [12]. While long-term effect has been found for duration of exclusive breastfeeding in neurodevelopment outcomes, other findings have not observed an effect on executive function, behavior or social-emotional development [89]. Indeed, higher proportion of breast milk intake in the first month of life was associated with deep nuclear gray matter and hippocampus volume at the first months of life (between 3 and 42 weeks postnatal age), but such findings did not appear at the age of 7 years [49]. In contrast, Luby et al. found increases in cortical and subcortical gray matter volume between the ages of 9 and 14 years when comparing those who were breastfed with those who were not [56]. Ou et al., using voxel-based morphometric analysis, found that breastfed infants presented greater gray matter in the inferior temporal and superior parietal lobes at age 8 compared with those fed cow-milk formula [57]. Of note, no previous studies have found an associations between any breastfeeding and gray matter volume in the inferior frontal gyrus and rolandic operculum;

however, the potential role of breastfeeding on speech and language during childhood has been previously reported [90], and it is possible that those regions may be implicated in these benefits. Thus, we highlight the importance of long-term breastfeeding for brain development, although future studies are needed to confirm these results. While no associations were found for duration of exclusive breastfeeding, we observed a high prevalence of participants exclusively breastfed for longer than six months. This is of particular interest since obese children have been reported to have received lower duration of exclusive breastfeeding [91,92].

Previous studies in different populations have demonstrated that volume and microstructure of gray matter volume is related to several aspects of executive function, and academic abilities [64,93]. In the present study, gray matter volumes (previously associated with early life factors) were not associated with academic performance in overweight/obese children. Obesity *per se* has been associated with poorer cognitive function compared with normal weight peers [94-96], and this may hinder the influence of early life factors with differences in gray matter volumes on academic performance in an overweight/obese population. For example, Wang et al. examined the association of gray matter and academic performance in a large sample of Chinese adolescents, and found that the left dorsolateral prefrontal cortex was related to academic performance [32]. In addition, in consonant with our current findings, Isaac et al., found that breastfeeding was not associated with cortical gray matter, but it was associated with WM; and in turn, WM was associated with intellectual quotient in children and adolescents [97]. To note, we included an important confounder that was not previously considered (i.e., CRF). Several sources of evidence, including a previous study with the present sample, supported the associations of CRF with academic performance [36,64], brain structure [36,47,98] and thus, CRF has been proposed as an important moderator/mediator of cognitive function related to obesity [99].

As such, the inclusion of important health-related factors as covariates, such as CRF, may attenuate the strength of the association between gray matter volume and academic performance. However, future research is needed to examine the influence of early life factors on gray matter, and in turn, on academic performance including both normal-weight and overweight/obese children.

There are several limitations that should be acknowledged. First, the use of a retrospective cross-sectional design does not allow attributing causality between variables. Second, participants potentially misunderstood the term or meaning of "exclusiveness" of breastfeeding, and such an issue may have been exacerbated by a lack of information regarding formula-fed for those who were not breastfed [100], which may explain, in part, the null association related to breastfeeding practices. Third, the inclusion of only overweight and obese children limits the generalizability of our findings. Nevertheless, obesity is an important public health concern during childhood, and examining health factors in in this population is of great importance to understand factors that influence brain development. Strengths of the present study include its relatively large sample of overweight/obese children, the use of birth records to assess early life factors and the adjustment for important confounders.

Conclusion

Our results support the influence of early life factors (i.e., birth weight, birth length and any breastfeeding) on gray matter volume of numerous cortical and subcortical brain structures in overweight/obese children. These results were maintained despite controlling for a wide number of health-related factors such as CRF and BMI. However, there were no associations between gray matter volumes associated with early life factors and academic performance in overweight/obese children. These results might have important practical

applications since obesity is epidemic in childhood and attention in prenatal nutrition during pregnancy may be necessary to avoid negative effects of early life factors on the offspring's brain development later in life. Therefore, interventions aiming at improving optimal intrauterine growth and development, and promoting breastfeeding in infancy may be of importance to achieve a healthy brain later in life.

5.2. Study 2: Early life factors and white matter microstructure in children with overweight/obesity: The ActiveBrains project

Introduction

The period between conception and 2 years of age has been recognized as a critical stage for later health [34] as well as for brain development [12,101]. Exposure to a suboptimal environment during the fetal and early infancy periods results in long-term consequences for brain morphology and function [12,23,102]. In this line, several markers of perinatal nutrition and growth such as anthropometric neonatal data (i.e. birth weight and birth length) and infant feeding practices (i.e., breastfeeding practices and formula feeding) have been associated with brain development during childhood [103-105], as well as with neurocognitive outcomes [49].

WM is a tissue from the brain that denotes axons of the neurons that connect nerve cell bodies. Diffusion tensor imaging (DTI) is a neuroimaging technique that allows characterizing water diffusion patterns for in-vivo investigation of brain WM microstructure [104]. Fractional anisotropy (FA) and mean diffusivity (MD) are the two main indicators derived from DTI. While FA is related to myelination and fiber organization of WM tracts, representing WM maturation; MD is an indicator that represents the average of water diffusion in all directions, representing poor WM maturation [106,107]. In turn, WM microstructure has been related to executive function and academic abilities in different populations [105,108,109]

Small size for gestational age, and short length at birth may be the result of intrauterine growth restriction, and have been related to poor WM maturation [101,110]. For instance, infants with low birth weight show lower values of FA and higher values of MD during the first day of life [111]. In addition, large size for gestational age at birth has been consistently related to higher values of FA and lower values of MD in several WM tracts (e.g., corticospinal, inferior fronto-occipital fasciculus, corona radiata) of infants across the wide spectrum of gestational age [101,110]. Of note, few studies have investigated the

associations of neonatal anthropometric indices with long-lasting WM microstructure later in life [106,112-117], and most of them focused on birth weight. For example, children born with low birth weight had lower FA values in specific projection or association tracts (e.g., forceps minor and superior longitudinal fasciculus), but not in global FA [15]. Likewise, young adults born preterm with low birth weight had regional reduced FA and increased MD [115]. Further, there is a lack of studies investigating the specific association of birth length with WM microstructure during childhood.

Breastfeeding provides important nutrients that are precursors of myelin and WM microstructure, and in turn, of cognitive development in the first stage of life [27,105]. The duration of breastfeeding has been associated with better regional, but no global WM maturation both in humans and non-human primates during childhood [62,118,119]. Despite this, only few studies have examined the long-lasting association of breastfeeding practices specifically on WM maturation, and robust conclusions have not been established so far.

Suboptimal early life factors are particularly interesting in children with overweight and obesity since birth weight has been related not only to WM maturation but also to BMI [106,112] and CRF during childhood [59,120]. Even, children with overweight/obesity showed reduced academic abilities and different patterns of WM maturation [121,122] compared to normal-weight children [62,123]. Interestingly, while there are previous studies showing the influence of CRF on WM maturation in normal weight and obese children [38,124], no studies have considered BMI or CRF when examining the influence of early life factors on WM maturation. Therefore, further studies investigating the association of early life factors, WM maturation and academic performance, including several confounder factors as BMI and CRF in children with overweight/obesity are needed.

The objectives were i) to investigate the associations of early life factors such as anthropometric neonatal data (i.e., birth weight and birth length) and breastfeeding

practices (i.e., exclusive and any breastfeeding) with WM microstructure, and ii) we test whether WM tract related to early life factors are associated with academic performance in children with overweight/obesity.

Method

Participants

We included 98 children with overweight/obesity (categorized based on the World Obesity Federation cut-off points) [65] aged 8-11 years from the ActiveBrains randomized clinical trial [68] (www.profith.ugr.es/activebrains). The present cross-sectional study was performed using baseline data prior to randomization. Measurements were carried out from November 2014 to February 2016. All included participants had valid data of early life factors, DTI metrics and academic performance variables (see **Table 1** for n in each variable). Parents or legal guardians were informed of the purpose of the ActiveBrains study and parental written informed consents were obtained. The ActiveBrains project was registered in ClinicalTrials.gov (identifier: NCT02295072) and was approved by the Ethics Committee on Human Research of the University of Granada.

Early life factors

Height (kg) and length (cm) at birth were collected from health records (i.e., physical medical record that parents have with the offspring's perinatal information). The duration of both exclusive and any breastfeeding in months was reported by parents. Parents were asked the question: *for how long (months) did the child receive only breast milk (neither formula or other liquid or solid)?* This was considered as exclusive breastfeeding. Furthermore, they were also asked *for how long (months) did the child receive any breast milk (combined with other liquid, formula, or solid)?* This was

considered as any breastfeeding. The responses were collected as continuous scale in months of breastfeeding.

Magnetic Resonance Imaging (MRI) procedure

Image acquisition

MRI data were acquired with a 3.0 Tesla Siemens Magnetom Tim Trio scanner (Siemens Medical Solutions, Erlangen, Germany). The DTI sequence consisted of a 128 direction echo planar imaging (EPI) sequence using the following sequence parameters: TR = 3,300 ms, TE = 90 ms, flip angle = 90, matrix=128 x 128, FOV= 230 mm x 230 mm, slice thickness = 4 mm, number of slices = 25 and voxel resolution= 1.8 x 1.8 x 4 mm^3. 30 volumes with diffusion weighting (b=1000s/mm^2) were collected and one volume without diffusion weighting (b=0s/mm^2).

Image quality assurance

Raw image quality was checked with a visual inspection. The sum of squares error (SSE) maps from the tensor estimation was calculated and visually inspected for structured noise. Image quality was rated using a 4-point scale, with 1="excellent", 2= "minor", 3="moderate", and 4="severe". Two subjects were manually excluded to be of insufficient quality (i.e., moderate and severe) due to poor image quality. Finally, probabilistic tractography data were visually inspected. First, the native space FA map registration was inspected to ensure images were all properly aligned to the template (masks were properly mapped to native space). Second, all tracts were visualized to ensure accurate path reconstruction.

Image preprocessing

Functional MRI of the Brain´s Software Library (FSL) (https://fsl.fmrib.ox.ac.uk) was used for image preprocessing [125,126]. First, image was adjusted for minor head motion and eddy-current induced artifacts [127,128]. In order to account for rotations applied to the image data [129,130], the resulting transformation matrices were used to rotate the diffusion gradient. Non-brain tissue was removed using the FSL Brain Extraction Tool [131]. Then, the diffusion tensor was fit, and common scalar maps (e.g., FA and MD) were subsequently computed. Detailed information about pre-processing steps is described elsewhere [132].

Probabilistic fiber tractography

Diffusion tensor data were first processed using the Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques (BEDPOSTx), accounting for two fiber orientations at each voxel [133,134]. Next, for each subject, the FA map was aligned to the FMRIB-58 FA template image with the FSL nonlinear registration tool (FNIRT). The inverse of this nonlinear warp field was computed and applied to a series of predefined seed, target, exclusion, and termination masks provided by the AutoPtx plugin. Probabilistic fiber tracking was then performed with the FSL Probtrackx module using these supplied tract-specific masks (i.e., seed, target, etc.) that were warped to the native diffusion image space of each subject [133]. The resulting path distributions were normalized to a scale from 0 to 1 using the total number of successful seed-to-target attempts and were subsequently thresholded, based on previous studies, to remove low-probability voxels likely related to noise. FA and MD values for these tracts were estimated for 15 large fiber bundles separately for left and right hemispheres (see **Table 2**) [135].

Academic performance

The Bateria III Woodcock-Muñoz Tests of Achievement was used to assess academic performance (i.e., Spanish version of the Woodcock-Johnson III) [69]. A trained evaluator individually administered the tests to each child. The full administration time was

between 100 to 120 min. In this study, we included standard score indicators of reading, writing mathematics and total achievement.

Covariates

Gestational age (weeks) was collected from birth records; pubertal maturity status was determined by peak height velocity (PHV) and was obtained through the Moore et al. equation for boys and girls [70]; PHV offset was calculated by the difference between PHV and chronological age. Parents were asked to report their maximum completed level of education and answers were categorized as: none of the parents had university degree, one of the parents had a university degree or both parents had a university degree. BMI was computed as weight in kilograms divided by height in meters squared (kg/m^2). CRF was assessed through the 20-meter shuttle-run test and maximal oxygen consumption (VO_2max, mL/kg/min) was calculated using the Lèger equation [72].

Statistical analysis

Participant's characteristics are shown as mean and standard deviation (SD) for continuous variables, and n and percentages for categorical variables. Multiple linear regressions were performed to test the associations between each early life factor such as anthropometric neonatal data (i.e., birth weight and birth length), and breastfeeding practices (i.e. exclusive and any breastfeeding) with FA and MD values of each WM tract, adjusted for sex, gestational age, PHV, parental education level, BMI and CRF. Since there were significant and robust correlations between hemispheres for most of the tracts (see correlation matrix in **figure 1 and 2**), we averaged FA and MD values across left and right hemispheres. Because of the number of comparisons (15 per DTI metric), a false discovery rate (FDR) [136] correction was applied to the results of each early life factors across both FA and MD simultaneously (30 comparisons for each early life factor), using the "p.adjust" function in R. Then, multiple linear regressions were performed for those tracts associated

with early life factors (without FDR correction) and academic performance, adjusting for sex, PHV, parental education level, BMI and CRF. Sensitivity analyses were carried out excluding children born preterm (gestational age < 37 weeks).

All statistical analyses were performed using R (version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria), and statistical significance was set at $p < .05$.

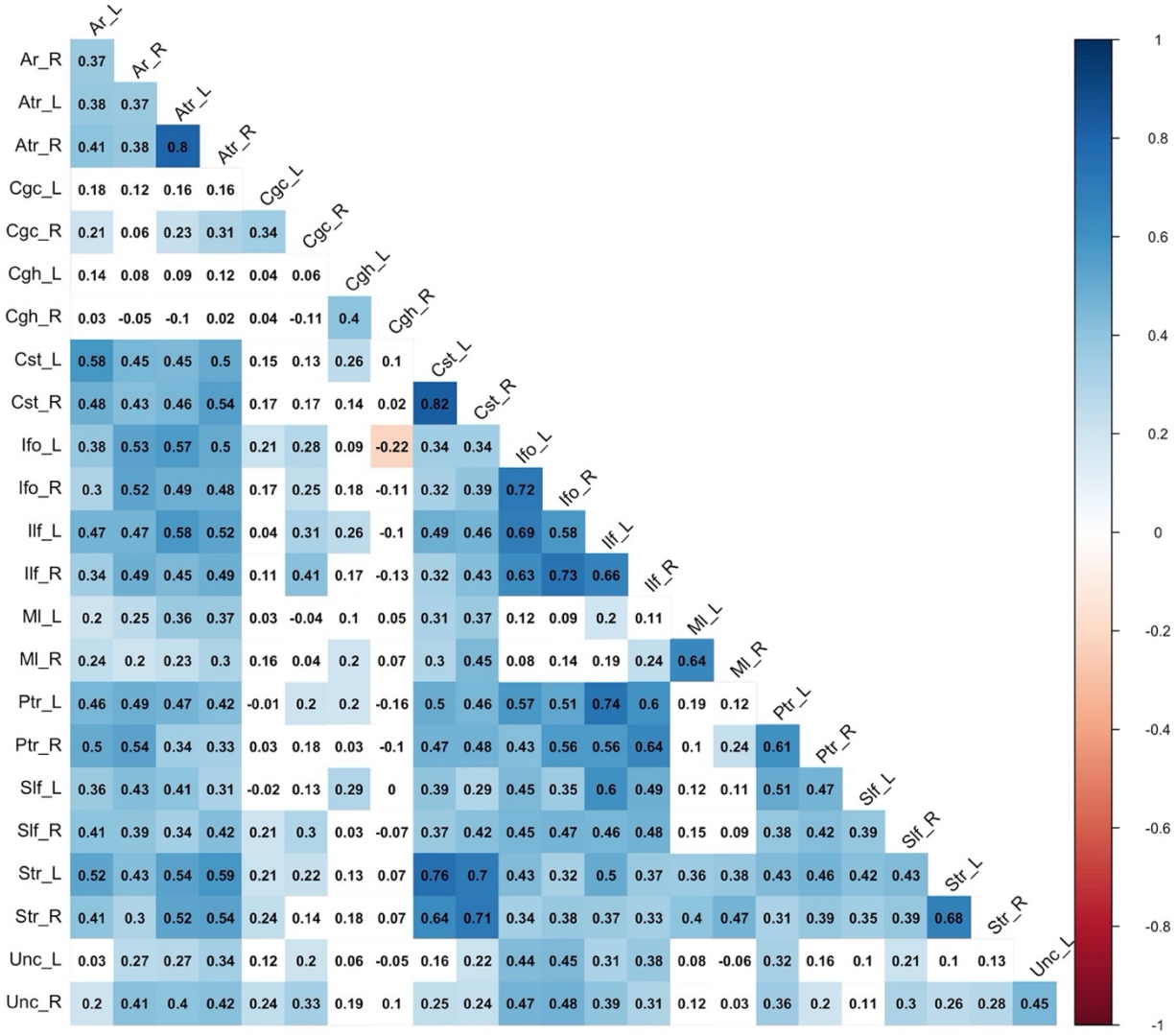

Figure 1. Correlation matrix between fractional anisotropy values of left and right tracts· Ar: Acoustic Radiation; Atr: Anterior thalamic radiation; Cgc: Cingulate gyrus part of cingulum; Cgh: Hippocampal part of the cingulum; Cst: Corticospinal tract; Ifo: Inferior fronto-occipital fasciculus; Ilf: Inferior longitudinal fasciculus; Ml: Medial lemniscus; Ptr: Posterior thalamic radiation; Slf: Superior longitudinal fasciculus; Unc: Uncinate fasciculus; _R: Right hemisphere; _L: left hemisphere· Numbers indicate r-values from pearson correlation· The color bar indicates the r-values ranging from -1 (red) to 1 (blue)· White backgrounds indicate non-significant correlation (p>0·05).

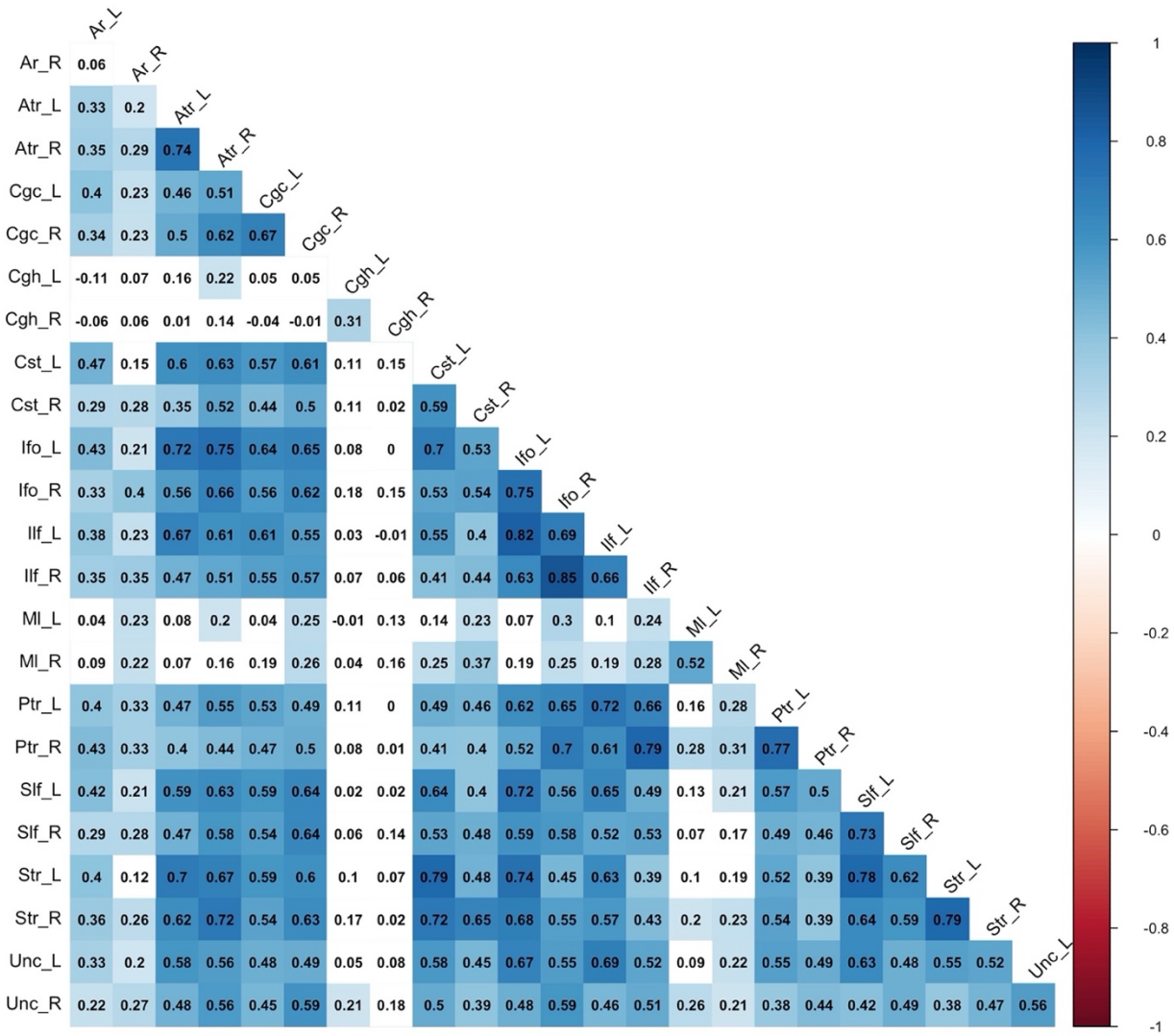

Figure 2. Correlation matrix between mean diffusivity values of left and right tracts·Ar: Acoustic Radiation; Atr: Anterior thalamic radiation; Cgc: Cingulate gyrus part of cingulum; Cgh: Hippocampal part of the cingulum; Cst: Corticospinal tract; Ifo: Inferior fronto-occipital fasciculus; Ilf: Inferior longitudinal fasciculus; Ml: Medial lemniscus; Ptr: Posterior thalamic radiation; Slf: Superior longitudinal fasciculus; Unc: Uncinate fasciculus; _R: Right hemisphere; _L: left hemisphere· Numbers indicate r-values from pearson correlation· The color bar indicates the r-values ranging from -1 (red) to 1 (blue)· White backgrounds indicate non-significant correlation (p>0·05)·

Results

Table 1 presents participant characteristics. **Table 2** presents the associations of anthropometric neonatal data with tract-specific FA and MD. Birth weight was positively associated with FA in the cingulate gyrus part of cingulum tract (β = 0.280 and p = 0.022). Birth length was positively associated with FA in the cingulate gyrus part of cingulum,

inferior fronto-occipital fasciculus and inferior longitudinal fasciculus tract (β ranging from 0.263 to 0.320 and $p \leq 0.030$), and negatively associated with MD in uncinate fasciculus tracts ($\beta = -0.255$ and $p = 0.032$). However, after FDR correction, none anthropometric neonatal data was associated to WM microstructure.

Table 1. Characteristics of the study sample.

		All		*Boys*		*Girls*
N	*n*		*n*		*n*	
Physical characteristics	98		60		38	
Age (yr)		10.03 ± 1.16		10.15 ± 1.18		9.81 ± 1.10
Weight (kg)		55.52 ± 11.22		56.55 ± 10.99		53.87 ± 11.53
Height (cm)		143.9 ± 8.56		144.78 ± 7.92		142.59 ± 9.44
Peak height velocity offset (yr)		-2.30 ± 0.98		-2.64 ± 0.82		-1.76 ± 0.99
Cardiorespiratory fitness (mL/kg/min)*		40.86 ± 2.72		40.85 ± 2.67		40.88 ± 2.73
Body mass index (kg/m^2)		26.58 ± 3.64		26.79 ± 3.76		26.24 ± 3.47
Body mass index category (%)	98		60		38	
Overweight		27.6		26.7		28.9
Obesity type I		42.9		46.7		36.8
Obesity type II		29.6		26.7		34.2
Parental education university level (%)	98		60		38	
None of the parents		65.3		70.0		57.9
One of the two parents		18.4		16.7		21.1
Both parents		16.3		13.3		21.1
Neonatal characteristics						
Birth weight (g)	96	3325.31 ± 549.13	59	3337.11 ± 597.30	37	3306.48 ± 469.41
Birth length (cm)	87	50.68 ± 2.67	57	50.60 ± 3.06	30	50.86 ± 1.77
Gestational age (week)	98	38.58 ± 2.63	60	38.50 ± 2.69	38	38.69 ± 2.56
Exclusive breastfeeding[†](%)	94		59		35	
Never		31.9		28.8		37.1
< 3 months		16.0		18.6		11.4
3-5 months		25.5		20.3		34.3
≥ 6 months		26.6		32.2		17.1
Any breastfeeding[‡] (%)	95		59		36	
Never		21.1		20.3		22.2
< 3 months		12.6		11.9		13.9
3-5 months		26.3		23.7		30.6
≥ 6 months		40.0		44.1		33.3
Academic performance (standard score)**	98		60		38	
Mathematics		102.49 ± 10.64		103.0 ± 11.1		10.00 ± 9.97
Reading		108.90 ± 12.89		109.0 ± 11.2		109.00 ± 15.3
Writing		114.70 ± 12.12		113.0 ± 11.8		117.0 0 ± 12.3
Total Achievement		110.10 ± 11.65		110.0 ± 10.7		111.00 ± 13.1

Values are mean ± SD or percentage. *Measured with the 20-m shuttle run test; [†]Months the child received only breast milk. [†]Months the child received breast milk combined with other liquids, or solids. **Measured by the Bateria III Woodcock-Muñoz Tests of Achievement.

Table 2. Association of birth wight and birth length with fractional anisotropy (FA) and mean diffusivity (MD) of the white matter microstructure.

White matter tract	*Birth weight*				*Birth length*			
	FA		*MD*		*FA*		*MD*	
	β	*p*	*β*	*p*	*β*	*p*	*β*	*p*
Acoustic Radiation	0.064	0.591	-0.08	0.498	0.234	0.052	-0.191	0.112
Anterior thalamic radiation	0.081	0.484	0.004	0.971	0.196	0.090	-0.204	0.08
Cingulate gyrus part of cingulum	**0.280**	**0.022**	-0.086	0.483	**0.276**	**0.030**	-0.199	0.115
Hippocampal part of the cingulum	0.046	0.704	-0.006	0.960	0.046	0.711	-0.012	0.918
Corticospinal tract	-0.045	0.695	-0.092	0.421	0.134	0.255	-0.171	0.141
Inferior fronto-occipital fasciculus	0.156	0.177	0.025	0.823	**0.320**	**0.006**	-0.166	0.149
Inferior longitudinal fasciculus	0.109	0.342	0.103	0.352	**0.263**	**0.023**	-0.094	0.407
Medial lemniscus	0.149	0.202	-0.209	0.08	0.142	0.241	-0.16	0.192
Posterior thalamic radiation	-0.074	0.518	-0.075	0.502	0.157	0.175	-0.193	0.092
Superior longitudinal fasciculus	-0.046	0.689	-0.087	0.450	0.121	0.305	-0.224	0.055
Superior thalamic radiation	-0.050	0.666	-0.068	0.55	0.153	0.188	-0.205	0.070
Uncinate fasciculus	0.162	0.173	-0.087	0.461	0.166	0.169	**-0.255**	**0.032**
Forceps major	-0.091	0.443	0.042	0.718	-0.048	0.692	-0.036	0.762
Forceps minor	0.022	0.848	-0.03	0.797	0.044	0.712	0.014	0.907
Middle cerebellar peduncle	0.014	0.905	-0.123	0.282	-0.053	0.654	-0.058	0.623

Linear regression models were adjusted for sex, peak height velocity, gestational age, parental education level, body mass index and cardiorespiratory fitness. FA= Fractional anisotropy (high FA corresponds to preferential diffusion along one direction indicating a high level of tissue organization), MD= mean diffusivity (high MD corresponds to relatively unimpeded water diffusion and indicates regions of low tissue organization). Values of $p < 0.05$ are in bold. β = Standardized values. No p value survived the FDR correction.

Early life factors and white matter

Table 3 presents the associations of exclusive and any breastfeeding with tract-specific FA and MD, adjusted for CRF and BMI. The only two significant associations between exclusive breastfeeding and FA in the inferior longitudinal tract and in the posterior thalamic radiation tract (β ranging from 0.221 to 0.259 and $p \leq 0.041$) disappeared after FDR correction. The results were repeated excluding children born preterm from the analyses, and the outcome did not substantially change (data not shown). **Table 4** shows the non-FDR corrected association between tract previously related to birth weight, birth length and breastfeeding and academic performance. No significant associations were found (all $p \geq 0.08$).

Table 3. Association of breastfeeding with fractional anisotropy (FA) and mean diffusivity (MD) of the white matter microstructure.

White matter tract	*Exclusive breastfeeding*				*Any breastfeeding*			
	FA		*MD*				*FA*	
	β	*p*	*β*	*p*	*β*	*p*	*β*	*p*
Acoustic Radiation	-0.047	0.678	0.097	0.379	0.086	0.420	0.042	0.687
Anterior thalamic radiation	0.005	0.964	0.023	0.832	0.046	0.666	0.079	0.447
Cingulate gyrus part of cingulum	0.055	0.633	0.084	0.459	0.134	0.226	0.098	0.366
Hippocampal part of the cingulum	0.009	0.939	0.017	0.879	0.123	0.258	-0.136	0.201
Corticospinal tract	0.133	0.219	-0.018	0.868	-0.009	0.93	0.114	0.262
Inferior fronto-occipital fasciculus	0.193	0.077	0.042	0.694	0.136	0.193	0.100	0.327
Inferior longitudinal fasciculus	**0.221**	**0.041**	0.080	0.443	0.187	0.071	0.139	0.162
Medial lemniscus	0.021	0.852	-0.037	0.744	-0.091	0.39	-0.014	0.895
Posterior thalamic radiation	**0.259**	**0.015**	0.042	0.689	0.135	0.191	0.128	0.196
Superior longitudinal fasciculus	0.206	0.058	0.002	0.986	0.142	0.174	0.043	0.674
Superior thalamic radiation	0.130	0.236	0.017	0.878	0.078	0.458	0.147	0.147
Uncinate fasciculus	0.029	0.797	-0.034	0.759	0.048	0.654	-0.045	0.672
Forceps major	0.206	0.065	-0.042	0.702	0.006	0.955	0.081	0.438
Forceps minor	-0.098	0.376	0.166	0.128	-0.121	0.252	0.143	0.171
Middle cerebellar peduncle	0.176	0.108	-0.025	0.819	0.107	0.308	-0.023	0.828

Linear regression models were adjusted for sex, peak height velocity, gestational age, parental education level, body mass index and cardiorespiratory fitness. FA= Fractional anisotropy (high FA corresponds to preferential diffusion along one direction indicating a high level of tissue organization), MD= mean diffusivity (high MD corresponds to relatively unimpeded water diffusion and indicates regions of low tissue organization). Values of $p < 0.05$ are in bold. No p value survived the FDR correction.

Table 4. Associations of white matter tract related to early life factors with academic performance.

White matter tract	*Mathematics*		*Reading*		*Writing*		*Total achievement*	
	β	*p*	*β*	*p*	*β*	*p*	*β*	*p*
Fractional Anisotropy								
Cingulate gyrus part of cingulum	-0.100	0.330	-0.100	0.350	-0.120	0.260	-0.127	0.223
Inferior fronto-occipital fasciculus	-0.140	0.150	-0.060	0.530	-0.110	0.260	-0.111	0.239
Inferior longitudinal fasciculus	-0.050	0.600	0.008	0.940	-0.110	0.270	-0.049	0.607
Posterior thalamic radiation	-0.170	0.080	-0.080	0.410	-0.090	0.350	-0.130	0.171
Mean Diffusivity								
Uncinate fasciculus	0.049	0.620	-0.080	0.410	-0.080	0.410	-0.058	0.537

Linear regression models were adjusted for sex, peak height velocity, parental education level, body mass index and cardiorespiratory fitness. Fractional anisotropy corresponds to preferential diffusion along one direction indicating a high level of tissue organization, and mean diffusivity corresponds to relatively unimpeded water diffusion and indicates regions of low tissue organization). Academic performance was measured with the Bateria III Woodcock-Muñoz Tests of Achievement. β = Standardized values

Discussion

The aim of the present study was to examine tract-specific associations between early life factors and WM maturation in children with overweight/obesity after adjusting for several covariates including BMI and CRF. Specifically, birth weight and birth length were only associated with higher FA in the cingulate gyrus part of cingulum, and birth length was selectively associated with higher FA in the inferior fronto-occipital fasciculus and inferior longitudinal fasciculus, and lower MD in the uncinate fasciculus. In addition, exclusive breastfeeding, but not any breastfeeding, was associated with higher FA in the inferior longitudinal fasciculus and the posterior thalamic radiation tract. However, WM tracts explained by birth weight, birth length and exclusive breastfeeding did not surpass FDR correction. Lastly, WM tracts related to birth weight, birth length and exclusive breastfeeding were not associated with academic performance. These results suggest that WM maturation during childhood could be partially influenced by the fetal environment in children with overweight/obesity, but the implicated behavioral consequences remain inconclusive.

Several mechanisms might be speculated to explain the present results. During the gestation period, several key processes help to develop WM microstructure, such as the growth and wiring of axonal fibers; indeed, a non-optimal pregnant environment might cause negative consequences for WM development. For example, oligodendrocytes sensitive to hypoxia, that are abundant after term and are precursors of myelin in axonal fibers, mostly occur during the second period of gestation. On the other hand, the pruning of useless connections rather starts during the first post-natal weeks along with external stimulations related to acute changes in myelination during the first post-natal months [137,138]. Of note, FA increases up to 44% during the first year, while the second year increases up to 9% over levels at age 1 year [12]. Although it is well established that WM is developed

during prenatal period, certain regions continue their development through childhood and adolescence [139]. Lastly, a recent work suggests that exposure to perinatal risk factors is mainly related to reductions in cross-section of WM fiber more than to alterations in fiber density [140].

Regarding specific tract associations, both birth weight and birth length were positively associated to FA in the cingulate gyrus part of cingulum. This tract has been associated with other anthropometric birth indicators both in the newborn, as well as during childhood and adulthood. In this line, very low birth weight infants showed poorer WM organization (increased MD) in the cingulate gyrus part of cingulum [141]. Our findings suggest that the association of anthropometric neonatal data with WM microstructure in this region persists until childhood. Similarly, Eikenes et al. demonstrated that the effect of anthropometric neonatal data on WM microstructure persists until adulthood, even affecting other peripheral tracts [115]. Importantly, the cingulate gyrus part of cingulum is a major limbic WM pathway linking cortical regions and amygdala, and it is involved in cognitive control, affect and emotion regulation, and face processing [141-143].

Specifically, birth length was associated with higher FA in the inferior fronto-occipital fasciculus, inferior longitudinal fasciculus and lower MD in the uncinate fasciculus. In this line, previous associations have been found with similar anthropometric neonatal data in neonates with different body size at birth [110,144] For instance, head circumference was associated with WM maturation in the inferior fronto-occipital fasciculus in preterm children at term-equivalent age [144]. In addition, Lepomaki found that small of gestational age was associated with poor WM in this tracts at term age [111]. Also, those born preterm showed lower FA in inferior fronto-occipital and uncinate fasciculus in comparison with term-born children at school age [117] as well as during adolescence [116]. This suggests that the inferior fronto-occipital and the uncinate fasciculus are especially

sensitive to insults of the gestational environment, and this is detectable even several years later. Previous studies suggest the possible implications of these tracts in several cognitive indicators. For instance, lower FA in the inferior fronto-occipital fasciculus has been related to poorer cognition [145], since it connects multiple regions involved in critical cognitive functions (i.e., language and reading development) [113], such as occipital to the temporal and frontal lobe [146]. Further, recent findings also suggest that the inferior fronto-occipital fasciculus and uncinate fasciculus play a crucial role in both verbal and non-verbal semantic language [117,147] as well as face processing [146] or goal-oriented behavior [148].

Although it is well established that breastfeeding is an important precursor of key nutrients associated with WM development, we did not find associations of any breastfeeding and WM maturation in children with overweight/obesity. Likewise, a previous work in a similar age range found that breastfeeding duration was not associated with WM volumes [56], and another work found a significant association between exclusive breastfeeding duration and WM microstructure in boys, but not in girls [149]. In this line, we found exclusive breastfeeding was positively associated with FA in the inferior longitudinal fasciculus and posterior thalamic radiation tract, although these associations did not surpass FDR correction. Interestingly, the implications of both tracts are similar to previous tracts, suggesting a key role in the language deficit. For example, both the inferior longitudinal fasciculus and inferior fronto-occipital fasciculus join the posterior occipitotemporal regions to the temporal pole, with evident role in semantic language [150]. Further, these tracts are inferior tracts that have shown associations with birth weight in adolescents [108], and indeed we also found birth weight was associated with the inferior longitudinal fasciculus in children with overweight/obesity. On the other hand, the posterior thalamic radiation tract is a thalamocortical tract involucrate in sensory motor function [151]. Moreover, those

born with very low birth weight has decreased FA values in the posterior thalamic radiation tract in comparison with a control group during adulthood [115].

Interestingly, previous studies reported that the development of WM starts from the center to the periphery of the brain and from the occipital lobe towards the frontal lobe, with a posterior to anterior development [152,153]. Indeed, these WM microstructural changes might be responsible for neurodevelopmental deficits in cognitive and motor functions [154]. Here, we tested the influence of WM maturation related to suboptimal early life factors on academic performance, according to previous studies suggesting its implication in executive function and academic abilities [105,108,109]. Conversely, WM tracts previously associated with early life factors were not related to academic performance in children with overweight/obesity; future studies in larger samples should examine other possible implications in language, sensory-motor function, emotion regulation or face recognition.

Limitations

The current study has some limitations that must be mentioned. The use of a retrospective cross-sectional design prevents us from inferring causal relationships. In addition, our analyses need replication in larger samples to elucidate the associations between the different early life factors and WM maturation in both children with overweight/obesity and normal-weight children. On the other hand, we did not collect information about the type of complementary feeding or feeding following the breast- or formula-feeding period, and null results about any breastfeeding might be explained by the differences in these dietary patterns and other lifestyle indicators not controlled during the first years of life. Further, although DTI-derived measurements provide valuable information about WM microstructure, caution needs to be taken when interpreting the results. It is well known that interpretation of FA is complex and diffusivity measurements

are at most indirect indicators of myelination, axon packing, membrane permeability or axon density [81,155], and diffusion can be influenced by numerous factors [156,157] that FA cannot differentiate. In this line, our resolution was 4 mm^3 and has been documented that in an isotropic resolution ≥ 2 mm, FA can be affected by crossing fibers [155]. Additionally, our tract-specific approach did not have tract-specific masks for all brain regions. For instance, we did not encompass the corpus callosum outsize of the forceps major and forceps minor fiber bundles. Despite the noted limitations, this study has several strengths such as the inclusion of BMI, CRF, socioeconomic status and pubertal maturity status as covariates, the use of a reliable measurement of academic performance, the relatively large sample of children with overweight/obesity with DTI measurements and its bounded range of age.

Conclusion

Our findings suggest that perinatal factors may influence to some extent WM microstructure in children with overweight/obesity. Specifically, among the 15 tracts examined, higher birth weight and birth length were only related to better WM microstructure in the cingulate gyrus part of cingulum, and birth length were related to inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, and the uncinate fasciculus. In addition, longer exclusive breastfeeding, but not any breastfeeding, was only associated to better WM microstructure in the inferior longitudinal fasciculus and the posterior thalamic radiation tract. Despite this, none of mentioned association survived multiple comparisons. Lastly, although WM tracts related to early life factors were not associated with academic performance, other behavioral implications remain a matter of speculation in this sample.

5.3. Study 3: Early life factors and structural brain network in children with overweight/ obesity: The ActiveBrains project

Introduction

Early life stage is a sensitive period related to brain health later in life [158]. Fetal and postnatal environment are crucial for the consolidation of brain structures with long lasting consequences. Early life factors such as anthropometric neonatal data (birth weight, birth length) and breastfeeding practices (exclusive and any breastfeeding) are indicators that represent fetal environment and the first stage of life. These factors have been associated with brain outcomes later in life such as, gray matter volumes [103,159], WM microstructure [160], and functional brain connectivity [161].

Previous studies with the present sample demonstrated that birth weight and length were extensively associated with gray matter volumes [103], and WM microstructure in children with overweight/obesity (submitted). Similarly, other previous studies showed that those born with low birth weight show reduction in gray matter volume in several cortical and subcortical regions including hippocampus, amygdala, cerebellum as well as lower academic abilities at childhood [162,163]. However, the early life programing of structural brain networks during childhood is still poorly understood. Unlike to traditional approaches such as brain region of interest or voxel-based morphometry (VBM), the structural covariance analysis provides information about interindividual differences in regional brain structure covarying with other brain structures as a complex network [164,165]. Structural covariance networks provide a most comprehensive approach of the interrelationship among different regions. This analysis solves some limitations related to VBM associated to type I error due to large number of comparisons [17]. Thus, the study of brain structural networks will provide a powerful approach to advance the knowledge on the relationship of early life factors and brain health.

Early life factors and structural brain networks

Structural brain network studies found that compared to term-born controls, premature-born individuals had alterations in several gray matter structural network during adolescence [16], and even during young adulthood, with implications in language development [166]. Similarly, lower gestational age was associated with reduced volumes in several structural brain networks, coupled with poorer executive function during childhood [17]. In addition, breastfeeding practices are powerful postnatal environmental factors related to brain development principally due to breast milk provides key nutrients necessary to myelinization as well brain maturation. In this line, while few studies have investigated its relationship with WM connectivity [167] and functional connectivity [161], no study has explored the relationship of breastfeeding practices with structural gray matter network in children with overweight or obesity.

Obesity has been linked to structural brain network abnormalities, mainly, to WM connectivity [31,168], and gray matter volume in several network, including medial frontal, temporal areas, limbic regions and memory-sensitive networks [169]. Further, obesity has detrimental effects on regional gray matter volumes, implicated in executive function and higher-order of cognitive processes, which in turn may influence academic performance [170]. Indeed, compared to normal-weight children, those with overweight and obesity showed lower academic performance [33]. To our knowledge, this is the first study investigating how early life factors may influence structural covariance network, and in turn academic performance in the context of childhood obesity.

Thus, the aims of the present study were (i) to investigate the association of early life factors such as anthropometric neonatal data (birth weight, birth length) and breastfeeding practices (exclusive and any breastfeeding) with structural brain network and

(ii) to test whether structural brain networks associated to early life factors are related to academic performance in children with overweight/obesity.

Methods

Participants

In this cross-sectional analysis, we used baseline from the ActiveBrains randomized clinical trial [68] (www.profith.ugr.es/activebrains). A total of 96 children (aged 8-11 years) with overweight/obesity [171] with valid data on early life factors, brain structural network and academic performance variables were included (see **Table 1** for n in each variable). Measurements were carried out from November 2014 to February 2016. Parents or legal guardians signed written informed parental consents were obtained and were informed about purpose of the Activebrain randomized clinical trial. The ActiveBrains project was approved by the Ethics Committee on Human Research (CEIH) of the University of Granada and was registered in ClinicalTrials.gov (identifier: NCT02295072).

Early life factors

Birth weight (kg) and birth length (cm) were obtained from the health records. Parents were asked to report their child's breastfeeding practices[103], namely, the duration in months (as continuous scale) of exclusive (neither formula or other liquid) and any (combined with other liquid, formula, or solid) breastfeeding.

Academic performance

Academic performance was assessed using the Bateria III Woodcock-Muñoz Tests of Achievement (i.e., Spanish version of the Woodcock- Johnson III) [172]. Each administration lasted between 100 and 120 min, and instructions were provided

individually during the session to each child. Indicators of reading, writing, mathematics, and a total achievement standard scores were used in this study.

Covariates

Participant's sex, gestational age, peak height velocity (PHV), parental educational level and CRF were included as covariates. Gestational age in weeks was collected from the birth records. PHV was calculated and used as pubertal maturity status [70]. The difference with chronological age was defined as a value of maturity offset. Both parents were asked to report their maximum completed level of education and answers were categorized as: (0) none of the parents had university degree, (1) one of the parents had a university degree or (2) both parents had a university degree [71]. CRF was obtained using 20-m shuttle-run test and maximal oxygen consumption (VO2max, mL/kg/min) and calculated by the Leger formulae [72]. We included CRF (a measure of the aerobic capacity) since a previous volumetric study with the present sample showed strong association with gray matter volume.

Magnetic resonance imaging (MRI)

Acquisition and processing

Details regarding MRI methods have been previously published [36]. Briefly, MRI data were obtained using a 3.0 Tesla Siemens Magnetom Tim Trio scanner (Siemens Medical Solutions, Erlangen, Germany). Whole-brain T1-weighted image dataset were acquired using a magnetization-prepared rapid gradient- echo (MPRAGE) sequence (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 = 394 s. The processing protocol include

quality control, alignment and segmentation into gray matter tissue, WM tissue and cerebrospinal fluid. In addition, Montreal Neurological Institute (MNI) space was used to spatial normalization and to create a template using Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL). Finally, a normalization to the DARTEL template using a non-linear transformation and modulated with Jacobian determinants were performed; and images were smoothed by convolving them with an isotropic Gaussian kernel of 8 mm full width at half maximum (FWHM).

Non-negative Matrix Factorization analysis

Non-negative Matrix Factorization (NNMF) analysis was used to identify structural networks. NNMF is a method for extracting structural networks where volume covaries across all participants [165]. An extended function of NNMF was used corresponding to the orthonormal projective non-negative matrix factorization (OPNMF), which was run using "*opnmf_mem*" in MATLAB with code available in https://github.com/asotiras/brainparts. This approach provided components that could be considered as a biologically more meaningful parts-based representation of the brain as compared to more standard approaches such as principal component analysis (PCA) or independent component analysis (ICA) [165,173]. In order to build more consistent network, available data from both time point (pre- and post) smoothed structural gray matter images for each subject were reshaped into a matrix (dimensions: 198 participants x 2122945 voxels). The local grey matter volumes with a threshold of 0.2 (i.e., to eliminate the voxels with partial volume effect) were then extracted in a whole-brain grey matter mask and used as input for OPNMF (dimensions: 198 participants x 470556 voxels) and approximates this matrix as a product of two matrices with non-negative elements.

The data were represented denoting the corresponding sparse components (W) and the subject specific loading coefficients (H). The first matrix, W, is of size V × K and contains the estimated non-negative networks and their respective loadings on each of the V voxels; and K is the specified number of networks. The W matrix, or "Network Components," is composed of coefficients that denote the relative contribution of each voxel in the network. The second matrix, H, is of size K × N and contains subject-specific loading coefficients for each network. These subject specific coefficients indicate the contribution of each network in reconstructing the original gray matter map. To obtain a range of possible solutions for comparison [165,174], we ran multiple NMF solutions requesting a K from 6 to24 networks in steps of two (i.e., K=6:2:24). We then calculated the reconstruction error for each solution as the Frobenius norm and plotted the reconstruction error for all solutions. The solution resulted in 20 networks showed in the **Figure 1.** Only baseline loading coefficients were used for the analyses

Statistical analysis

Participant's descriptive characteristics are presented as mean and standard deviation (SD) for continuous variables, and absolute (n) and relative frequencies (%) for categorical variables. As first step, multiple linear regressions using each early life factor such as anthropometric neonatal data (i.e., birth weight, birth length), and breastfeeding practices (i.e., exclusive and any breastfeeding) as explicative variable and each structural network solution (specific loading coefficient -H- for each network) as outcome. Covariates used in all models were sex, gestational age, PHV, parental education level and CRF. Because of the number of comparisons (20 network for each explicatory variable), a false discovery rate (FDR) [136] correction ($p < .05$) was applied to the results of each early life factors across structural networks (40 comparisons for anthropometric neonatal data and

40 for breastfeeding practices), using the "p.adjust" function in R. In a second step, for those networks associated with early life factors after FDR correction, multiple linear regressions were performed to examine the associations between each structural network solution and academic performance adjusting for the abovementioned covariates. A FDR correction was also applied to the association of network associated with academic performance (corresponding to 28 comparisons). All statistical analyses were performed in R (version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria), and statistical significance was set at $p < .05$.

Results

Table 1 presents a description of the participant characteristics. The 20 structural covariance networks delineated by NNFM are shown in **Figure 1**. **Table 2** presents the associations of each early life factor with the structural networks. Results showed that both birth weight and birth length were positively associated with the same 5 structural networks following FDR correction. In addition, birth weight was related to network 4 and birth length with network 7 **(Figure 2)**.

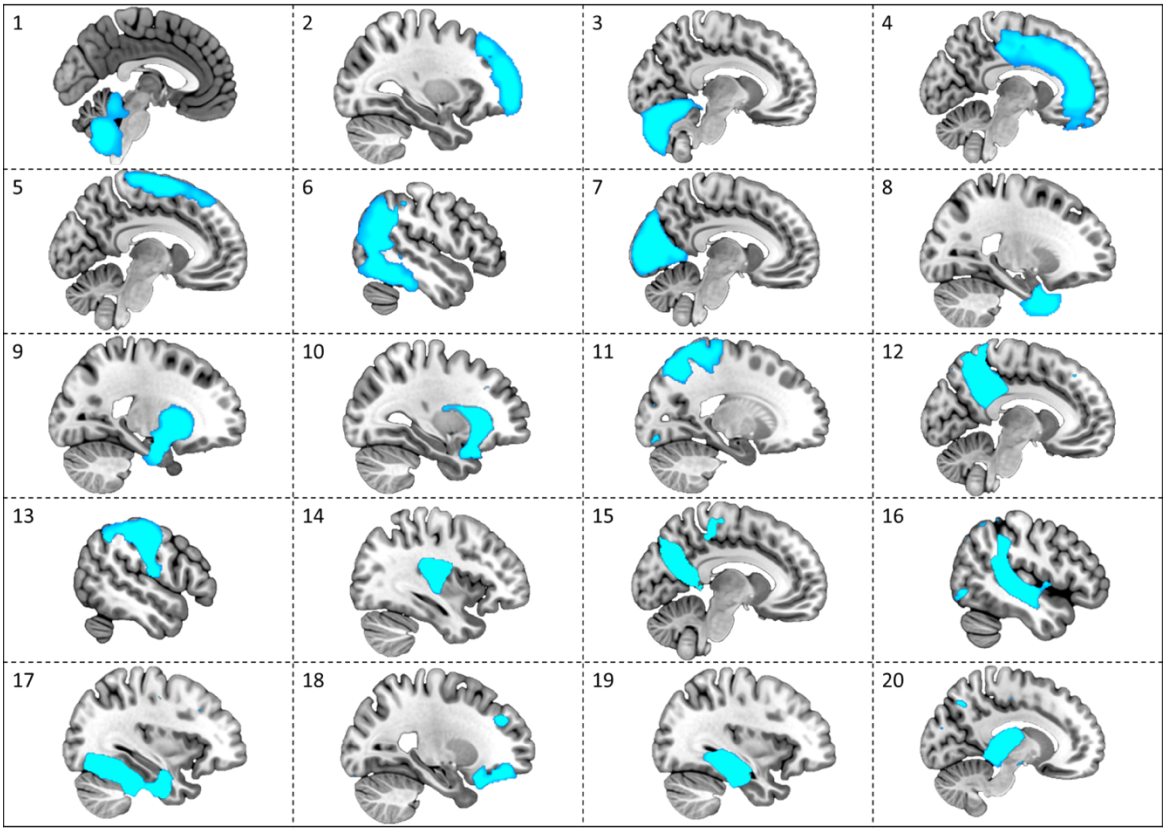

Figure 1. Structural covariance networks delineated by Non-negative Matrix Factorization analysis. Structural covariance networks are shown for the 20-network solution. The blue color represents the spatial distribution of each network. For each network, we show the sagittal view (left hemisphere) that best captures the main areas of coverage. The anatomical coverage of each structural covariance network was a follows:(1) cerebellum I-IV, VIIIa, VIIb, crus II, vermis VIIIb to vermis IX; (2) frontal pole; (3) cerebellum V, VI, crus I, vermis VI to vermis VIIIa; (4) frontal medial cortex, paracingulate gyrus to anterior cingulate gyrus; (5) superior frontal gyrus, supplementary motor cortex to precentral gyrus; (6) lateral occipital cortex, angular gyrus to temporooccipital parts of middle and inferior temporal gyri; (7) occipital pole, supracalcarine cortex, intracalcarine cortex to lingual gyrus; (8) temporal pole to temporal fusiform cortex; (9) caudate, putamen ,pallidum, accumbens to amygdala; (10) frontal operculum cortex to insular cortex; (11) superior postcentral gyrus to superior lateral occipital cortex; (12) posterior cingulate gyrus to anterior precuneus cortex; (13) inferior postcentral gyrus to central opercular cortex; (14) anterior supramarginal gyrus to parietal operculum cortex; (15) posterior precuneus cortex to cuneal cortex; (16) posterior supramarginal gyrus to posterior superior temporal gyrus; (17) occipital fusiform gyrus to temporal occipital fusiform cortex; (18) frontal orbital cortex; (19) hippocampus to parahippocampal gyrus; and (20) thalamus.

Table 1. Characteristics of study sample.

	All		*Boys*		*Girls*	
N	n		n		n	
Physical characteristics	96		60		36	
Age (yr)		10.01 ± 1.14		10.16 ± 1.14		9.78 ± 1.13
Weight (kg)		55.65 ± 11.15		56.66 ± 10.69		53.97 ± 11.85
Height (cm)		143.81 ± 8.32		144.69 ± 7.37		142.34 ± 9.64
Peak height velocity offset (yr)		-2.33 ± 0.96		-2.65 ± 0.78		-1.80 ± 1.01
Cardiorespiratory fitness (mL/kg/min)*		40.86 ± 2.77		40.84 ± 2.77		40.90 ± 2.82
Body mass index (kg/m^2)		26.70 ± 3.69		26.90 ± 3.79		26.36 ± 3.53
Body mass index category (n,%)	96		60		36	
Overweight		25 (26.0)		16 (26.7)		9 (25.0)
Obesity type I		41 (42.7)		27 (45.0)		14 (38.9)
Obesity type II		30 (31.2)		17 (28.3)		13 (36.1)
Parental education university level (n,%)	96		60		36	
None of the parents		64 (66.7)		43 (71.7)		21 (58.3)
One of the two parents		17 (17.7)		10 (16.7)		7 (19.4)
Both parents		15 (15.6)		7 (11.7)		8 (22.2)
Neonatal characteristics						
Birth weight (g)	94	3343.72 ± 542.25	59	3358.98 ± 579.32	35	3318.00 ± 480.28
Birth length (cm)	85	50.69 ± 2.68	57	50.61 ± 3.02	28	50.86 ± 1.84
Gestational age (week)	96	38.62 ± 2.59	60	38.57 ± 2.59	36	38.70 ± 2.63
Breastfeeding practices (months)	92		59		33	
Exclusive breastfeeding†		3.19 ± 3.24		3.53 ± 3.53		2.58 ± 2.59
Any breastfeeding‡		7.06 ± 8.03		6.81 ± 7.18		7.50 ± 9.44
Academic performance (standard score)**	96		60		36	
Mathematics		102.02 ± 10.68		102.37 ± 11.23		101.44 ± 9.82
Reading		108.55 ± 12.92		108.33 ± 11.08		108.92 ± 15.67
Writing		113.99 ± 11.99		112.55 ± 11.91		116.39 ± 11.91
Total Achievement		109.49 ± 11.66		108.98 ± 10.67		110.33 ± 13.25

Values are mean ± SD or percentage. *Measured by the 20-m shuttle run test; †Months the child received only breast milk. ‡Months the child received breast milk combined with other liquid, or solid. **Measured by the Bateria III Woodcock-Muñoz Tests of Achievement.

Table 2. Association of early life factors with structural networks

Network	*Birth weight*		*Birth length*		*Exclusi*
	β	*p*	*β*	*p*	*β*
Network 1: Cerebellum I-IV, VIIIa, VIIb , crus II, and vermis VIIIb and IX	0.309	**0.001**	0.359	**<0.001**	0.16
Network 2: Frontal pole	0.158	0.103	0.117	0.281	0.06
Network 3: Cerebellum V, VI, crus I, and vermis VI and VIIIa	0.260	**0.009**	0.314	**0.003**	0.15
Network 4: Frontal medial cortex, paracingulate and anterior cingulate gyrus	0.245	**0.010**	0.237	0.023	0.03
Network 5: Superior frontal, supplementary motor cortex and precentral gyrus	0.094	0.318	0.071	0.499	0.07
Network 6: Lateral occipital cortex, angular gyrus and temporooccipital parts of middle and inferior temporal gyri	0.207	0.033	0.225	0.035	0.11
Network 7: Occipital pole, supracalcarine cortex, intracalcarine cortex and lingual gyrus	0.212	0.037	0.288	**0.009**	0.19
Network 8: Temporal pole and temporal fusiform cortex	0.194	0.043	0.145	0.169	0.01
Network 9: Caudate, putamen, pallidum, accumbens and amygdala	0.252	**0.008**	0.304	**0.004**	-0.01
Network 10: Frontal operculum cortex to insular cortex	0.199	0.031	0.196	0.058	0.01
Network 11: Superior poscentral gyrus to superior lateral occipital cortex	0.150	0.149	0.133	0.240	0.04
Network 12: Posterior cingulate gyrus and anterior precuneous cortex	0.240	**0.014**	0.266	**0.013**	0.04
Network 13: Inferior poscentral gyrus to central opercular cortex	0.127	0.229	0.131	0.256	0.04
Network 14: Anterior supramarginal gyrus to parietal operculum cortex	0.126	0.190	0.149	0.157	0.04
Network 15: Posterior precuneus cortex and cuneal cortex	0.198	0.040	0.237	0.025	0.07
Network 16: Posterior supramarginal to posterior superior temporal gyrus	0.212	0.032	0.242	0.021	0.13
Network 17: Occipital fusiform gyrus to temporal occipital fusiform cortex	0.095	0.307	0.187	0.069	0.09
Network 18: Frontal orbital cortex	0.155	0.096	0.109	0.293	0.04
Network 19: Hippocampus and parahippocampal gyrus	0.252	**0.011**	0.327	**0.002**	0.05
Network 20: Thalamus	0.189	0.062	0.174	0.118	0.03

Analyses were adjusted for sex, peak height velocity offset (years), parent education university level (neither/one/both), gestational
(mL/kg/min). †Months the child received only breast milk. ‡Months the child received breast milk combined with other liquid
networks are depicted graphically in Figure 1. Values in bold indicated that p value survived the false discovery rate correction fo

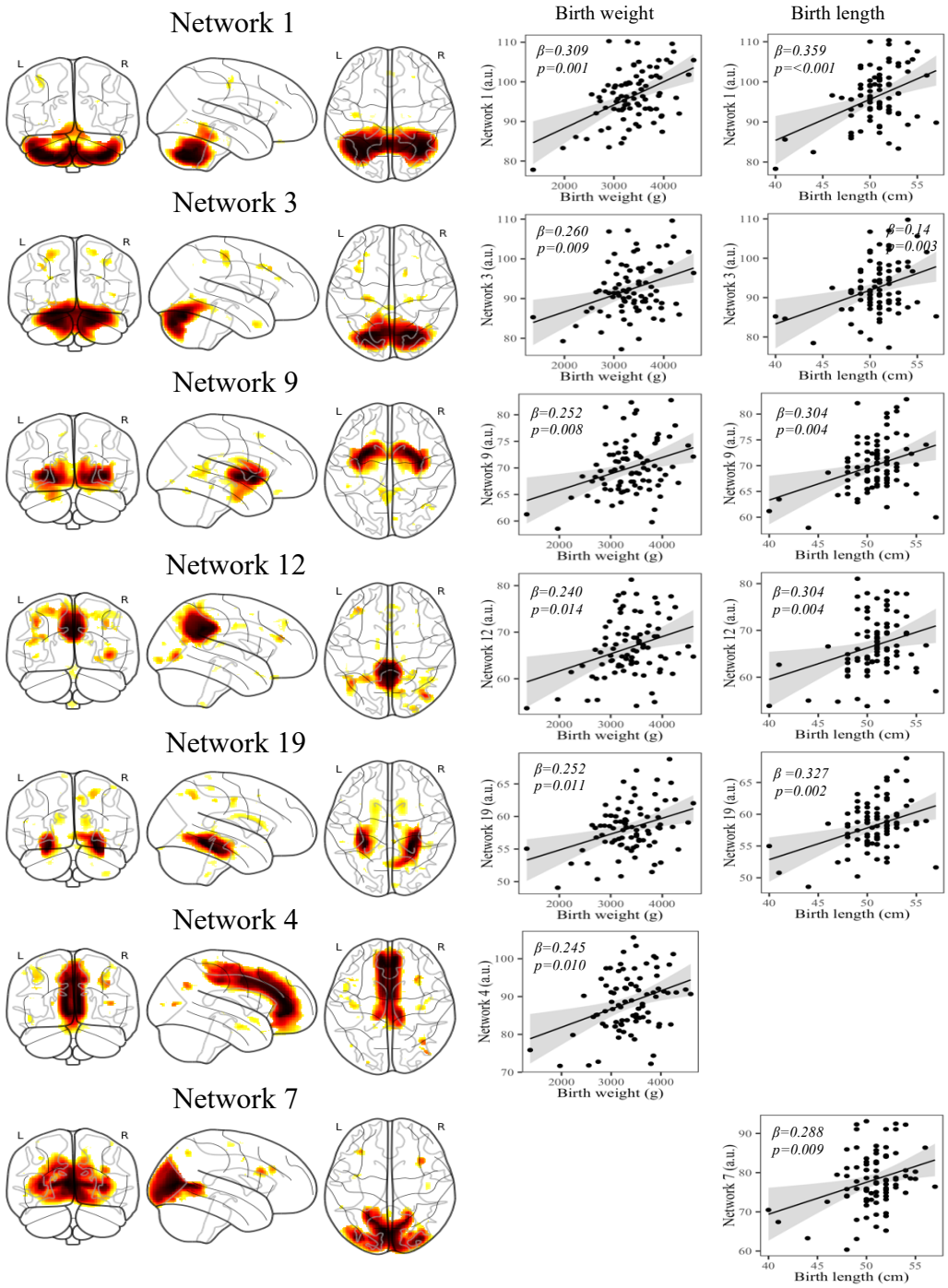

Figure 2. Structural brain network associated to birth weight and birth length after FDR correction. **Network 1:** Cerebellum I-IV, VIIIa, VIIb, crus II, and vermis VIIIb and IX; **Network 3:** Cerebellum V, VI, crus I, and vermis VI and VIIIa: **Network 4:** Frontal medial cortex, paracingulate and anterior cingulate gyrus; **Network 7:** Occipital pole, supracalcarine cortex, intracalcarine cortex and lingual gyrus; **Network 9:** Caudate, putamen, pallidum, accumbens and amygdala; **Network 12:** Posterior cingulate gyrus and anterior precuneous cortex; **Network 19:** Hippocampus and parahippocampal gyrus. β = Standardized values. a.u. = arbitrary units.

Early life factors and structural brain networks

Exclusive or any breastfeeding practices were not associated with structural networks after FDR correction (**Table 2**). **Table 3** shows the association between the networks previously related to anthropometric neonatal data and academic performance. Only an association between the network 12 and reading ($\beta = 0.245$; p = 0.025) was found, though this association disappear after FDR correction.

Table 3. Associations of early-life factor-related structural networks with academic performance*.

Network	*Writing*		*Reading*		*Mathematics*		*Total achievement*	
	β	*p*	*β*	*p*	*β*	*p*	*β*	*p*
Network 1	-0.027	0.813	-0.068	0.546	-0.098	0.387	-0.076	0.488
Network 3	0.024	0.829	-0.021	0.845	-0.12	0.273	-0.049	0.644
Network 4	0.095	0.412	0.017	0.881	-0.074	0.512	0.020	0.856
Network 7	0.166	0.121	0.021	0.840	-0.036	0.731	0.073	0.473
Network 9	0.069	0.546	0.040	0.715	-0.19	0.086	-0.023	0.831
Network 12	**0.245**	**0.025**[#]	0.159	0.138	0.064	0.559	0.196	0.060
Network 19	0.128	0.251	0.039	0.716	-0.08	0.466	0.042	0.690

Values are standardized regression coefficients (β). Analyses were adjusted for sex, peak height velocity offset (years), parent education university level (neither/one/ both) and cardiorespiratory fitness (mL/kg/min). *Measured with the Bateria III Woodcock-Muñoz Tests of achievement. [#]This association disappears after false discovery rate correction. Network are depicted in Figure 1.

Discussion

In this cross-sectional study, we examined the associations of the early life factors with structural covariance network in children with overweight or obesity. Our main finding was that both birth weight and birth length were associated with 5 overlapped structural brain networks, however breastfeeding practices were not related to brain structural networks. Those 5 networks covered regions on cerebellum (networks 1 and 3), posterior cingulate gyrus and anterior precuneus cortex (network 12), and subcortical structures as hippocampus and parahippocampal gyrus (network 19) and caudate, putamen, pallidum, accumbens and amygdala (network 9). In addition, the network 12 was related to reading, although this association disappeared after correcting for multiple comparison. Therefore, our study do not support that the influence of early life factors observed on

structural networks is translated into a worse academic performance at least in this sample of children with overweight/obesity. Future studies will investigate the potential impact of these early life factors-related structural networks on other health outcomes.

Our and other previous studies supported the selective vulnerability of cerebellum explained by early life factors[103,175,176]. We found that both birth weight and birth length were associated with 2 structural networks in the cerebellum (networks 1 and 3) in children with overweight or obesity. This is particularly important in the context of overweight/obesity since early life factors may predict future childhood obesity, and the cerebellum is a region linked to vulnerability in obese population with implication in motor as well as other non-motor domains [176-178]. In addition, we found that birth weight and birth length had overlapped influenced in three networks in the cerebrum, namely, two of them in subcortical regions (networks 9 and 19) and one in cortical regions (network 12). The networks involving deep gray matter structures covered caudate, putamen, pallidum, accumbens and amygdala (network 9); these regions participated in sensorimotor, limbic, and cognitive information processing [179]. In this line, a recent study showed that compared to term-born controls, premature-born individuals had altered deep grey matter structures, such as thalamus and basal ganglia volumes, at 8 years of age [180]. The other subcortical network covered hippocampus and parahippocampal gyrus (network 19), characterized as memory-related regions and also known as specific areas involved in disruptions produced by the early life stress [181]. Lastly, the cortical network covered the posterior cingulate gyrus and anterior precuneus cortex it has been associated default mode network with implication in self-referential and internal and external mentation processing [182-184]. However, breastfeeding practices were not associated with structural networks in children with overweight or obesity. Therefore, while early life programing of structural network was mainly driven by birth weight and birth length in this population, future studies should

examine the early life factors-structural network association comparing normal-weight children and those with overweight/obesity.

On the other hand, birth weight and birth length were selectively associated with 2 cortical structural networks. Birth weight was related to a network covering frontal regions (network 4: frontal medial cortex, paracingulate and anterior cingulate gyrus), which has been related to early life factors [81] and recently highlighted as reduced in obese population [185]; regions of this network are implicated in emotional processes including social behaviors, decision-making and emotional responses [186,187]. However, birth length was associated with a network covering occipital regions (network 7: occipital pole, supracalcarine cortex, intracalcarine cortex and lingual gyrus); these occipital regions had shown individual vulnerability to birth length in a previous study with the present sample, reinforcing the idea of the long-term consequence of fetal environment on brain structure related to visual areas [87,88,103,188]. Importantly, as the birth weight and birth length are consequences of poor fetal nutrition and other stressor during pregnancy, mechanisms underlying the association of these indicators with structural gray matter network are diverse. Ischemia, inflammation, excitotoxicity due to poor nutrition (producing intrauterine growth restriction) or stressing during gestation may affect the neuron production, differentiation as well as consolidation of brain gray matter regions, and its consequences may reach even later ages [17,181].

To our knowledge, only one study used our similar approach of structural networks analysis related to early life factors, thought this analysis was focused only in gestational age and cortical thickness of adolescents [17]. Nassar et al. used the NNMF approach, the one that we applied in the present study, to define structural networks; they found that gestational age was related to 11 (of 26) structural networks in regions including orbitofrontal, parietal, temporal as well as hippocampus, amygdala and caudate, among

others [17]. However, they did not examine the potential behavioral implication of this association. In the present study, we examined the academic implications of the early life factors-structural networks associations, but this should be considered with caution. To note, most networks previously associated with early life factors were not related to academic achievement; specifically, only the network corresponding to posterior cingulate gyrus and anterior precuneus (network 12) was related to better reading, although this association did not pass multiple comparison. Regions covering this network have been previously shown to be implicated in reading skills in children and adults[189,190]. Future research is needed to address the academic and other behavioral implications of early life programing of structural networks during childhood.

The present study has both strengths and limitations. An intrinsic limitation is its cross-sectional design which precludes determination of causality Also, breastfeeding practices were retrospectively recorded, and the misunderstanding meaning of "exclusiveness" of breastfeeding, as well the lack of information about complementary feeding may explain, in part, the null association related to breastfeeding practices [100,103]. In addition, an important strength is the relative large sample with MRI data providing an important understanding of the relationship of early life factors and brain development in children with overweight or obesity. Lastly, we included important covariables as PHV, gestational age or CRF in our statistical models, although other potential variables of fetal environment such as nutrition indicators, maternal stress or smoke exposure which may impact brain outcomes were not available [191,192]

In summary, these results suggest that birth weight and birth length were associated with structural brain networks covering the cerebellum and cortical (i.e., posterior cingulate gyrus and anterior precuneus cortex) and subcortical (i.e., hippocampus and

parahippocampal gyrus; and caudate, putamen, pallidum, accumbens and amygdala) regions of the cerebrum. However, breastfeeding practices were nor related to brain structural networks. In addition, the structural networks do not have implication on academic performance in the present sample of children with overweight/obesity. Importantly, the current results suggest that early life factors are related with several structural brain networks, and it is necessary to pay attention in early life stage to normal brain development during childhood, though the behavioral consequences of these networks are need to further studies.

5.4. Study 4: Early life factors and hippocampal functional connectivity in children with overweight/obesity

Introduction

Early life environment is particularly important for brain development [193]. Early life factors such as anthropometric neonatal data (birth length, birth weight) and breastfeeding practices (exclusive and breastfeeding) might have long lasting consequences in the key cognitive-related brain regions. Specifically, the hippocampus undergoes protracted functional and structural development after birth, which may have consequences for memory and learning, and in turn academic performance during childhood [193-196].

There is extensive evidence on early life factors and morphologic changes of the hippocampus [194]. For example, low birth weight has been associated with hippocampal shape and volume in school-children [15,197]. In this line, Anne et al. found that children that born preterm and with very low birth weight had smaller hippocampal subfield volume than age-matched term-born controls at 9 years [198]. Similarly, neonatal stress was associated with smaller regional volumes in the limbic system including hippocampus in preterm children at 8 years [180]. Interestingly, breastfed children showed larger hippocampal volume at term equivalent age, but this association were not evident at 7 age [49]. To our knowledge, only a recent study was to investigate the association of birth weight and limbic areas (which include hippocampus), indicating that birth weight was related to more efficient limbic communication at rest in adolescents [199]. Unlike to structural evidence of hippocampus, previous studies did not examine the relationship of early life factors with the function of hippocampus at childhood.

The study of resting state functional connectivity (rsFC) is sensitive to early life environment, with alterations evident at term equivalent age in network related to motor, language, and executive functions[200]. Previous studies on early life factors-related rsFC

have focused on amygdala, considering its long-lasting consequences on affective and cognitive profile later in life [201,202]; however, its influence on rsFC of hippocampus is poorly understood [194]. The rsFC of hippocampus has been related to different health states at childhood and adulthood [203-206]. In addition, the rsFC of hippocampus may relate to several behavioral changes [207], which may have implication for academic performance during childhood [178,208]. Therefore, these findings highlight the importance of examining the early life programming of hippocampal rsFC and its academic implication in children, and particularly in children with overweight/obesity, who have shown altered between-network connectivity and worse executive function and academic skills.

Thus, we investigated the associations of early life factors such as anthropometric neonatal data (i.e., birth length and birth weight) and breastfeeding practices (i.e., exclusive and any breastfeeding) with hippocampal rsFC, and ii) we tested whether connectivity related to early life factors are associated with academic performance in children with overweight/obesity.

Methods

Participants

We included 96 overweight/obese children (categorized based on World Obesity Federation cut-off points)[65,66] aged 8-11 years from the ActiveBrains project[68] (www.profith.ugr.es/activebrains) with valid measures of early life factors, brain and academic performance variables. This study is a cross-sectional analysis of baseline data prior to randomization to an exercise intervention. Parents or legal guardians were informed of the purpose of the ActiveBrains study and its written informed consents were obtained.

The project was approved by the Human Research Ethics Committee of the University of Granada, and was registered in ClinicalTrials.gov (identifier: NCT02295072).

Early life factors

Weight (kg) and length (cm) at birth were collected from health records (i.e., physical medical record that parents have with the offspring's perinatal information). In addition, parents were asked the questions: *(i) for how long (months) did the child receive only breast milk (neither formula or other liquid or solid)?*, as an indicator of exclusive breastfeeding, and (ii) *for how long (months) did the child receive any breast milk (combined with other liquid, formula, or solid)?*, as an indicator of any breastfeeding [103].

Resting state magnetic resonance imaging (MRI)

MRI data acquisition information and processing and seed creation

Images were collected using a 3.0 Tesla Siemens Magnetom Tim Trio system (Siemens Medical Solutions, Erlangen, Germany) with a 32-channel head coil. The complete procedure was published in a previous work of the present sample [39]. High-resolution T1-weighted images were acquired using a 3D magnetization-prepared rapid gradient-echo (MPRAGE) protocol. The parameters were as follows: 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 40,216. The resting-state fMRI was composed of a series of 160 scans acquired using a Gradient Echo Pulse Sequence while participants rested with eyes closed. The parameters were as follows: TR = 1000 ms, TE =

25 ms, flip angle = 80º, FOV = 240 mm, acquisition matrix= 240 x 240, 35 slices, resolution = 3.5 x 3.5 x 3.5 mm, and scan duration of 5 min and 25 s.

Preprocessing steps were carried out in FMRIB's Software Library (FSL) version 5.0.7. The following steps were applied: (i) skull-stripping using brain extraction tool (BET), (ii) spatial normalization of structural image to Montreal Neurological Institute (MNI) space, (iii) alignment of all rsfMRI frames to correct for head motion during the scan, (iv) co-registration to each participant's structural image and spatial normalization to MNI space, (v) the rsfMRI time courses were then band-pass filtered (0.1–0.01 Hz) to attenuate respiration and other physiological noise and to focus on signal frequencies associated with intrinsic connectivity, (vi) six affine transformation parameters from the alignment process, as well as the mean time courses from the brain parenchyma including WM tissue and ventricles were included as covariates to further account for motion and physiological noise. We visually checked each individual image for acquisition artifacts, and one child was excluded due to visual image corruption. The residualized parameter estimate maps were converted to z scores (via Fishers r to z transformation) to achieve normality and were entered into higher level analyses.

Seed creation

FMRIB's Integrated Registration and Segmentation Tool (FIRST) in FSL 5.0.7 was used for seed creation. FIRST is a semi-automated model based subcortical segmentation tool that use 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 217. Briefly, two-stage affine registration are run to a standard MNI space using 12 degrees of freedom with 1 mm resolution and uses a

subcortical mask to exclude voxels which not corresponding (outside) to subcortical regions. Then, subcortical regions, including hippocampus, are separately segmented for each hemisphere. Manual volumetric region labels are parameterized as surface meshes and modeled as a point distribution model. The final segmentations of the hippocampus seeds were visually checked as quality control.

Academic performance

The Bateria III Woodcock-Muñoz Tests of Achievement was used as a measure academic performance (i.e., Spanish version of the Woodcock-Johnson III) [69]. A trained member of the research staff applied individually the test for each child. The full administration time was between 100 to 120 min. We included standard score indicators of reading, writing mathematics and total achievement in the present analysis [36].

Covariates

Gestational age (weeks) was collected from health records; peak height velocity (PHV) was used as indicator of pubertal maturity status and was obtained through the Moore et al. equation[70]; PHV offset was computed by the difference between PHV and chronological age. Parents reported their maximum completed level of education and answers were categorized as: none of the parents had university degree, one of the parents had a university degree or both parents had a university degree. CRF was estimated through the 20-meter shuttle-run test and maximal oxygen consumption (VO_2max, mL/kg/min) was calculated using the Lèger equation [72].

Statistical analysis

Descriptive data by sex are presented as mean and standard deviation (SD) for

continuous variables as well as n and percentage (%) for categorical variables. In a first-level (individual-level), voxel-wise functional connectivity network maps were created for left and right hippocampal seed, for each participant using the pre-processed rsfMRI data. Then, in a second-level, we conducted the group-level of analysis for resting-state functional connectivity (rsFC) to identify regions where connectivity with the seed is explained by each early life factor (birth weight, birth length, exclusive and any breastfeeding), including the first-level seed map into separate linear regression models. Sex, gestational age, PHV, parental educational level and CRF were included as covariates in all models of group-level analysis. All the variables were mean centered prior to include into group-level models. Multiple comparisons were perform using FSL's automatic FEAT cluster-based thresholding at $p<.05$, which is a method of Family-Wise Error correction based on Gaussian Random Field Theory. Sensitivity analyses were carried out excluding children born preterm (gestational age < 37 weeks, n=17). All clusters with most of the region out of the brain were excluded. In addition, we extracted the blood oxygen level dependent (BOLD) signals for each significant region to perform post-hoc analyses. Lastly, we examined the associations between BOLD-related to early life factors and academic performance; a false discovery rate was applied using the Benjamini and Hochberg method ($q<.05$). Descriptive and statistical analysis corresponding to extracted BOLD signal were perform in R (version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria), and statistical significance was set at $p < .05$)

Results

Table 1 shows the characteristics of study sample. Higher birth weight was associated with increased rsFC between left hippocampus and 2 clusters corresponding to left precentral and postcentral gyri **(Table 2, Figure 1).**

Table 1. Characteristics of study sample.

		All		*Boys*		*Girls*
N	n		n		n	
Physical characteristics	96		60		36	
Age (yr)		10.01 ± 1.14		10.16 ± 1.14		9.78 ± 1.13
Weight (kg)		55.65 ± 11.15		56.66 ± 10.69		53.97 ± 11.85
Height (cm)		143.81 ± 8.32		144.69 ± 7.37		142.34 ± 9.64
Peak height velocity offset (yr)		-2.33 ± 0.96		-2.65 ± 0.78		-1.80 ± 1.01
Cardiorespiratory fitness (mL/kg/min)*		40.86 ± 2.77		40.84 ± 2.77		40.90 ± 2.82
Body mass index (kg/m^2)		26.70 ± 3.69		26.90 ± 3.79		26.36 ± 3.53
Body mass index category (n,%)	96		60		36	
Overweight		25 (26.0)		16 (26.7)		9 (25.0)
Obesity type I		41 (42.7)		27 (45.0)		14 (38.9)
Obesity type II		30 (31.2)		17 (28.3)		13 (36.1)
Parental education university level (n,%)	96		60		36	
None of the parents		64 (66.7)		43 (71.7)		21 (58.3)
One of the two parents		17 (17.7)		10 (16.7)		7 (19.4)
Both parents		15 (15.6)		7 (11.7)		8 (22.2)
Neonatal characteristics						
Birth weight (g)	94	3343.72 ± 542.25	59	3358.98 ± 579.32	35	3318.00 ± 480.28
Birth length (cm)	85	50.69 ± 2.68	57	50.61 ± 3.02	28	50.86 ± 1.84
Gestational age (week)	96	38.62 ± 2.59	60	38.57 ± 2.59	36	38.70 ± 2.63
Breastfeeding practices (months)	92		59		33	
Exclusive breastfeeding†		3.19 ± 3.24		3.53 ± 3.53		2.58 ± 2.59
Any breastfeeding‡		7.06 ± 8.03		6.81 ± 7.18		7.50 ± 9.44
Academic performance (standard score)**	96		60		36	
Mathematics		102.02 ± 10.68		102.37 ± 11.23		101.44 ± 9.82
Reading		108.55 ± 12.92		108.33 ± 11.08		108.92 ± 15.67
Writing		113.99 ± 11.99		112.55 ± 11.91		116.39 ± 11.91
Total Achievement		109.49 ± 11.66		108.98 ± 10.67		110.33 ± 13.25

Values are mean ± SD or percentage. *Measured by the 20-m shuttle run test; †Months the child received only breast milk. †Months the child received breast milk combined with other liquid, or solid. **Measured by the Bateria III Woodcock-Muñoz Tests of Achievement.

Table 2. Significant clusters in the association of early life factors associated with hippocampal connectivity

Explicative variable	Seed	Nature of association	Cluster location	Cluste
Birth weight	Left hippocampus	Positive	L precentral gyrus	26
		Positive	L postcentral gyrus	38
	Right hippocampus	Positive	L postcentral gyrus	42
		Positive	R postcentral gyrus	40
		Positive	L cerebellum	25
Exclusive breastfeeding	Left hippocampus	Negative	L angular gyrus	36
	Right hippocampus	Negative	L primary motor cortex	25
Any breastfeeding	Right hippocampus	Positive	R middle temporal gyrus	32

R: Right; L: Left; MNI: Montreal neurologic institute. Analyses were adjusted for sex, peak height velo university level (neither/one/both), gestational age (weeks) and cardiorespiratory fitness (mL/kg/min). hippocampal resting state functional connectivity.

In addition, higher birth weight was associated with increased rsFC between right hippocampus and 3 clusters corresponding to left and right postcentral gyri and cerebellum **(Table 2, Figure 1).** Birth length was not associated with connectivity of the hippocampus.

Longer exclusive breastfeeding was associated with decreased rsFC of left hippocampus with left angular gyri **(Table 2, Figure 1.C)** and of right hippocampus with primary motor cortex **(Table 2, Figure 1.D)**. Any breastfeeding was not associated with rsFC of left hippocampus, while was positively associated with rsFC between right hippocampus and middle temporal gyrus **(Table 2, Figure 1.D)**.

Table 3 shows the association between BOLD signal of cluster previously associated between early life factors and hippocampal rsFC. There was a positive association between the connectivity of the right hippocampus with left primary motor cortex and mathematics (β= 0.225; p=0.019), although this association disappears after FDR correction.

Table 3. Associations of early-life factor-related hippocampal connectivity with academic performance*.

Early life factor	Seed	Cluster location	Mathematics		Reading		
			β	p-value	*β*	p-value	
Birth weight	L hippocampus	L precentral gyrus	-0.006	0.949	-0.05	0.614	-0
		L postcentral gyrus	0.185	*0.051*	0.117	0.228	-0
	R hippocampus	L postcentral gyrus	0.072	0.445	0.069	0.475	-0
		R postcentral gyrus	0.098	0.309	0.092	0.347	-0
		L cerebellum	0.079	0.412	-0.078	0.429	-0
Exclusive breastfeeding	L hippocampus	L angular gyrus	-0.050	0.608	-0.037	0.711	0.
	R hippocampus	L primary motor cortex	0.225	0.019[#]	0.176	0.075	0.
Any breastfeeding	R hippocampus	R middle temporal gyrus	0.084	0.383	0.095	0.338	0.

Values are standardized regression coefficients (β). Analyses were adjusted for sex, peak height velocity offse level (neither/one/ both) and cardiorespiratory fitness (mL/kg/min). L: Left, R: Right *Measured with the B achievement. [#] This association disappears after FDR correction.

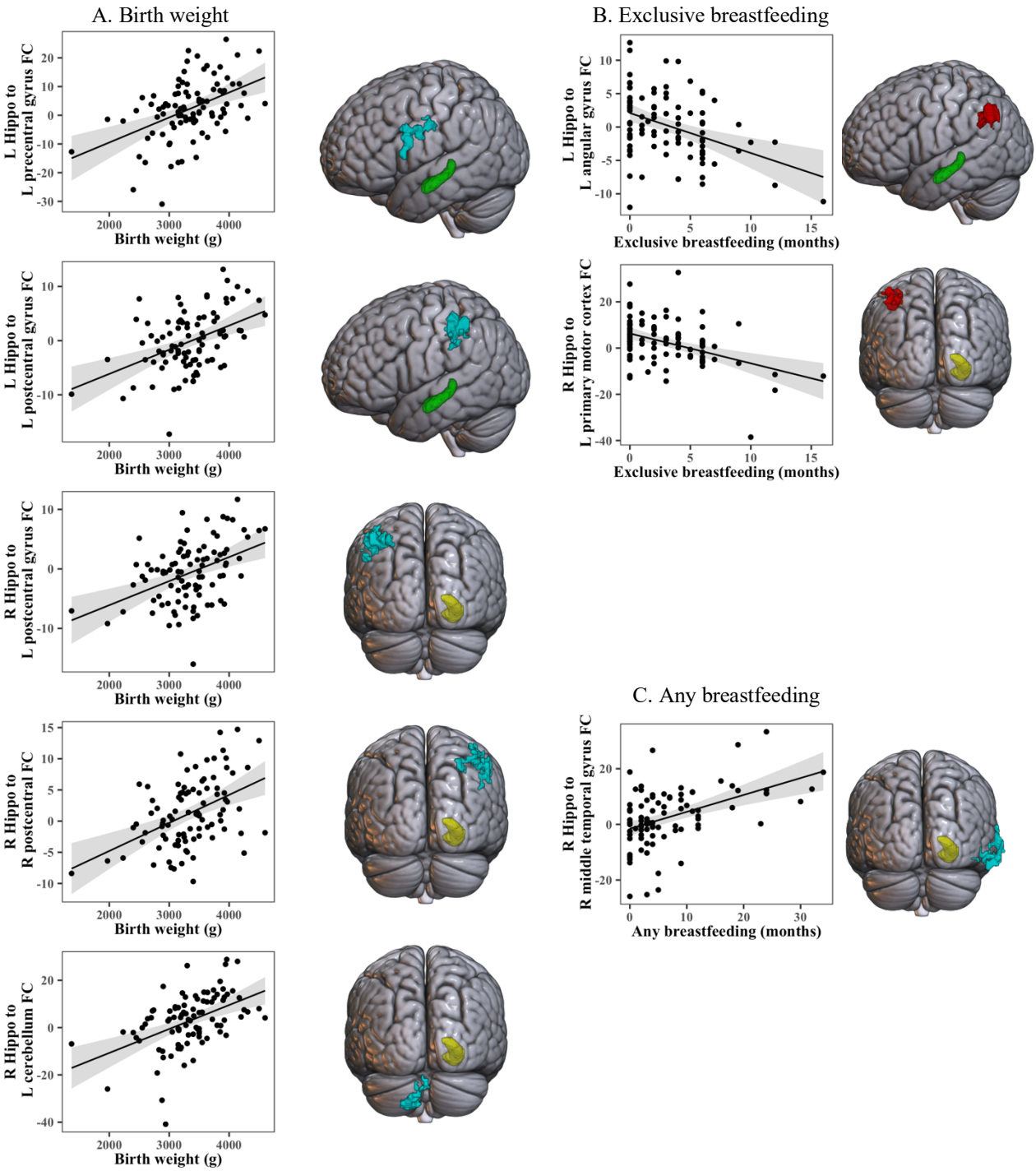

Figure. 1. Clusters showing association between early life factors and left and right hippocampal resting state functional connectivity. The color represent seed for left (green) and right hippocampi (yellow), as well as positive (light blue) and negative (red) association. Figures A shows birth weight cluster associated with left and right hippocampal seed. Figure B shows exclusive and any breastfeeding with left and right hippocampal seed. L: left, R: Right, FC: functional connectivity.

Discussion

Our main finding suggests that early life factors were related to hippocampal connectivity in children with overweight/obesity. Specifically, birth weight, but not birth length, was associated with greater hippocampal rsFC. In addition, longer exclusive breastfeeding was associated with diminished hippocampal rsFC, and any breastfeeding was related to greater hippocampal rsFC. However, hippocampal rsFC was not coupled with better academic abilities. The present study expands the knowledge about the influence of early life environment on hippocampal structure by showing that birth weight and breastfeeding may relate to hippocampal functional connectivity later in life, but its behavioral implications remain unknown.

Some potential mechanisms may be implicated in our results. Birth weight has been associated with hippocampal gene expression which may explain its influence on its connectivity [209]. In addition, as glucocorticoid receptors are located selectively in the hippocampus, exposure to glucocorticoid may predict hippocampal functional connectivity at later in life with consequences in memory processing [210-212]. Indeed, offspring hippocampus is more vulnerable than other brain areas by hypothalamic–pituitary–adrenal dysregulation and cortisol exposure due to maternal stress [195,213]. Also, exacerbated hippocampal neuronal growth in utero suggest the hippocampus as a typical region susceptible to fetal environment with functional consequences even when hippocampal growth peak, around 9-11 years [194,214-216].

The hippocampus is a key region involved in learning and memory as well as in higher order cognitive processes. Previous structural brain studies showed that compared with term-born controls, premature-born individual had reduced hippocampal volume

during childhood [15,197], and even during adulthood, coupled with cognitive consequences [217,218] . Functional studies showed that the activation of hippocampus at rest is disrupted in obesity showing worse decision making [219]. In addition, disrupted hippocampal connectivity during adolescence, mainly within the limbic network, was partially explained by birth weight [199]. In the present study, birth weight was mainly associated with greater functional connectivity between the hippocampus and frontal regions, namely, precentral and postcentral gyri. Both precentral and postcentral gyri are related to sensorimotor and somatosensory systems and have recognized as obesity-disrupted regions [220,221]. Specifically, while the precentral gyrus participated in planning and execution, the postcentral gyrus is a sensory region implicated in sense of touch. Decreased activation in precentral and postcentral have been associated with loneliness [220] and cue reactivity scores [222], both important suggested obesity-related behaviors in children [223,224]. In addition, we found birth weight was related to greater rsFC between the hippocampus and cerebellum, and we previously showed that both birth weight and birth length were also associated with cerebellar volume [103]. The hippocampal-cerebellar functional connectivity has a key role in spatial navigation in different neurological disorders, suggesting its clinical implication [225]. Collectively, we support the role of birth weight on functional connectivity between hippocampus and sensorimotor and motor regions in children with overweight/obesity. However, future larger studies should examine the influence of anthropometric neonatal indicators (i.e., birth weight/length) on functional connectivity using graph- based network analysis of rsfMRI to expand our understanding of early life programming of brain as a complex network.

Surprisingly, we found exclusive breastfeeding was associated with diminished connectivity of the hippocampus with the angular gyrus and the primary motor cortex.

Early life factors and hippocampal rsFC

Greater hippocampal-angular gyrus connectivity has been related to episodic memory, and reduced communication between these regions lead to reduced ability to think creatively and to imagine an episodic future event [226]. However, we did not find any negative academic implication in relation to the lower hippocampal-angular gyrus connectivity. Consequently, these unexpected results might explained by the possible functional reorganization in response to early life adversity [227,228]. In addition, any breastfeeding was associated with greater connectivity between hippocampus and middle temporal gyrus. A recently work show that connectivity between hippocampus and medial temporal gyrus is a critical neural basis for novelty and usefulness processing during concept construction, perceptual motor system (including precentral and postcentral gyrus) [229], and linguistic and nonlinguistic semantic-level processes or memory [82]. However, we neither found a behavioral coupled relationship in this sample of children with overweight/obesity, since hippocampal functional connectivity was not related to any indicators of academic achievement, suggesting that these networks could be implicated in other different processes.

Collectively, we did not find any academic implication for the association between early life factors and hippocampal connectivity. Similarly, a previous study with the present sample on early life factors and gray matter volume was not coupled with academic improvements. Thus, it is possible that in our specific sample of children with overweight or obesity, adiposity may mask the association of functional network with academic achievement. Further research examining the academic implications of the early life factors-hippocampal rsFC comparing normal-weight children and those with overweight/obesity is needed.

The present study expands the existing literature, which has mainly examined morphologic long-lasting changes of hippocampus explained by early life factors, by showing the early life programming of hippocampal connectivity in children with overweight/obesity. However, some limitations should be acknowledged. The retrospective cross-sectional design, precludes our ability to draw causal interpretations. Parents self-report of breastfeeding practices did not collect information about formula-fed, and even, potentially misunderstood the meaning of "exclusiveness" of breastfeeding [100], which may explain, in part, the unexpected association of breastfeeding practices. Lastly, we focused on a specific population (i.e, overweight/obesity) and bounded age range (i.e., children aged 8-11y), and the results could not be generalized to population of other age ranges or weight status. In addition, we do no collect information about feeding or hunger status during MRI session, and different pattern of feeding has been demonstrated altered acutely brain connectivity in obese population. The current findings provide new insights and prompt questions for future studies to further understand hippocampal functional connectivity in this population. The study has several strengths, including the relatively large sample with MRI, and the unique contribution to the literature on early life programming of hippocampal functional connectivity in children with overweight or obesity.

In conclusion, our finding suggest that early life factors are associated with long lasting effects on hippocampal functional connectivity in children with overweight or obesity. Future research on the association of these changes in hippocampal functional connectivity and its implication in other behavioral outcomes is needed.

6. General discussion

Integrated summary of the main findings of the Doctoral Thesis

The present doctoral thesis attempted to advance the current understanding of the role of early life factors on brain development in children with overweight/obesity, using different analytical brain approaches. First, we investigated the association of early life factors with gray matter brain volume using a voxel-based morphometric whole-brain approach. With this approach, we attempted to understand the influence of early life environment on regional gray matter volume **(study 1)**. Second, we investigated the association of early life factors with WM microstructure, using FA and MD values derived by tractography. With this approach, we attempted to study WM microstructure of 15 tracts, to understand the influence of fetal environment on water diffusivity molecules in WM **(study 2)**. Third, we used a NNMF analysis to reconstruct gray matter structural network. Thus, factor loadings of specific network were used to study the association of early life factors with structural gray matter network **(study 3)**. Fourth, we studied the association between early life factors and connectivity of the hippocampus with other brain regions using a hippocampal seed-based rsFC approach. This last approach provides an understanding on how early life factors may predict functional connectivity in a key memory-related region, the hippocampus **(study 4)**. Finally, in order to understand the behavioral implication of early life factors on different brain outcomes, we included in each chapter the potential coupled academic implication for each brain outcome **(studies 1-4),** yet other behavioral outcomes should be study in the future.

In general, the results of the present doctoral thesis showed that early life indicators were associated with selective brain outcomes **(Table 1)**. Gray matter volume **(study 1)**, structural brain networks **(study 3)** and rsFC of hippocampus **(study 4)** were explained by early life factors, however these factors did not predict WM microstructure later in life after FDR correction **(study 2)**. In addition, among the studied early life factors, anthropometric

neonatal data (i.e., birth weight and birth length), seem to have greater influence on brain health outcomes later in life than breastfeeding practices. In this context, birth weight the most studied early factor, as it is the result of intrauterine growth restriction with several consequences for the brain development [53,216], while birth length is far less studied, yet has also has been demonstrated to be associated with brain outcomes [230,231]. Intrauterine growth restriction is the major cause of perinatal morbility reaching approximately in the 5-10% of pregnancies [232]. The brain of the offspring is especially vulnerable to intrauterine growth restriction [233], increasing the risk of several neurological disorders or learning difficulties later in life [232]. Interestingly, one of the principal regions that seems to be affected according our results is the cerebellum. The cerebellum is the regions with most increased of volume in the first year of life, being a region susceptible to the consequences of poor fetal environment, and this can lead to motor and learning problem in the future [11]. In addition, our results also showed specific influence on precentral regions, hippocampus and other regions related to language and emotion regulation.

Table 1. Summary of main findings of the present Doctoral Thesis.

		Anthropometric neonatal data		Breastfeeding practices	
	Outcome	Birth weight	Birth length	Exclusive breastfeeding	Any breastfeeding
Study 1	Gray matter volumes	Positively associated	Positively associated	Not associated	Positively associated
Study 2	White matter microstructure	Not associated*	Not Associated*	Not associated*	Not associated
Study 3	Structural covariance networks	Positively associated	Positively associated	Not associated	Not associated
Study 4	resting state hippocampal functional connectivity	Positively associated	Not Associated	Negatively associated	Positively associated

* It did show some significant associations but did not survived the false discovery rate correction.

On the other hand, breastfeeding practices were inconsistently associated with brain outcomes in the present thesis. While only any breastfeeding was associated with greater gray matter volumes **(study 1)** and with greater rsFC between the hippocampus and middle temporal gyrus **(study 4)**, exclusive breastfeeding was associated with decreased rsFC of the hippocampus with angular gyrus and primary motor cortex **(study 4)**. Exclusive or any breastfeeding practices were not associated with WM microstructure (after FDR correction) **(study 2)** or structural brain networks **(study 3).** Thus, although it have been suggested that breast milk have key components to brain development [23], our results support its influence on specific gray matter regions and hippocampal connectivity, but not on WM microstructure **(study 2)** nor on structural brain networks **(study 3)** . These results show the complexity of the brain, and the numerous and key process can took place during prenatal stage affecting differentially to brain dimensions. For, example, the number of neurons growth a 10-fold during the third trimester of gestation, unlike to the stabilization in neurons numbers occurring during the first week of life [10-12]. Therefore, interventions aiming at improving optimal intrauterine growth and development may be of importance to achieve a healthy brain later in life.

Overall limitation and strengths of this thesis

The present doctoral thesis has limitations and strengths that should be recognized. The anthropometric neonatal data and breastfeeding practices were retrospectively collected. Parents self-report of breastfeeding practices did not collect information about complementary feeding or any other potential nutritional implication during early stage which may explain, in part, the inconsistent associations of these indicators with brain outcomes among studies.

General discussion

Strengths of the present doctoral thesis include its relatively large sample of children with overweight/obesity with MRI data. Besides, the broad approaches including different analyses of brain outcomes provide consistent evidence on long lasting influence of early life factors on brain health. Finally, the inclusion of important confounders, including CRF that has been also related to brain health, should be acknowledged.

7. Concluding remarks

Overall conclusion

The findings of the present doctoral thesis support the long-lasting associations of early life factors with brain development in children with overweight/obesity. Overall, early life factors (i.e., birth weight, birth length, exclusive and any breastfeeding) were related to most of the brain health outcomes studied (i.e., gray matter volumes, structural networks and rsFC of hippocampus), except for WM microstructure. Specifically, anthropometric neonatal data seems more consistently associated with brain health than breastfeeding practices, supporting importance of fetal environment on brain development at childhood. However, brain outcomes analyzed in the present doctoral thesis did not show any relationship with academic achievement, suggesting that behavioral consequences other than the one studied here merit further investigations.

Specific conclusions

Study 1

Birth weight and birth length were positively associated with the gray matter volume in 9 brain regions including the middle frontal gyrus, rectal gyrus, thalamus, putamen, middle temporal gyrus, lingual gyrus, middle occipital gyrus, calcarine cortex and cerebellum. Longer exclusive breastfeeding was associated with greater gray matter in inferior frontal gyrus and rolandic operculum, while any breastfeeding was not associated with gray matter volumes. None of the gray matter regions were associated with academic performance. Thus, birth weight, birth length, and breastfeeding are predictor of gray matter volume of several brain regions, while its academic implication remained unknown.

Study 2

Birth weight, birth length and exclusive breastfeeding, but not any breastfeeding, were associated with better WM microstructure, although did not remain significant after

FDR correction. In addition, the non-FDR corrected association between WM tracts previously related to early life factors were not associated with academic performance.

Study 3

Greater birth weight and birth length were associated to structural connectivity in networks covering cerebellum and cortical (i.e., posterior cingulate gyrus and anterior precuneus cortex) and subcortical (i.e., hippocampus and parahippocampal gyrus; and caudate, putamen, pallidum, accumbens and amygdala) regions of the cerebrum. Breastfeeding practices were not associated to structural brain networks. None network was related to academic performance. This result support long-lasting associations of the pregnant environment on structural brain connectivity at childhood, but its academic implications remained unknown.

Study 4

Birth weight was positively associated with rsFC of hippocampus with cerebellum and precentral and postcentral gyri, while birth length was not associated with hippocampus rsFC. Exclusive breastfeeding was negatively associated with rsFC of hippocampus with angular gyrus and primary motor cortex, while any breastfeeding was positively associated with rsFC of hippocampus with middle temporal gyrus. The connectivity of hippocampus with the regions were not associated with academic performance. Future research on the association of these differences in hippocampal functional connectivity and its implication in other behavioral outcomes is needed.

8. Anexes

Patricio Solis Urra – **Short Curriculum Vitae**

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2017-2020 PhD student in Biomedicine, University of Granada, Granada, Spain., España.

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Publications (sort by date):

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Congress communications as first author

Sep 7-10, 2019 Tract-specific white matter microstructure and its association with attention in children with overweight/obesity born preterm and term: the Activebrains project. **32nd ECNP Congress, Copenhagen.** Modality: Poster.

Feb 13-15, 2019 Factores perinatales e integridad de la sustancia blanca cerebral en niños con sobrepeso/obesidad: Proyecto Activebrains. **I Congreso national de investigadores del PTS.** Modality: Poster.

Oct 19-20, 2018 Asociación entre el peso al nacer y el volume de materia gris regional en niños con sobrepeso/obesidad: el rol de la capacidad cariorrespiratoria. **VI Simposio EXERNET. Investigación en Ejercicio, Salud y Bienestar. "Exercise is Medicine", Pamplona.** Modality: Poster.

March 8, 2018 Early life factors and brain structure in overweight/obese children. **The ActiveBrains-SmarterMove International Seminar: Exercise, Cognition and Brain in Childhood and Older Age, Granada.** Modality: Oral presentation.

Dec 13-14, 2017 Body size at birth, brain structure, executive function and Academic performance in overweight/obese children: The ActiveBrains project. **Jornadas Internacionales en investigación en actividad física y salud, Cuenca.** Modality: Oral presentation.

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“Mientras el cerebro sea un universo, el universo continuará siendo un misterio”

Santiago Ramón y Cajal