José Mora González

PHYSICAL FITNESS, EXERCISE AND BRAIN HEALTH IN CHILDREN WITH OVERWEIGHT/OBESITY: THE ACTIVEBRAINS RANDOMIZED CONTROLLED TRIAL

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PHYSICAL FITNESS, EXERCISE AND BRAIN HEALTH IN CHILDREN WITH OVERWEIGHT/OBESITY: THE ACTIVEBRAINS RANDOMIZED CONTROLLED TRIAL

Condición física, ejercicio y salud cerebral en niños con sobrepeso/obesidad: Ensayo aleatorizado controlado ActiveBrains

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José Mora González

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“Reza como si todo dependiera de Dios.
Trabaja como si todo dependiera de ti.

Ama y haz lo que quieras”

San Agustín

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ABSTRACT

Childhood obesity has been catalogued as one of the most serious public health problems. Among the most important consequences of childhood obesity we can find its negative influence on brain health. Childhood is a critical period for neurodevelopment, and the presence of obesity could negatively alter it. In this context, health-related factors such as physical fitness or physical activity may help to counteract this negative influence. Although there is emerging evidence on this respect, it is still not sufficient to draw firm conclusions and several limitations have been identified in this body of literature and must be addressed.

Therefore, the overall aim of the present International Doctoral Thesis is to study the role of physical fitness, physical activity, and sedentary time in brain health outcomes (**SECTION 1**), and to investigate the effect of a 4.5-month physical exercise program on brain health (**SECTION 2**) in children with overweight/obesity.

The results of the present Thesis show that cardiorespiratory fitness and speed-agility are both associated with cognitive flexibility (**Study 1**), working memory and the neuroelectric activity underlying it (**Study 2**), and inhibitory control and underlying neuroelectric activity (**Study 3**). Vigorous physical activity shows a consistent association with both working memory (**Study 2**) and inhibitory control (**Study 3**) and the neuroelectric activity. None associations are observed for sedentary time with any of the outcomes. From all the fitness components, cardiorespiratory fitness is the only one positively associated with the brain activity (i.e., current source density) of regions of the frontal, limbic and occipital lobes during the encoding and maintenance phases of working memory, and of regions of the temporal lobe during the retrieval phase of working memory (**Study 4**). The fitness-related association with brain activity of the posterior cingulate and parahippocampal gyri is further related to better working memory performance. Further, physical activity, mainly of moderate intensity, and step-related behaviors, but not sedentary time, are positively associated with the brain-derived neurotrophic factor (**Study 5**). Lastly, a 4.5-month exercise program induces alterations of the brain activity of temporal and frontal brain regions underlying the encoding phase of working memory and, to a lesser extent, the brain activity of frontal areas underlying information processing phase of inhibitory control (**Study 6**). This exercise program has also a beneficial effect on intelligence, cognitive flexibility, and academic performance in mathematics, and a slight effect (yet non-significant) on hippocampal brain volumes in children with overweight/obesity (**Study 7**).

Collectively, the results of the present International Doctoral Thesis enhance our understanding about the implications that health-related factors such as physical fitness, physical activity, sedentary time and exercise have for brain health in children with overweight/obesity.

RESUMEN

La obesidad infantil ha sido catalogada como uno de los problemas de salud pública más serios. Entre sus consecuencias más importantes podemos encontrar la influencia negativa que podría ejercer sobre la salud cerebral. La infancia es una etapa de la vida clave para el neurodesarrollo, y la presencia de la obesidad durante ella podría afectar negativamente a dicho neurodesarrollo. En este contexto, factores relacionados con la salud tales como la condición física

o la actividad física podrían ser de ayuda para contrarrestar la influencia negativa de la obesidad sobre el cerebro. A pesar de la creciente evidencia al respecto, no se puede considerar ésta como suficiente para sacar conclusiones firmes. Además, se han identificado numeras limitaciones en la literatura al respecto que deben ser abordadas.

Por consiguiente, el objetivo de la presente Tesis Internacional es estudiar el rol que juegan la condición física, actividad física y sedentarismo en diversas variables de salud cerebral (**SECCIÓN 1**), así como investigar el efecto de un programa de ejercicio físico de 4.5 meses de duración sobre la salud cerebral (**SECCIÓN 2**) en niños con sobrepeso/obesidad.

Los resultados de la presente Tesis muestran que la capacidad cardiorrespiratoria y la velocidad-agilidad se encuentran asociadas a la flexibilidad cognitiva (**Estudio 1**), a la memoria de trabajo y la actividad neuroeléctrica subyacente a ésta (**Estudio 2**), y al control inhibitorio y a la actividad neuroeléctrica subyacente a éste (**Estudio 3**). La actividad física vigorosa muestra una asociación consistente tanto con la memoria de trabajo (**Estudio 2**) como con el control inhibitorio (**Estudio 3**) y la actividad neuroeléctrica. No se observan asociaciones para el tiempo de sedentarismo con ninguna de las variables. De todos los componentes de la condición física, la capacidad cardiorrespiratoria es el único que se asocia positivamente con la actividad del cerebro (densidad de corriente eléctrica) de regiones de los lóbulos frontal, límbico y occipital durante las fases de almacenamiento y mantenimiento de la memoria de trabajo, y con regiones del lóbulo temporal durante la fase de recuperación de ésta (**Estudio 4**). La actividad cerebral del cíngulo posterior y el giro parahipocampal, previamente asociadas a la capacidad cardiorrespiratoria, se asocian a su vez con una mejor memoria de trabajo. Además, la actividad física, principalmente de moderada intensidad, y los pasos, pero no el tiempo de sedentarismo, se encuentran positivamente asociados al factor neurotrófico derivado del cerebro (**Estudio 5**). Por último, un programa de ejercicio físico de 4.5 meses de duración induce alteraciones en la actividad cerebral de regiones cerebrales frontales y temporales que subyacen a la fase de almacenamiento de la memoria de trabajo y, en menor medida, de regiones frontales que subyacen a la fase de procesamiento de la información del control inhibitorio (**Estudio 6**). Este programa de ejercicio tiene un efecto beneficioso también sobre la inteligencia, la flexibilidad cognitiva y el rendimiento académico en matemáticas, y un ligero efecto (aunque no significativo) sobre volúmenes cerebrales del hipocampo en niños con sobrepeso/obesidad (**Estudio 7**).

De manera colectiva, los resultados de la presente Tesis Internacional mejoran nuestro entendimiento sobre las implicaciones que ejercen factores relacionados con la salud, tales como la condición física, la actividad física, el tiempo de sedentarismo y el ejercicio físico sobre la salud cerebral en niños con sobrepeso/obesidad.

GENERAL INTRODUCTION

CHILDHOOD OBESITY: A PANDEMIC AFFECTING BRAIN HEALTH

The prevalence of overweight and obesity is rising problematically in developed and developing countries worldwide.[1] In fact, childhood obesity has been catalogued as one of the most serious public health problems due to its epidemic proportions.[2,3] The pandemic of pediatric obesity has risen dramatically from just 4% in 1975 to over 18% in 2016, with over 340 million children and adolescents identified with overweight or obesity in 2016.[3] Among the most important consequences of childhood obesity, the following have been highlighted: (i) gaining excess weight in childhood is likely to lead to overweight/obesity later in life;[4] (ii) pediatric obesity is associated with future morbidity and mortality, as well as with greater risk and earlier onset of chronic diseases such as cardiovascular diseases, type 2 diabetes, and cancer;[5–7] (iii) obesity at early stages has been considered as a risk factor for lifelong psychiatric disorders;[8,9] (iv) obesity is associated to higher risk of chronic diseases leading to disability pension in the future;[10] and (v) obesity may have an important influence on the health of children's brains.[11–13]

The term "Brain health" has been defined by the 2018 Physical Activity Guidelines Advisory Committee Scientific Report as the optimal or maximal functioning of behavioral and biological measures of the brain and the subjective experiences arising from brain function (e.g., mood).[14] This includes measurements of biological markers of the brain (e.g., structural brain morphology) or the subjective manifestations of brain function, including mood and anxiety, perceptions of quality of life, cognitive function (e.g., attention and memory), and sleep.[14] For the present International Doctoral Thesis, the term "brain health" will refer to measurements of intelligence, cognitive performance, academic performance, brain structure, and brain activity underlying tasks of executive function.[14] Particularly, childhood obesity has been associated with detectable structural brain abnormalities, specifically with smaller gray matter volume in brain regions that underlie aspects of executive functioning, such as the prefrontal cortex, the hippocampus or the thalamus.[11,12,15] Therefore, these structural changes in the brain as consequence of an obesity condition, may also tend to produce detrimental changes in the executive function.[16] Executive functions are defined as the higher cognitive processes that enable forethought and goal-directed action.[17] The three main aspects of executive function are: (1) cognitive flexibility, which refers to the ability to shift attention as well as mental sets or rules when situationally appropriate, (2) working memory, which refers to the ability to temporary storage information and manipulate and update information in memory as required, and (3) inhibitory control, which refers to the ability to suppress irrelevant information and impulsive or automatic responses in order to give a correct response.[17] A recent meta-analysis supports the existence of broad executive function deficits in individuals with overweight/obesity.[13] Indeed, obesity is also clearly associated with a decreased ability to modulate the executive function networks, as reflected by cognitive task performance and neuroelectric activity.[18]

Collectively, at the same time that the prevalence of obesity increases during childhood, this period is considered a critical one for neurodevelopment, in which the brain matures, develops, learns, and forms connections.[19,20] Indeed, children are sensitive to experience changes in their brain's structure and function, as well as in the executive function.[19–21] In the present International Doctoral Thesis, children with overweight/obesity are presented as the target population on which the study of the role of several health-related factors in brain health is crucial.

PHYSICAL FITNESS AND PHYSICAL ACTIVITY: HEALTH-RELATED FACTORS TO COUNTERACT THE NEGATIVE INFLUENCE OF OBESITY ON BRAIN HEALTH

Being childhood obesity a pandemic that affects brain health, the investigation of factors that may help to counteract this negative influence becomes of relevance. In this context, there exists a fundamental neurobiological principle that states that cellular and molecular events in the brain are amenable to modification by environmental enrichment.[22] Here is where factors such as physical fitness and physical activity become important as they may favorably influence brain health.[23,24]

Physical fitness and physical activity are both markers of children's physical health.[25,26] Physical fitness is defined as the capacity to perform physical activities and is composed of a set of physical components such as cardiorespiratory fitness (i.e. the capacity of the cardiovascular and respiratory systems to carry out prolonged strenuous exercise), speed-agility (i.e. the ability to move the body as fast as possible), and muscular strength (i.e. the capacity to exert work against a resistance).[27] Specifically, these three main physical fitness components have shown differential influence on several cardiovascular and metabolic health outcomes,[26,28,29] and on few brain outcomes.[30,31] However, there is still scarce evidence on how the physical fitness components differ in their relationship with brain health.

Physical activity is defined as any body movement produced by skeletal muscles that result in an energy expenditure above the basal levels (i.e., 1.5 metabolic equivalents).[27] On the contrary, physical inactivity is defined as the level of activity in which the International Physical Activity Guidelines are not met, and sedentary behavior is considered any waking behavior with an energy expenditure ≤1.5 metabolic equivalents (METs, ml/kg/min) while in a sitting or reclining position.[32] Another related term is exercise, defined as the subset of physical activities that are planned, structured and systematic. The benefits of physical activity on cardiovascular health has been widely investigated,[33] however evidence on its relationship with cognition is still emerging in youth.[34] In this context, apart from being a sensitive period for cognitive and brain stimulation, childhood is also considered a crucial period for the establishment of healthy habits, such as a physically active lifestyle. Furthermore, since childhood overweight/obesity is a marker of chronic inactivity and sedentarism,[35] children with overweight/obesity as well as sedentary children may be more likely to benefit from physical activity than lean children. Collectively, stimulating brain health of those who need it most (i.e., children with overweight/obesity) through health-related factors such as physical fitness and physical activity could have an impact in future societies, making them healthier and, as a consequence, smarter and more successful in several scenarios of life. Therefore, in the present Doctoral Thesis we examine the influence of all health-related factors (i.e., physical fitness, physical activity, and sedentary time) on brain health in children with overweight/obesity.

ROLE OF PHYSICAL FITNESS, PHYSICAL ACTIVITY AND SEDENTARY TIME IN THE EXECUTIVE FUNCTION AND THE UNDERLYING NEUROELECTRIC ACTIVITY

In the last decade, physical fitness and physical activity have emerged as promising factors related to executive function in children.[23,34] As mentioned previously, executive function comprises processes such as cognitive flexibility, working memory, and inhibitory control, which are vital to success in school, vocation, and life.[17] With the aim of ensuring a proper cognitive development during childhood, many schools' administrations have decided to maximize the time spent on instrumental school subjects while, consequently, time devoted to physical activity in the school curriculum tends to decline.[36] Whereas this happens, physical inactivity and sedentarism have been associated to deficits in children's cognitive development.[37] Furthermore, children with normal-weight showing higher levels of physical fitness and physical activity have shown better performance in several executive functions.[34,38] Given the high prevalence of overweight and obesity during childhood and its detrimental effects on executive function,[15,18] it is of high relevance to test the role of physical fitness, physical activity, and sedentary time in the executive function of children with overweight/obesity.

Indeed, consistent with such an assertion, the existing literature in this area has generally observed a positive association of both physical fitness and physical activity with the executive function in children with overweight/obesity.[39–41] For example, cardiorespiratory fitness has been associated with better planning and attention,[39] and with higher response accuracy in tasks measuring cognitive flexibility and inhibitory control.[41] In regards with physical activity, children with normal-weight either active or inactive have shown higher scores of planning and attention than their inactive peers with overweight.[40] Beyond the assessment of specific executive function outcomes, prior studies have also included a neuroelectric activity measurement (i.e., electroencephalography, EEG). Neuroelectric measurements and more specifically the event-related brain potentials (ERPs), have been especially useful for achieving a better understanding of the neural and executive function correlates of physical fitness, physical activity, and sedentary time.[42] Particularly, the P3 is a positive-going ERP component observed in the stimulus-locked ERP waveform and is believed to represent the updating of memory once sensory information has been analyzed.[43,44] Importantly, its amplitude is thought to be proportional to the amount of attentional resources allocated during stimulus engagement, and its latency is believed to be proportional to the information processing speed.[44] The amplitude and latency of P3 component have been the neuroelectric activity parameters most widely used in relation to physical fitness and physical activity.[34] Overall prior observational investigations have shown that children with higher cardiorespiratory fitness levels exhibited larger P3 amplitude and shorter P3 latency compared to their lower-fit peers during tasks specifically measuring different aspects of inhibitory control.[45–47]

To date, the literature on the relationship of physical fitness, physical activity, and sedentary time with executive function and also with neuroelectric activity underlying it have mainly focused on the role of a singular physical fitness component (i.e. cardiorespiratory fitness) or on self-reported physical activity.[34] In this context, accumulating evidence has suggested that other physical fitness components, such as speed-agility or muscular strength may be also beneficial for cognition.[30,31,34] However, no previous studies have examined these other fitness components with the executive function and neuroelectric activity in children with overweight/obesity. Furthermore, self-reported methods to assess physical activity and sedentary time (e.g., questionnaires) have been shown poorly reliable and valid, especially in younger populations, and therefore may produce erroneous values.[48,49] In contrast to this, the use of accelerometers to assess physical activity and sedentary time[50] has been shown as an objective and feasible alternative[48] and the recent improvements in these methods must be taken into account when studying the relationship of physical activity and sedentary time with neuroelectric indices of executive function. Finally, the body of research including neuroelectric measurements has not considered the weight class of the children, solely focusing on children with normal-weight.[34] On the basis of the above, in the present Thesis we address the role of physical fitness, physical activity, and sedentary time in neuroelectric indices of executive function domains (i.e., working memory and inhibitory control), taking into account the main health-related physical fitness components, using a valid, objective and feasible measurement of physical activity and sedentary time (i.e., accelerometers), and including a sample of children with overweight/obesity.

BRAIN SOURCE ANALYSIS: AN ALTERNATIVE IN THE STUDY OF NEUROELECTRIC ACTIVITY IN RELATION TO PHYSICAL FITNESS AND EXERCISE

The rapid growth of neuroelectric and neuroimaging techniques in the 21st century has favored the advance of research to the understanding of the neural foundations of physical activity-induced benefits to brain health.[51] As we specified in the previous section, the ERPs have been the most prominent approach derive from the neuroelectric system (i.e., EEG) to address the relationship between physical fitness and neuroelectric activity as well as the exercise-induced changes in brain activity.[42] However, an important aspect to take into consideration is to assign the relation of each fitness component or the effects of exercise to specific brain regions. In this sense, despite the excellent temporal resolution of the ERP (i.e., in the range of milliseconds), a fundamental limitation of this approach is the inverse solution, that is, the challenge in determining activated brain regions by scalp-recorded EEG activity.[52] An alternative may be the use of functional magnetic resonance imaging (fMRI). fMRI yet has a better spatial resolution than EEG, is inherently slow (i.e., on the scale of seconds). It has been suggested that the low temporal resolution of this technique may mask temporally separated activations into a single, more spread activity.[53] Hence, the spatial extend of the recovered activation may be much larger than the real anatomical activation, degrading the actual spatial resolution of fMRI.[53] In sum, the impossibility to separate the different activations on the scale of millisecond degrades the spatial resolution of this technique. For those investigations in which an EEG measurement is carried out, the brain source localization algorithms have emerged as an alternative approach that inherit the high temporal resolution of EEG and generate a more direct presentation in the brain activity space.[54] Specifically, standardized low-resolution brain electromagnetic tomography (sLORETA) has become an accepted EEG-based tool that offers a reliable spatial and temporal detection of brain cortical activity together with a simple, an economical, and a noninvasive use.[55] Further, this software provide a measurement of current source density which has been shown to provide a rich and accurate view of the spatial and temporal dynamics of brain activity.[53,56] Current source density estimation allows to reach a spatial resolution of 2–3 cm, which comes close to the size of brain areas.

The present Doctoral Thesis provides, for the first time, an observational perspective of the relationship between the health-related physical fitness components (i.e., cardiorespiratory fitness, speed-agility and muscular strength) and the brain activity (i.e., current source density) underling a working memory task. Furthermore, and also for the first time, this Thesis will shed light on the effects of a long-term exercise program on the brain current source density underlying working memory and inhibitory control operations based on a brain source analysis that takes advantage of the high temporal resolution of the EEG at the same time that provides a spatial representation of the brain activity.

NEUROTROPHIC FACTORS AS NEUROBIOLOGICAL MECHANISMS RESPONSIBLE FOR BRAIN HEALTH: ROLE OF PHYSICAL ACTIVITY AND SEDENTARY TIME

Neurotrophic factors are a class of growth factor consisting of proteins that bring about the survival, development, and function of neurons, and are through to provide neuroprotective benefits.[57] Among all the neurotrophic factors, the brain-derived neurotrophic factor (BDNF) has received the most of attention since it has been suggested as a potential mechanism underlying the relationship between physical activity and brain health.[58,59] Animal models have supported that BDNF promotes brain development and cognitive benefits in several ways, including neural development and cell survival,[60] enhanced synaptic plasticity,[61] neurogenesis stimulation (i.e., the formation of new neurons), and changes in brain structure.[62] Furthermore, many studies in humans have demonstrated the effect of higher BDNF levels on numerous cognitive processes, such as better spatial[63] and verbal memory,[64] as well as better hippocampal functioning.[65] Apart from this, BDNF has been linked to metabolic disorders, including obesity, based on the fact that BDNF missense mutations in its receptor, TrkB, have been associated with weight gain in humans.[66] Therefore, it is of important to focus on individuals with overweight/obesity when studying the role of physical activity in BDNF.

The BDNF is produced in the brain and in selected peripheral tissues such as platelets.[67] Additionally, BDNF may be released from the brain to the periphery during the practice of physical activity.[68]

Apart from BDNF, other neurotrophic factors such as vascular endothelial growth factor (VEGF) or insulin growth factor-1 (IGF-1) are important for neural growth and neuron survival.[59] However, in humans, there is inconsistent evidence on the role of physical activity in neurotrophic factors.[69–72] On the basis of previous literature supporting that a physically active lifestyle together with decreased sedentary time are protective factors against weight gains over childhood,[35] and taking into account that BDNF plays a key role in the regulation of obesity (i.e., energy homeostasis and appetite regulation),[73] examining the potential role of physical activity and sedentary time in neurotrophic factors in the context of obesity during childhood become of even more relevance.

EFFECTS OF EXERCISE PROGRAMS ON BRAIN HEALTH

So far, the present introduction has been mainly focused on describing the role of physical fitness, physical activity, and sedentary time in children's brain health more from an observational perspective, setting causal evidence based on the effects of exercise apart. It is well established the potential beneficial effects of exercise, as a programmed and structured physical activity practiced with a certain regularity, on physical health, but there is still a need for shedding light into the effects on brain health.[23] In this context, investigations demonstrating the positive effects of exercise on the brain in rodents has guided questions about its potential for positive effects in humans.[23] However, a recent review of this literature concluded that there is overall moderate evidence that exercise positively influences brain in humans.[23] In accordance with this review, the "moderate" grade of the evidence indicates that the effect of exercise on brain health remains still unclear due to methodological heterogeneity between studies. This heterogeneity refers to: (i) inconsistencies in the parameters used to design the exercise program across studies; (ii) the differences between tests used to assess executive function domains; (iii) a poor description of whether the interventions were successful at maintaining moderate-intensity physical activity through the course of the program; and (iv) the quality of the study designs.[23] As a result, the present Thesis tries to clarify some of these gaps using a well-designed randomized controlled trial (RCT) (i.e., the highest quality design) under which a 4.5-month exercise program including 1 hour of aerobic training plus 30 min of resistance training was built to study its effects on several aspects of brain health (i.e., brain activity underlying executive function operations, intelligence, cognitive and academic performance, and brain structure).

Most of recent systematic reviews and meta-analyses have supported a beneficial effect of exercise interventions on executive functions and academic performance, with the strongest evidence supporting a positive impact on inhibitory control and mathematics.[74–78] While there is large body of literature focused on executive function domains (i.e., cognitive flexibility, working memory and inhibitory control) and academic achievement, only 3 of them did on intelligence.[79–81] To the best of our knowledge, none of them included a sample of children with overweight/obesity nor included an indicator of crystalized and fluid intelligence. Further, only 3 RCTs in children have analyzed the effects of exercise on brain structure and function measured by magnetic resonance imaging (MRI and fMRI).[82–88] However, no previous exercise RCT examined the effects on gray matter volume. More concretely, it is surprising that, whereas previous studies in animals and older adults have shown the hippocampus as the most affected region from exercise,[89] no previous study carried out in children have addressed the effects of exercise on this specific brain region, so far.

One of the limitations of this literature that have been previously commented and that will be covered in the present dissertation is the poor or null description made in the studies on whether the interventions were successful at maintaining moderate-intensity physical activity.[23] Together with this, there is a need of ensuring whether the intervention program have a compensatory effect in the exercise group, reflected as a no real increase of their weekly physical activity levels, or in the control group, reflected as an increase of their activity levels as a consequence of enrolling in the trial.[90,91] For this purpose, we objectively monitored physical activity using accelerometer before and during the intervention program in both exercise and control groups reporting therefore this compensatory effect in the present dissertation. Apart from monitoring the physical activity levels before and during the intervention, the well documented evidence on the association between cardiorespiratory fitness and brain health in children[30,31,34] rises the importance of testing whether exercise-induced cardiorespiratory fitness improvements lead to improvements in brain health.

SUMMARY OF EXISTING EVIDENCE, GAPS IN THE KNOWLEDGE AND HOW THIS THESIS ADDRESS THEM

Summing up, in the past decade numerous studies have emerged affirming that physical fitness, physical activity, sedentary time, and exercise might have an important implication for the health of children's brains.[23,34] As commented in previous sections, preceding studies have shown an overall positive association of physical fitness and physical activity with key aspects of executive function such as cognitive flexibility,[41] working memory[92] and inhibitory control,[38,93] as well as with the neuroelectric activity underlying, preferably, inhibitory control.[45–47] Furthermore, in an attempt to explain these relationships, several investigations have focused on studying the role of physical activity in the underlying mechanisms that may explain these positive findings. For instance, neurotrophic factors that promotes brain development and cognitive benefits[60,61] such as the BDNF, have been identified as potentially related to physical activity.[59,94] Beyond observational literature, there is also growing evidence showing that long-term exercise programs induce positive changes in neuroelectric indices of executive function,[88,95] as well as in academic performance[96] and brain structure (i.e., white matter integrity)[82–84] in children.

Despite the positive trend followed by the findings of previous literature, recent systematic reviews and meta-analyses have detected many limitations and, therefore, have called for further studies addressing them.[23,34] First, whereas physical fitness is composed by several components (i.e., cardiorespiratory fitness, speed-agility and muscular strength) that have shown important implications for physical and brain health,[26,29,30] vast majority of previous studies have focused their attention on cardiorespiratory fitness, forgetting about the other two components. In line with this, a recent Position Stand[34] highlighted the fact that most of them, mainly including neuroelectric and executive function measurements, split the sample into two extreme fitness groups (i.e., high- and low-fit children) excluding those with middle level and therefore no covering the whole cardiorespiratory fitness spectrum of the targeted population. Second, studies including physical activity and sedentary time measurements in relation to brain health have mainly used self-reported methods such as questionnaires, although they have been shown poorly reliable and valid in youth.[48,49] Third, research using neuroelectric measurements such as EEG have not taken into account the inverse problem of this measurement which is the challenge in determining activated brain regions by scalp-recorded EEG activity.[52] Therefore, to date, no previous studies have raised an alternative that allows to measure brain activity in both temporal and spatial terms. Fourth, with respect to intervention studies addressing the effects of long-term exercise programs on brain health, a recent meta-analysis has identified many limitations such as the low quality of study designs or the methodological heterogeneity between studies.[23]

To shed light on these research limitations, the present International Doctoral Thesis seeks to answer to all of the gaps in the literature presented above on physical fitness, physical activity, sedentary time, exercise and brain health in children with overweight/obesity as a risk group for impaired brain health.[12,13] For this purpose, this Thesis is structured in two different sections preceded by an overview of all the methodologies followed in the studies included. **SECTION 1** includes the cross-sectional studies of the role of main health-related physical fitness components, and objectively-measured physical activity and sedentary time in executive function (**Study 1**), and the neuroelectric activity underlying working memory and inhibitory control operations (**Study 2, 3**), including, for the first time in this field, a brain source analysis (**Study 4**). Furthermore, this section includes also the study of the relationship of physical activity and sedentary time with neurotrophic factors (**Study 5**). **SECTION 2** includes intervention studies that address the study of the effects of a randomized controlled trial-based exercise intervention on brain source analysis-based brain activity during tasks (**Study 6**), and on intelligence, cognitive performance, academic performance and brain structure (**Study 7**) in children with overweight/obesity.

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AIMS

AIMS

Overall aim

The overall aim of the present International Doctoral Thesis is to study the role of physical fitness, physical activity, and sedentary time in brain health outcomes, and to study the effects of a 4.5-month exercise program on brain health in children with overweight/obesity. This overall aim is addressed in seven specific aims (i.e., seven studies):

Specific aims

SECTION 1. Cross-sectional studies: Role of physical fitness, physical activity and sedentary time in executive function, brain activity during task performance and neurotrophic factors

- Specific aim 1: To examine the association of each physical fitness component (i.e., muscular strength, speed-agility, and cardiorespiratory fitness), an overall fitness score, physical activity and sedentary time with indicators of the executive function (i.e., cognitive flexibility, inhibition, and planning ability) in children with overweight/obesity (**Study 1**).
- Specific aim 2: To investigate the association of different physical fitness components (i.e., muscular strength, speed-agility, and cardiorespiratory fitness), sedentary time, and physical activity with working memory and neuroelectric activity in a sample of children with overweight/obesity (**Study 2**).
- Specific aim 3: To analyze the association of different physical fitness components (i.e., muscular strength, speed-agility, and cardiorespiratory fitness), various physical activity intensities, and sedentary time with behavioral and neuroelectric (i.e., P3 amplitude and latency) concomitants of inhibitory control in children with overweight/obesity (**Study 3**).
- Specific aim 4: (i) To investigate the association of the various physical fitness components (i.e., muscular strength, speed-agility, and cardiorespiratory fitness) with brain current source density during a working memory task; and (ii) to examine whether fitness-related associations in current source density are related to working memory performance and academic performance in a sample of children with overweight/obesity (**Study 4**).
- Specific aim 5: To analyze the association of sedentary time, physical activity and step-related behaviors with BDNF and other neurotrophic factors (i.e., VEGF and IGF-1) in children with overweight/obesity (**Study 5**).

SECTION 2. Intervention studies: Effects of exercise on brain activity during task performance, cognitive and academic performance, and brain structure

- Specific aim 6: To investigate whether a 4.5-month exercise program induces significant changes from pre- to post-intervention in spatial-temporal brain current source density while performing tasks of working memory and inhibitory control in children with overweight/obesity (**Study 6**).
- Specific aim 7: (i) To investigate the effects of a 4.5-month exercise program on brain health, and specifically on intelligence, executive function (i.e., cognitive flexibility, inhibition and working

memory), academic performance and gray matter volumes in the whole brain, with hippocampus as main candidate region, in children with overweight/obesity; and (ii) to analyze the effects of the exercise program on cardiorespiratory fitness and whether changes in cardiorespiratory fitness mediate the main effects observed in this intervention study (**Study 7**).

METHODS, RESULTS AND DISCUSSION

Studies' methodological overview

STUDIES'S METHODOLOGICAL OVERVIEW

The present International Doctoral Thesis contains a total of 7 studies. They are classified in two different sections depending on the study design (i.e., cross-sectional or intervention). All studies contain data from children with overweight/obesity enrolled in the ActiveBrains project. **Table 1** shows an overview of the design, participants and variables included in every study contained in this Thesis.

Table 1. Studies' methodology overview.

Studies	General aim	Design	Participants	Variables studied
Study 1	To study the association of physical fitness, PA and sedentary time with executive function	Cross-sectional	100 children (10.1 ± 1.1 years; 58% boys)	*Predictors*: CRF (20mSRT), muscular strength (handgrip and SLJ) and speed-agility (4x10m), PA and sedentary time (ACC) *Dependents*: Cognitive flexibility (DFT and TMT), inhibition (Stroop), planning (Zoo test)
Study 2	To study the association of physical fitness, PA and sedentary time with working memory and neuroelectric activity	Cross-sectional	79 children (10.2 ± 1.1 years; 65% boys)	*Predictors*: CRF (20mSRT), muscular strength (handgrip and SLJ) and speed-agility (4x10m), PA and sedentary time (ACC) *Dependents*: RT and response accuracy (DNMS working memory task), P3 amplitude and latency (EEG)
Study 3	To study the association of physical fitness, PA and sedentary time with inhibitory control and neuroelectric activity	Cross-sectional	84 children (10.1 ± 1.1 years old; 56% boys)	*Predictors:* CRF (20mSRT), muscular strength (handgrip and SLJ) and speed-agility (4x10m), PA and sedentary time (ACC) Dependents: RT and response accuracy (MFT inhibitory control task), P3 amplitude and latency (EEG)
Study 4	To study the association of physical fitness with brain current source density during working memory and the association of fitness-related current density with working memory and academic achievement	Cross-sectional	85 children (10.1 ± 1.1 years; 62% boys)	*Predictors:* CRF (20mSRT), muscular strength (handgrip and SLJ) and speed-agility (4x10m) *Dependents*: Brain current source density (sLORETA), response accuracy (DNMS working memory task), academic performance (Woodcock-Johnson III battery)
Study 5	To study the association of PA, sedentary time and steps with neurotrophic factors	Cross-sectional	97 children (10.0 ± 1.2 years; 58% boys)	*Predictors*: PA, sedentary time and steps (ACC) *Dependents*: BDNF, VEGF, IGF-1
Study 6	To study the effect of the exercise program on brain current source density during working memory and inhibitory control tasks	RCT	67 children (10.00 ± 1.10; 69% boys). Exercise group, N = 35; Control group, N = 32)	*Intervention program (Predictor):* 4.5-month exercise program. 5 sessions/week, 90 min/session. Based on high intensity active games. *Dependents:* Brain current source density (sLORETA), RT and response accuracy (DNMS working memory task and MFT inhibitory control task)
Study 7	To study the effect of the exercise program on brain health and cardiorespiratory fitness and its mediation role on the main effects	RCT	89 children (10.02 ± 1.10; 66% boys). Exercise group, N = 47; Control group, N = 42	*Intervention program (Predictor):* 4.5-month r exercise program. 5 sessions/week, 90 min/session. Based on high intensity active games. *Dependents:* intelligence (K-BIT), cognitive flexibility (DFT and TMT), inhibition (Stroop), working memory (response accuracy from DNMS), academic performance (Woodcock-Johnson III battery), brain structure (MRI, whole brain and hippocampal volume), CRF (20mSRT)

ACC = Accelerometry; BDNF = Brain-derived neurotrophic factor; CRF = Cardiorespiratory fitness; DFT = Design Fluency Test; DNMS = Delayed non-matched-to-sample task; EEG = Electroencephalography; IGF-1 = Insulin growth factor-1; K-BIT = Kaufman Brief Intelligence Test; MFT = Modified flanker task; MRI = Magnetic resonance imagining; PA = Physical activity; RCT = Randomized controlled trial; RT = Reaction time; SLJ = Standing long jump test; sLORETA = Standardized low-resolution brain electromagnetic tomography; SRT = Shuttle run test; TMT = Trail Making Test; VEGF = Vascular endothelial growth factor. Following confounders were included in cross-sectional analyses: Sex (Studies 1–5), age (2, 3, 5), peak height velocity (1, 3–5), puberty stage (2), body mass index (3, 4), fat mass index (1, 5), wave of participation (1–5), parental educational level (1–5), intelligence (2–5).

SECTION 1

Cross-sectional studies: Role of physical fitness, physical activity and sedentary time in executive function, brain activity during task performance, and neurotrophic factors

Study 1

Physical fitness, physical activity, and the executive function in children with overweight/obesity

BACKGROUND

Physical fitness and physical activity (PA) are markers of children's physical health.[1,2] However, evidence on the relationship with cognition is still emerging.[3] Among all aspects of cognition, executive function seems to be the most closely linked function to physical fitness and PA.[3] Executive function is composed of cognitive flexibility, inhibitory control, planning, working memory, and decision making, which are particularly important for the performance of daily activities, motor development, and social relationships.[4] A recent systematic review supported a beneficial relationship between physical fitness or PA and the executive function in children.[3] In general, children with normal-weight with higher levels of any fitness component have shown better performance in several executive functions, such as working memory, inhibition, and cognitive flexibility.[3,5] Because the vast majority of the present literature has focused on children with normal weight, the potential associations between physical fitness, PA, and executive function are less known in children with overweight and obesity.

Obesity has been associated with detectable structural brain abnormalities during childhood, specifically with decreases in brain regions that underlie as- pects of executive functioning.[6] PA-based programs may improve executive function in the obese young population.[7] High levels of physical fitness and PA may serve to counteract the negative influence of overweight and obesity on brain and cognition.[8]

The aims of the present study were to examine the association of each physical fitness component (i.e., muscular strength, speed-agility, and cardiorespiratory fitness) and an overall fitness score with indicators of the executive function (i.e., cognitive flexibility, inhibition, and planning ability), and to examine the association of objectively measured PA and sedentary time with indicators of executive function in children with overweight/obesity. Given previous research, we hypothesize that physical fitness components, an overall fitness score, PA, and sedentary time would relate to indicators of executive function.

METHODS

Participants

The present cross-sectional study was conducted under the framework of the ActiveBrains project (http://profith.ugr.es/activebrains).[9] An initial sample of 110 Spanish children with overweight and obesity aged 8-11 years old were recruited from Granada, Spain, after meeting the defined inclusion criteria: (1) overweight or obese based on World Obesity Federation cut-off points, (2) to be 8-11 years old, (3) no physical disabilities or neurologic disorder, (4) for girls, not to have started the menstruation at the moment of the assessments, and (5) to be right handed.[9] Recruitment was done at the Unit of Pediatrics of the University Hospitals San Cecilio and Virgen de las Nieves of Granada (Spain). Additionally, the head teacher of both public and private schools of Granada were contacted and advertisements in the local media were published. Any child meeting the inclusion criteria was invited to participate. The study was conducted in 3 waves of participation. For the present study, a sample of 100 children with overweight and obesity (10.1 ± 1.1 years old; 58.0% boys; 91% participation rate) was included with complete baseline data on physical fitness and executive function variables. For PA and sedentary time variables, we additionally excluded 4 participants because they did not have accelerometer data (n =

96). The baseline data collection took part from November 2014 to February 2016. The ActiveBrains project was approved by the Human Research Ethics Committee of the University of Granada, and it was registered in ClinicalTrials.gov (identifier: NCT02295072).

Descriptive measurements

Body weight (kilograms) was measured with an electronic scale (SECA 861, Hamburg, Germany), and height (centimeters) was measured using a precision stadiometer (SECA 225, Hamburg, Germany), both were measured twice and the average score was computed. We calculated the body mass index (BMI; kg/m2), and we defined BMI categories (i.e., overweight, obesity grade I, II, III) according to Cole and Lobstein.[10]

Physical fitness components

The ALPHA health-related physical fitness test battery for children and adolescents was used to assess physical fitness. A detailed description of the validity and reliability of the ALPHA battery has been provided elsewhere.[11] Briefly, muscular strength was assessed using the maximum handgrip strength test and the standing long jump test. In the handgrip test, each child performed the test twice with each hand, and the maximum value of each hand was taken and averaged. In the standing long jump test, the longest attempt from 3 was recorded (centimeters) and multiplied by the body weight to obtain an absolute measurement as in previous research in children with obesity.[12] The speed-agility was assessed twice using the 4×10-meter shuttle-run test and the fastest completion time (seconds) was recorded and inverted by multiplying by –1. Cardiorespiratory fitness was assessed through the 20-meter shuttle-run test and the total number of completed laps was registered.

The Z-score of muscular strength was calculated as the mean of the 2 standardized by sex scores (Z-standardized value = [absolute value – the sample mean] / SD) of the absolute handgrip strength and standing long jump tests. An overall physical fitness Z-score was then calculated as the mean of the standardized scores of each physical fitness component.

Physical activity and sedentary time

PA and sedentary time were assessed by accelerometer (GT3X+, ActiGraph, Pensacola, FL, USA). Participants were instructed to remove the accelerometers only for water activities (i.e., bathing or swimming). The raw accelerations collected at a sampling frequency of 100 Hz were processed in R (v. 3.1.2, https:// www.cran.r-project.org/) using the GGIR package (v. 1.5-12, https://cran.r-project.org/web/packages/GGIR/)[13] to calculate the Euclidean Norm Minus One (ENMO) metric after auto-calibration of the acceleration signal.[13,14] The mean of ENMO with negative values rounded to zero was calculated over 5s epochs. Additionally, the participants recorded information on wake up and sleep times and any removal times on a diary during the 7 days. Processing of the ENMO in GGIR included (1) non-wear time detection by the approach proposed by Van Hees et al.[15] (2) Detection of abnormally high acceleration values (i.e., clipped time). (3) Replacement of the non-wear and clipped time for the mean of the activity performed within the same time frame on the rest of the days.[15] If no data were collected for a specific time frame for the rest of the days, it was replaced by 0 for all metrics. (4) Identification of waking and sleeping hours based on an automatized algorithm guided by the diaries completed by the participants.[16] We considered a valid day when the wearing time was ≥600 min/day of waking hours and ≥240 min/day of sleeping hours. A minimum of four valid days (three weekdays and one weekend day) per week was required to be included in the analyses. The compliance wearing the accelerometer was high, with 98% of sample wearing the accelerometers for ≥ 6 days.

Children wore two accelerometers located on the non-dominant wrist and right hip simultaneously for 7 consecutive days (24h). For the present study, the data from the non-dominant wrist were used in order to be consistent with some major projects, such as the NHANES (https://www.cdc.gov/nchs/nhanes/index.htm), but analyses were replicated using the hip data (data not shown). Therefore, PA was classified into moderate and vigorous intensities using the wrist-based cut-off points defined by Hildebrand et al. for the ENMO metric.[17,18] The variables included in this study were total minutes per day at moderate-to-vigorous physical activity (MVPA), sedentary time, and minutes accumulated in sustained bouts of 1, 5, and 10 minutes of MVPA with a drop tolerance of the 20% of the time.

Executive function

Executive function was assessed for the domains of cognitive flexibility, inhibition, and planning ability. Cognitive flexibility and inhibition were assessed through different tests from the 9 subscales of the Delis–Kaplan Executive Function System.[19] All tests were given to the participants by 3 different examiners and always in the same order. All the examiners were trained before the beginning of the study to perform the test in a coordinated matter and to be able to provide the same standardize instructions. This battery has a test–retest reliability ranging from 0.62 to 0.80.[20] Planning ability was assessed using the Zoo Map Test from the Behavioral Assessment of Dysexecutive Syndrome.[21]

The Design Fluency Test and the Trail Making Test were used as indicators of cognitive flexibility. The Design Fluency Test assesses several executive functions such as problem-solving behaviors, fluency in generating visual patterns, creativity, and inhibitory control.[22] This test comprises three conditions: filled dots, empty dots, and switching.[19] In each condition, the participants were instructed to connect the dots using only four straight lines to design as many novel shapes as possible. For each of the three conditions, the child had 60 seconds to draw as many different designs as possible. The total number of correct drawn designs from all 3 conditions was registered.

The Trail Making Test comprises five different conditions. The first three conditions and the fifth one assess visual-perceptual abilities, whereas condition 4 is an indicator of cognitive flexibility.[22] We used condition 2 and condition 4, known as Part A and Part B of the Trail Making Test.[23] Condition 2 (i.e. Number Sequencing) assesses visual-perceptual abilities, and the participants have to draw lines to connect numbers 1–25 in ascending order as fast as possible. Condition 4 (i.e. Number-Letter Switching) assesses cognitive flexibility and consists in drawing a line to connect the numbers numerically and the letters alphabetically as fast as possible, switching each time from a number to a letter (e.g., 1–A–2–B). In order to remove the motor component from the score of the Trail Making Test-B and to obtain a more objective and valid measure of cognitive flexibility, we subtracted the total completion time of Trail Making Test-A from the total completion time of Part B.[23] For both conditions a maximum limit completion time was determined a priori (i.e., 2.30 min for Part A, and 4 min for Part B). Furthermore, the examiner pointed out errors as they occurred so that the participants could always complete the test. A smaller B – A difference indicates better cognitive flexibility, and since we had calculated an inversed B – A difference variable by multiplying this score by –1 for analyses purposes, higher scores indicated better cognitive flexibility.

To assess inhibition, we used a modified version of the Stroop test with a more demanding modified interference subtask that has been previously reported.[24] This version called the Stroop Color Word Test included four conditions and provided a measurement of inhibition. We recorded the completion time in all conditions. For the present study, we only used condition 1 and condition 3. Condition 1 is the baseline condition that measures fundamental linguistic skills (i.e., namely speed of naming), and consists in naming color of filled rectangles. Condition 3 is an indicator of inhibition where color-words are printed in a color that differs from their meaning (e.g. the word "red" printed in green) and the task consists in naming the color of the word (i.e., green in the example) and avoid reading the word. An interference score was obtained by subtracting condition 3 completion time – condition 1 completion time as previously reported.[25] A smaller Stroop interference score indicates better inhibition, and since we had calculated an inversed Stroop interference score variable by multiplying this score by –1 for analytic purposes, higher scores indicated better inhibition.

The Zoo Map Test particularly assesses planning abilities and behavioral regulation.[21] In this test, the participants completed two different conditions. In both conditions, they had to plan a route through a map while complying with a set of rules. Condition 1 was more difficult with no instructions about which path should be followed, whereas condition 2 included instructions that allowed the participants to know which places they should visit and in which order. A sequence score of each condition (i.e. 0-8 points) was based on the sum of the locations visited in the correct order and points were deducted if an error was made. An error was made when: A path was used more than once; there was a deviation from the path; there was a failure to draw a continuous line, such as jumps; an inappropriate location was visited. The total sequence score was used as the sum of the sequence scores of conditions 1 and 2 of Zoo Map Test (i.e. up to 16 points).

Potential confounders

Sex, age, puberty stage, wave of participation, parental educational level, and the intelligence quotient (IQ) were used as potential confounders in the analyses. Puberty stage was assessed by a physical examination carried out by a medical doctor and based on sexual maturation status (i.e., Tanner stages I-III).[26] The wave of participation was a categorical variable describing which wave of the study (1, 2, or 3) the child participated in. The parental educational level was assessed by a self-report questionnaire completed by the mother and father, and we combined the responses and classified them as none of them had a university degree (coded as 1), one of them had a university degree (coded as 2), or both of them had a university degree (coded as 3).[27] The IQ of the participants was measured by the Spanish version of the Kaufman Brief Intelligence Test.[28]

Statistical analysis

The characteristics of the study sample are presented as means and standard deviations (SD) or percentages. Before all analyses, all outcomes were checked for normal distribution. Interaction analyses were performed between sex and BMI, physical fitness components, PA, and sedentary time on the executive function indicators. No significant interactions with sex or BMI were observed (all $P \geq 0.10$); therefore, the analyses were performed for all participants together. We performed linear regression analyses to examine the association of each physical fitness component and an overall physical fitness score with executive function indicators adjusting by sex, age, puberty stage, wave of participation (entered as dummy variables), parental educational level, and IQ. We also performed linear regression analyses to examine the association of PA (i.e., MVPA, and bouts of MVPA) and sedentary time with the executive function indicators adjusting for the previous confounders. Each predictor was analyzed in a separate regression model for each dependent variable adjusting by all confounders.

A significance level of $P < 0.05$ was set. Additionally, all analyses were corrected for multiple comparisons using the Benjamini-Hochberg method.[29] All the statistical procedures were performed using the SPSS software for Windows (version 22.0, IBM Corporation).

RESULTS

Table 1 presents the descriptive characteristics of the study sample and by sex as means and standard deviations, unless otherwise indicated. **Table 2** shows the associations of each physical fitness component with indicators of executive function, adjusted for potential confounders. Handgrip strength showed a significant association with planning ability ($P = 0.025$). Speed-agility was positively associated with cognitive flexibility assessed by the Design Fluency Test as well as with inhibition ($P \leq 0.021$). Finally, cardiorespiratory fitness was significantly related to cognitive flexibility, specifically with the Design Fluency Test and the Trail Making Test ($P \leq 0.033$). No associations were found for standing long jump or the muscular strength score with any of the executive function indicators ($P > 0.05$).

Figure 1 presents the association between overall physical fitness and indicators of executive function, adjusting for potential confounders. Overall physical fitness showed a significant association with both measures of cognitive flexibility, namely the Design Fluency Test and the Trail Making Test ($P \leq 0.029$). No significant associations were found for inhibition and planning ability ($P > 0.05$). After correcting for multiple comparisons, speed-agility, cardiorespiratory fitness, and the overall physical fitness score remained significantly related to cognitive flexibility (i.e., Design Fluency Test), and speed-agility also to inhibition (all $P < 0.05$).

No significant associations were found between PA, sedentary time and any of the executive function indicators ($P > 0.05$; **Table 3**). Consistent results were obtained when performing the same analyses with hip placement PA and sedentary time (data not shown).

DISCUSSION

The main findings of the present study indicate that speed-agility, cardiorespiratory fitness, and an overall physical fitness score were positively related to cognitive flexibility. Particularly, cardiorespiratory fitness and the overall fitness score were related to both indicators of cognitive flexibility (ie, Design Fluency Test and Trail Making Test). In addition, speed-agility was the only fitness component positively associated with inhibition, and muscular strength was the only component related to planning ability.

Table 1. Descriptive characteristics of the study sample (n=100)

	All (n=100)	Boys (n=58)	Girls (n=42)
Age (years)	10.1 ± 1.1	10.2 ± 1.1	9.9 ± 1.1
Anthropometric characteristics			
Weight (kg)	56.1 ± 11.0	57.0 ± 10.5	54.9 ± 11.6
Height (cm)	144.1 ± 8.2	144.7 ± 7.3	143.3 ± 9.3
Puberty stage (%)			
Tanner I	38	37.9	38.1
Tanner II	44	50.0	35.7
Tanner III	18	12.1	26.2
BMI (%)			
Overweight	25	24.1	26.2
Obesity grade I	44	46.6	40.5
Obesity grade II	20	17.2	23.8
Obesity grade III	11	12.1	9.5
Wave of participation, (%)			
First	17	10.3	26.2
Second	42	48.3	33.3
Third	41	41.4	40.5
Parental university level, (%)			
None of them	68	75.9	57.1
One of them	18	15.5	21.4
Both of them	14	8.6	21.4
IQ			
K-BIT (Typical punctuation)	99.8 ± 18.6	97.1 ± 18.9	102.5 ± 18.1
Physical fitness			
Handgrip strength (kg)	16.6 ± 3.7	17.2 ± 3.7	15.9 ± 3.5
Standing longitudinal jump × Weight (cm × kg)	5872.7 ± 1415.2	6075.9 ± 1415.9	5592.1 ± 1381.9
Muscular strength (Z-score)*	0.0 ± 0.9	0.0 ± 0.9	0.0 ± 1.0
4 × 10-m shuttle-run test (sec)†	15.1 ± 1.6	14.9 ± 1.6	15.5 ± 1.4
20-m shuttle-run test (laps)	16.0 ± 7.9	17.3 ± 8.3	14.3 ± 7.0
Physical activity and sedentary time (min/day)‡			
MVPA	50.9 ± 19.8	58.7 ± 20.7	40.0 ± 11.9
Sedentary time	520.6 ± 55.0	523.0 ± 52.3	517.2 ± 59.0
1-min bouts of MVPA	12.3 ± 6.2	15.0 ± 6.0	8.4 ± 4.1
5-min bouts of MVPA	3.0 ± 3.2	3.9 ± 3.5	1.6 ± 1.9
10-min bouts of MVPA	3.2 ± 5.3	4.6 ± 6.2	1.3 ± 2.6
Cognitive flexibility			
Design Fluency Test (Total correct designs)	20.3 ± 6.6	20.9 ± 7.0	19.5 ± 6.0
Trail Making Test (B-A conditions time difference)†	91.9 ± 42.6	89.1 ± 43.5	95.8 ± 41.5
Inhibition			
Stroop Test (3-1 conditions time interference score)†	41.1 ± 17.3	39.4 ± 15.0	43.5 ± 20.0
Planning ability			
Zoo Map Test (Total sequence score)	11.6 ± 3.8	11.7 ± 3.8	11.4 ± 3.7

BMI = Body Mass Index; K-BIT = Kaufman Brief Intelligence Test; MVPA = Moderate-to-Vigorous Physical Activity; ENMO = Euclidian norm minus one.

Values are expressed as means ± standard deviations, unless otherwise indicated. *The muscular strength Z-score was computed based on the Z-scores from absolute measurements of the Handgrip strength and Standing long jump tests (i.e., Standing long jump × Weight). †Lower values indicate better results. ‡The sample for physical activity variables was n=96 (n=56 boys; n=40 girls). Speed-agility is indicated by 4 × 10-m shuttle-run test and cardiorespiratory fitness by 20-m shuttle-run test.

Table 2. Associations of physical fitness components with the executive function (n=100)

	Cognitive flexibility				Inhibition		Planning ability	
	Design Fluency Test (Total correct designs)		Trail Making Test (B-A conditions difference)*		Stroop Test (3-1 conditions interference score)*		Zoo Map Test (Total sequence score)	
	β	*P*	β	*P*	β	*P*	β	*P*
Muscular strength								
Handgrip strength (kg)	0.197	0.059	0.132	0.263	−0.053	0.628	0.263	**0.025**
Standing long jump × Weight (cm × kg)	0.190	0.096	0.131	0.303	−0.018	0.881	0.163	0.205
Muscular strength (Z-score)†	0.215	0.051	0.148	0.233	−0.040	0.733	0.241	0.053
Speed-agility								
4 × 10-m shuttle-run test (sec^{-1})*	0.303	**0.002**‡	0.179	0.101	0.233	**0.021**‡	−0.084	0.449
Cardiorespiratory fitness								
20-m shuttle-run test (laps)	0.250	**0.009**‡	0.228	**0.033**	0.140	0.164	−0.044	0.684

β values are standardized. These analyses were adjusted for the following covariates: sex, age, puberty stage, wave of participation, parental educational level, and intelligence quotient. The bold font is used to highlight significance level at *P* < 0.05. *The values were inverted so that higher values indicate better results. †The muscular strength Z-score was computed based on the Z-scores from absolute measurements of the Handgrip strength and Standing long jump tests (i.e., Standing long jump × Weight). ‡These association remained significant after adjustment for multiple comparisons using the Benjamini-Hochberg method.

Previous studies have mainly focused on children with normal weight or overweight, and either on only 1 physical fitness component (i.e., cardiorespiratory fitness)[30,31] or on self-reported PA,[32] and the results of the present study are, generally, in line with the findings of these investigations. For cognitive flexibility, one of the studies showed that higher levels of cardiorespiratory fitness were related

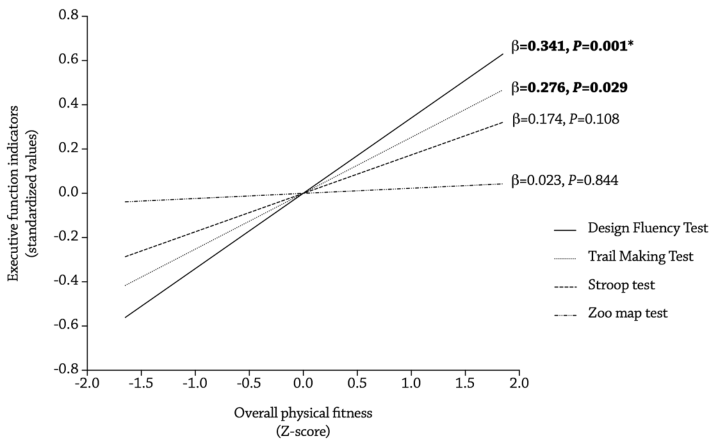

Figure 1. Associations between an overall physical fitness Z-score with executive function indicators (n = 100). β values are standardized. Regression analyses were adjusted for the following covariates: sex, age, puberty stage, wave of participation, parental educational level, and IQ. The bold font is used to highlight significance level at *P* < 0.05 and an asterisk (*) is used to highlight statistically significant values after an adjustment for multiple comparisons using the Benjamini-Hochberg method. The overall physical fitness Z-score was calculated as the mean of the Z-scores of each physical fitness component. The values were inverted for Trail Making Test and Stroop Test so that higher values indicate better results.

Table 3. Associations of physical activity, measured by ENMO metric in non-dominant wrist, with the executive function (n=96)

	Cognitive flexibility				Inhibition		Planning ability	
	Design Fluency Test (Total correct designs)		Trail Making Test (B-A conditions difference)*		Stroop Test (3-1 conditions interference score)*		Zoo Map Test (Total sequence score)	
	β	P	β	P	β	P	β	P
Physical activity and sedentary time (min/day)								
MVPA	0.100	0.351	−0.007	0.954	−0.032	0.767	−0.127	0.289
Sedentary time	−0.033	0.735	−0.025	0.818	0.026	0.786	0.109	0.310
Bouts of MVPA (min/day)								
1-min bouts	0.111	0.312	−0.055	0.653	−0.075	0.503	−0.142	0.248
5-min bouts	0.101	0.319	0.089	0.431	−0.046	0.654	−0.069	0.542
10-min bouts	0.066	0.502	0.067	0.540	0.062	0.528	−0.112	0.306

MVPA=Moderate-to-vigorous physical activity. β values are standardized. These analyses were adjusted for the following covariates: sex, age, puberty stage, wave of participation, parental educational level, and intelligence quotient. *The values were inverted so that higher values indicate better results.

to higher cognitive flexibility toward the correct target during a color-shape switch task in children with over- weight.[31] Another study, also in children with overweight, found that measures of peak VO2 and treadmill time were positively associated to planning and attention scores.[30] In children and adolescents with normal weight, there are many studies supporting our associations between cardiorespiratory fitness and cognitive flexibility. For example, those with higher fitness levels (ie, cardiorespiratory fitness) had a superior capability to flexibly allocate cognitive control processes.[33–36] Our findings can be also reconciled by previous randomized controlled trials in children with overweight and obesity that found dose–response benefits of an exercise program for physical fitness, executive function, and the areas of the brain that manage executive function.[30,37,38] We also found a positive relationship between speed-agility and cognitive flexibility, which concurs with a study showing a statistically significant association be- tween different motor skills and cognitive flexibility already in children with normal weight.[39] Taking into account that cognitive flexibility is regulated by the prefrontal cortex, the anterior cingulate cortex, and the basal ganglia, and the consistent positive findings between physical fitness and cognitive flexibility may be explained by the fact that increased levels of fitness are beneficial for these neural regions.[40]

With regard to inhibition, we found a positive association between speed-agility and the interference score on the Stroop test. A recent systematic review showed that complex motor skills are strongly related to higher-order cognitive skills such as inhibition.[41] Motor skills, such as the ones required for a proper performance in the speed-agility test used in the present study, involve precise execution movements based on an elaborated coordination of processes of motor movement. Thus, our findings may be explained by the fact that motor skills may activate a neuronal network that connects brain regions associated with both motor and cognitive functions.[42]

Regarding planning ability, we also found a positive association between handgrip strength and planning ability. However, this finding should be interpreted with caution because, after correcting for multiple comparisons, the significant association disappeared. To the best of our knowledge, there is no evidence concerning muscular strength and planning ability, which hampers comparisons with other studies.

This investigation also used an overall approach to quantify physical fitness (i.e., an integration of every fitnes component in a composite score) when analyzing its relationship with the executive function. Consistent with this approach, a recent study reported a significant association between an overall score of physical fitness and the executive function measured in its 3 dimensions.[43] This is in agreement with our findings in the present study, and highlights the importance of being physically fit in all fitness components, because it is related to better cognitive flexibility.

Although research on the association between physical fitness and executive function in children has consistently delivered overall positive results,[3] findings regarding PA, sedentary time, and executive function are more inconsistent. Contrary to the existing literature,[32] we did not find any significant association between PA and the executive function. However, our nonsignificant associations are in line with previous studies showing that PA is not necessarily associated with all domains of executive function in children with normal weight.[44] The inconsistency between our nonassociations of PA and sedentary time with executive function and the beneficial results of existing trials on cognition can be explained by previous literature declaring that cross-sectional associations do not necessarily indicate that the improvement of an outcome, such as MVPA, will result in improvements in outcomes previously associated with it (i.e., executive function).[30,45]

The main limitation of the present study was its cross-sectional design, which prevents us from drawing causal associations. Another limitation may be the lack of some unmeasured social factors such as the family income that may influence both fitness and executive function.

CONCLUSION

The results of the present study suggest that not only cardiorespiratory fitness, but also muscular strength and speed agility are positively associated with executive function in childrenwith overweight and obesity. Furthermore, cognitive flexibility seems to be the executive function indicator with a higher association with physical fitness, whereas planning ability and inhibition might be fitness component specific. Public health policies should promote not only physical health, but cognitive health as well. However, exercise-based randomized controlled trials are needed to extend our results.

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Study 2

Fitness, physical activity, working memory and neuroelectric activity in children with overweight/obesity

BACKGROUND

Apart from the well-known benefits of physical fitness and physical activity (PA) on youth's physical health,[1,2] low levels of both fitness and PA might be further related to poorer executive function and brain health in children.[3] These detrimental associations have been also found in individuals with obesity.[4–6] In fact, childhood obesity has been negatively associated with the structure and function of several brain regions that underlie executive function,[6,7] as well as with impairments in executive function processes per se, particularly working memory.[8] These associations, together with the fact that fitness and PA may be protective factors against the development of obesity, suggest that optimal levels of physical fitness and PA might attenuate the adverse influence of obesity on executive function.

In particular, within the various executive function domains, working memory is of high importance for learning and academic performance in children.[9] There are only three studies examining the cross-sectional association between physical fitness components and working memory in children with normal weight.[10–12] While two of them focused on cardiorespiratory fitness showing that higher levels of this component were associated with better performance during a working memory task; only one examined both cardiorespiratory fitness and muscular strength and showed that only muscular strength was related to better working memory.[12] In terms of sedentary time and PA, higher amounts of self-reported sedentary time were cross-sectionally associated with lower performance during a working memory task in children,[13] whereas total daily PA and moderate-to-vigorous PA (MVPA) were not associated with working memory.[14] However, no studies included speed-agility, a key component for executive function,[4,15] in relation to working memory, neither different PA intensities (i.e., light, moderate, or vigorous) or PA estimations from different accelerometer locations (e.g., hip or wrist) and cut-points which might influence its association with the outcome.[16,17]

Event-related brain potentials (ERPs) (e.g., P3 component) during a cognitive task may afford us to a better understanding of the neural and executive function correlates of physical fitness and PA.[18] Specifically, previous studies have shown that higher-fit children, in term of cardiorespiratory fitness, have larger amplitude (i.e., increased attentional resource allocation during stimulus engagement) and shorter latency (i.e., faster processing speed) of the P3 component than their lower-fit peers while performing an attentional inhibition task.[19,20] However, no previous studies examined other physical fitness components (i.e., muscular strength or speed-agility) or sedentary time and PA in relation to the neuroelectric activity underlying working memory in children.

Importantly, all the aforementioned studies have focused on healthy children with normal weight. Based on previous research declaring the negative influence of childhood obesity on executive function,[7,8] the aim of the present study was to investigate the association of different physical fitness components (i.e., muscular strength, speed-agility, and cardiorespiratory fitness), sedentary time and PA with working memory and neuroelectric activity in a sample of children with overweight/obesity. Given previous research on the association between physical fitness, physical activity, and executive functions,[3] we hypothesized that physical fitness components and PA, but not sedentary time, would positively relate to working memory and neuroelectric activity.

METHODS

Participants

Participants in this study were recruited from the ActiveBrains project (http://profith.ugr.es/activebrains). The complete methodology, procedures and inclusion/exclusion criteria for the project has been described elsewhere.[21] Briefly, the study was conducted in three waves of participation and, initially, a total of 110 children with overweight/obesity (i.e., defined as such according to sex-and-age specific international World Obesity Federation cut-off points) aged 8–11 years were recruited from Granada (Spain). The present study focuses only upon the baseline assessment data prior to randomization. A final sample of 79 children with overweight/obesity (10.2 ± 1.1 years old; 64.6% boys) with complete baseline data for physical fitness, sedentary time, PA, working memory (i.e., > 15 trials completed per task condition), and ERPs (i.e., neuroelectric activity non-artifacted) were included in this study.

Description and characteristics of the study were given to parents or legal guardian and a written informed consent was provided by them. The ActiveBrains project was approved by the Ethics Committee on Human Research of the University of Granada, and was registered in ClinicalTrials.gov (identifier: NCT02295072).

Physical fitness components

Components of physical fitness (i.e., muscular strength, speed-agility and cardiorespiratory fitness) were assessed using the ALPHA (Assessing Levels of Physical fitness and Health in Adolescents) health-related physical fitness test battery for children and adolescents which has been shown to be valid, reliable and feasible for the assessment of physical fitness in youth.[22]

Briefly, upper-limbs muscular strength and lower-limbs muscular strength were assessed using the maximum handgrip strength test and the standing long jump test, respectively. A digital hand dynamometer with an adjustable grip (TKK 5101 Grip D, Takei, Tokyo, Japan) was used to assess the handgrip strength. Each child performed the test twice, and the mean score of the maximum score of left and right hands was calculated as an absolute measurement of upper-limbs muscular strength (kg). The standing long jump test was performed three times and the longest jump was recorded in centimeters (cm) as a relative measurement of lower-limbs muscular strength. For exploratory analyses, we computed a relative-to-body weight measurement from upper-limbs muscular strength (kg / body weight) and an absolute measurement from lower-limbs muscular strength (cm × kg).

Speed-agility was assessed using the 4×10-meter shuttle-run test (4×10m SRT). The test was performed twice and the fastest time was recorded in seconds. Since a longer completion time indicates a lower fitness level, for analyses purposes we inverted this variable by multiplying test completion time (sec) by −1. Thus, higher scores indicated higher speed-agility levels.

Cardiorespiratory fitness was assessed through the 20-meter shuttle-run test (20m SRT).[23] This test was performed once and always at the end of the fitness battery testing session. The total number of completed laps was registered.

Sedentary time and physical activity

Sedentary time and PA were assessed by accelerometer (GT3X+, ActiGraph, Pensacola, FL, USA). Children wore simultaneously two accelerometers located on the right hip and non-dominant wrist during 7 consecutive days (24h/day), and they were instructed to remove them only for water activities (i.e., bathing or swimming). Data was presented from the hip-location and also from non-dominant wrist as supplementary. Raw data collected at a sampling frequency of 100 Hz were loaded in ActiLife (ActiGraph, Pensacola, FL, USA) and then processed in R (v. 3.1.2, https:// www.cran.r-project.org/) using the GGIR package (v. 1.5-12, https://cran.r-project.org/web/packages/GGIR/).[24] We calculated the Euclidean Norm Minus One G metric (ENMO, 1 G ~ 9.8 m/s2) after auto-calibrating the acceleration signal.[24,25] The mean of ENMO with negative values rounded to zero was calculated over 5s epochs. Accelerometric information processing in GGIR consisted in: 1) Non-wear time detection by the Van Hees et al. approach;[26] 2) detection of abnormally and sustained high acceleration values (i.e., clipped time); 3) replacement of the non-wear and clipped time by the mean acceleration recorded within the same time frame for the rest of the measurement.[26] A replacement by 0 for all metrics was performed if no data were collected for a specific time frame for the rest of the days; 4) identification of waking and sleeping hours based on an automatized algorithm guided by the diaries completed by the participants.[27] The inclusion criterion for a valid day was wearing the accelerometer with ≥600 min/day of waking hours and ≥240 min/day of sleeping hours. A minimum of four valid days (three weekdays and one weekend day) per week was required to be included in the analyses. The compliance wearing the accelerometer was high, with 98% of the sample wearing the accelerometers for ≥ 6 days.

Total minutes per day of sedentary time, light PA, moderate PA, vigorous PA, and MVPA were calculated following Hildebrand et al. hip- and wrist-based cut-off points for the Euclidean Norm Minus One (ENMO) metric.[28,29]

Working memory

All participants completed a modified version of the Delayed Non-Match-to-Sample (DNMS) computerized task to assess working memory.[30] All trials were presented focally on a computer screen using E-Prime software (Psychology Software Tools, Pittsburgh, PA). Each trial consisted of two phases: sample and choice. The sample phase included a memory set of four sequential stimuli. We adapted stimuli for children, and thus *Pokemon* cartoons were presented on a blue background. Participants were asked to remember 4 stimuli displayed for 500 ms with a 1000 ms inter-stimulus interval between them. After the presentation of the 4 stimuli and after a 4000 ms delay interval, a target consisting in 2 different cartoons presented together was shown during the choice phase for 1800 ms (**Figure 1**). During this phase, participants were asked to select the cartoon that was not shown on the 4 previous stimuli.

A total of 16 practice trials plus 140 experimental trials were presented. The practice phase was carried out before the presentation of experimental trials to make sure that all participants were familiarized with the cartoons and started the experimental task in equal conditions. Then, the 140 total trials were shown in 4 blocks of 35 trials each in a randomized order. For the low memory load condition (i.e., 40 trials), the 4 stimuli presented during the sample phase were repeated, whereas for the high memory load condition (i.e., 100 trials) 4 different

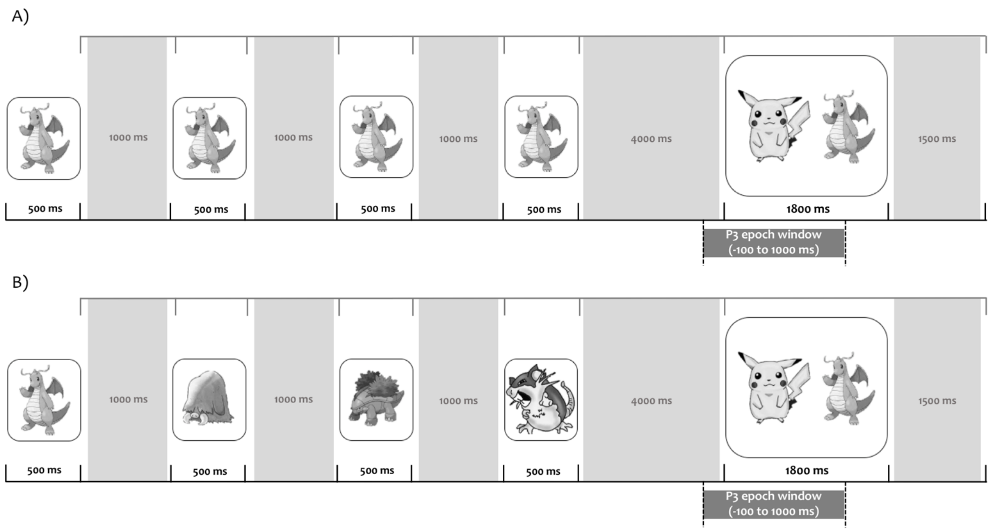

Figure 1. Low load (A) and high load (B) delayed non-match-to-sample working memory task, and epoch window for the extraction of P3 amplitude and latency.

stimuli were presented before the choice phase demanding greater working memory capacity. Duration of the task ranged from 35 to 45 min. Mean reaction time (RT) and response accuracy (%) were registered.

Neuroelectric activity

Neuroelectric activity was recorded from 64 electrode sites (Fpz, AFz, Fz, FCz, Cz, CPz, Pz, POz, Oz, Iz, Fp1/2, AF3/7/4/8, F1/3/5/7/2/4/6/8, FC1/3/5/2/4/6, FT7/8, T7/8, C1/3/5/2/4/6, TP7/8, CP1/3/5/2/4/6, P1/3/5/7/9/2/4/6/8/10, PO3/7/4/8, O1/2) arranged in an extended montage based on the International 10–10 system[31] using the ActiveTwo System of BioSemi (24-bit resolution, biopotential measurement system with Active Electrodes; Biosemi, Amsterdam, Netherlands). Prior to electroencephalography (EEG) recordings, inter-electrodes impedance was < 10kΩ with CMS and DRL sites serving as online (active and passive) ground electrodes. Details of the online BioSemi reference method can be found at http://www.biosemi.com/faq/cms&drl.htm. The EEG was conducted by using a 64-channel Active Two BioSemi EEG recording system (BioSemi; Amsterdam, Holland) using a sampling rate of 1024 Hz and a 100-Hz low-pass filter.

The EEG data were pre-processed using EEGLab version 13.5.4b,[32] and ERPLAB[33] toolbox version 6.02 and consisted of: (1) Down-sampling to 256Hz, (2) band-pass filtering between 0.1 and 30Hz (24 dB/octave), (3) application of artifact subspace reconstruction (ASR) to eliminate artifact channels, (4) removing eye-blink-related components by using an independent component analysis (ICA) with Multiple Artifact Rejection Algorithm (MARA),[32,34] (v) interpolation of the previously deleted channels by ASR, (vi) application of ICA with all channels. After the pre-processing, behavioral data were merged with the EEG data. Epochs were created for correct trials from -100 to 1000 ms surrounding the onset of the choice trial, baseline corrected using the −100 to 0 ms period. Trials with artifact exceeding ± 100 µV were rejected. To ensure the integrity of the signal, epochs were visually inspected blind to the experimental condition and participant prior to computing mean waveforms for each participant. The P3 component was defined as the largest positive-going peak within a 300 to 700 ms latency window. Data were then averaged across a 9-electrode site region of interest over the parietal and occipital regions (P1/Z/2, PO3/Z/4, O1/Z/2). Amplitude was measured as the difference between the mean pre-stimulus baseline and mean peak-interval amplitude; while peak latency was defined as the time point corresponding to the maximum peak amplitude.

Potential confounders

Sex, age, peak height velocity (PHV), body mass index (BMI), wave of participation, parental educational level and intelligence quotient (IQ) were used as potential confounders in the analyses. PHV is an indicator of maturity during childhood and adolescence and we used age and anthropometric variables to calculate it following Moore's equations.[35] Wave of participation was a categorical variable according to the moment of participation (wave 1, 2, or 3) of each child in the study. Parental educational level was assessed by a self-report questionnaire filled by the parents and we combined responses of both parents as: neither of them had a university degree; one of them had a university degree; both of them had a university degree.[36] The total composite IQ was assessed by the Spanish version of a valid and reliable tool named The Kaufman Brief Intelligence Test (K-BIT).[37]

Statistical analysis

The characteristics of the study sample are presented as means and standard deviations (SD) or percentages. Prior to all analyses, the extreme values were winsorized to limit their influence; this method allows replacing extreme high/low values for the closest (highest/lowest) valid value.[38] After checking for normal distribution, response accuracy from the low working memory load was normalized since it showed skewed distribution. Interaction analyses were performed between sex and physical fitness, sedentary time and PA on the outcomes. No significant interactions with sex were found (all Ps≥0.10) so analyses were carried out for the whole sample. Paired sample t-test was used to analyze differences in working memory (i.e., mean RT and response accuracy) and neuroelectric (i.e., P3 amplitude and latency) outcomes between low and high working memory loads. Bivariate Pearson correlations were performed to test the associations between potential confounders and working memory and neuroelectric outcomes. Statistical summary of these correlations is provided in **Table 1**.

Hierarchical linear stepwise regression analyses were performed to examine the associations of physical fitness, sedentary time and PA (i.e., data from both hip and wrist locations) with working memory and neuroelectric measurements. Stepwise method was used and all potential confounders (i.e., sex, age, PHV, BMI, wave of participation, parental educational level and IQ) were included into step 1 to test their association to the outcomes (working memory or neuroelectric activity). This step was performed to select the potential confounders that explain the higher amount of variance of their association with working memory and neuroelectric outcomes (see tables footnotes). Subsequently, hierarchical regressions were carried out entering each physical fitness, sedentary time and PA variable into step 2 in separate regression analyses after the inclusion of confounders previously found significantly associated to the outcomes in step 1.

Additionally, computation of the median for all the predictors was performed in order to visually

Table 1. Bivariate correlations between potential confounders and working memory task and neuroelectric measurements.

	Sex*	Age	PHV	BMI	Wave dummy†	Wave dummy 2‡	Parental educational level**	IQ
Working memory task								
Low working memory load								
Mean RT (ms)††	−0.134	−0.157	−0.179	0.089	0.189	−0.093	**−0.234**	−0.195
Response accuracy (%)ǂǂ	0.086	0.197	0.189	−0.148	−0.166	0.044	**0.305**	**0.231**
High working memory load								
Mean RT (ms)††	−0.028	−0.120	−0.083	0.068	0.206	−0.163	−0.194	−0.220
Response accuracy (%)	−0.069	**0.307**	0.198	−0.065	**−0.354**	**0.238**	0.207	**0.252**
Neuroelectric measurements								
Low working memory load								
P3 amplitude (μV)	−0.021	0.169	0.068	−0.164	−0.116	**0.228**	−0.124	0.018
P3 latency (ms)††	−0.037	**−0.323**	**−0.252**	0.115	0.026	−0.005	−0.057	**0.223**
High working memory load								
P3 amplitude (μV)	0.057	0.099	0.050	−0.145	−0.080	0.129	−0.098	−0.040
P3 latency (ms)††	−0.140	**−0.264**	**−0.273**	0.112	0.134	−0.016	−0.164	0.068

BMI = Body mass index; IQ = Intelligence quotient; PHV = Peak height velocity; RT = Reaction time. The bold font is used to highlight significance level at $P < 0.05$. *Sex was a categorical variable: 0 = Boys, 1 = Girls. †Wave dummy was a categorical variable: 0 = To belong to 1st or 3rd waves, 1 = To belong to 2nd wave. ‡Wave dummy 2 was a categorical variable: 0 = To belong to 1st and 2nd waves, 1 = To belong to 3rd wave. **Parental educational level was a categorical variable: 1 = None of the parents had a university degree, 2 = One of the parents had a university degree, 3 = both of parents had a university degree. ††Lower values indicate better performance. ǂǂNormalized values were used in the analysis.

represent the relationship of physical fitness, sedentary time and PA with P3 amplitude and latency. A significance level of $P < 0.05$ was set. Additionally, multiple comparisons correction was performed by independent variables (i.e., physical fitness, and sedentary time and PA) using the Benjamini-Hochberg method.[39] All the statistical procedures were performed using the SPSS software for Mac (version 22.0, IBM Corporation).

RESULTS

Working memory: Reaction time and response accuracy

Paired samples t-test showed a significant difference between low and high working memory loads with respect to response accuracy (82.99 ± 12.21% and 68.92 ± 13.47%, respectively; $P < 0.001$). For mean RT, no significant difference was observed between low and high working memory loads (920.28 ± 143.25 ms and 907.80 ± 142.74 ms, respectively; $P = 0.174$).

Physical fitness

The descriptive characteristics of the study sample can be found in **Table 2**. Higher upper-limbs absolute strength was associated with lower response accuracy in the high load (β = −0.270, *P* = 0.023; **Table 3**). This association remained significant after exploratory analysis with relative-to-body weight upper-limbs strength fitness (β = −0.223, *P* = 0.032). Higher speed-agility was associated with shorter mean RT in both the low and high loads (β = −0.231 and β = −0.251, respectively; both *P* < 0.05). Higher cardiorespiratory fitness was also associated with a shorter mean RT in the high load (β = −0.243, *P* =

Table 2. Descriptive characteristics of the study sample.

	All (n=79)	Boys (n=51)	Girls (n=28)
Sociodemographic characteristics			
Age (years)	10.2 ± 1.1	10.3 ± 1.1	10.0 ± 1.1
Peak height velocity offset (years)	−2.2 ± 1.0	−2.6 ± 0.8	−1.5 ± 1.0
Weight (kg)	57.4 ± 10.1	57.4 ± 10.0	57.5 ± 10.4
Height (cm)	145.3 ± 7.6	145.5 ± 7.5	144.9 ± 7.9
Body mass index (kg/m^2)	27.1 ± 3.5	27.0 ± 3.6	27.2 ± 3.2
Intelligence quotient (typical punctuation)	99.5 ± 12.0	97.9 ± 12.1	102.0 ± 11.6
Wave of participation, (%)			
First	14	8	25
Second	42	47	32
Third	44	45	43
Parental university level, (%)			
None of them	63	68	54
One of them	20	16	28
Both of them	17	16	18
Physical fitness			
Upper-limbs absolute strength (kg)	17.2 ± 4.2	17.7 ± 4.4	16.5 ± 3.7
Lower-limbs relative strength (cm)	106.1 ± 18.6	107.4 ± 16.8	103.7 ± 21.6
Speed-agility (sec)*	14.9 ± 1.6	14.7 ± 1.5	15.2 ± 1.6
Cardiorespiratory fitness (laps)	16.7 ± 8.1	17.7 ± 8.2	14.9 ± 7.8
Sedentary time and PA (min/day)†			
Sedentary time	827.2 ± 44.5	821.4 ± 45.9	837.7 ± 40.3
Light PA	65.8 ± 15.6	67.3 ± 15.7	63.2 ± 15.3
Moderate PA	34.3 ± 14.8	38.5 ± 15.8	26.8 ± 9.2
Vigorous PA	3.3 ± 2.8	4.0 ± 3.1	2.1 ± 1.5
MVPA	37.6 ± 16.8	42.5 ± 17.9	28.9 ± 10.1
Working memory task			
Low working memory load			
Mean reaction time (ms)*	920.3 ± 143.3	934.4 ± 156.9	894.6 ± 112.4
Response accuracy (%)	83.0 ± 12.2	81.6 ± 13.5	85.6 ± 9.0
High working memory load			
Mean reaction time (ms)*	907.8 ± 142.8	912.0 ± 153.7	900.2 ± 122.6
Response accuracy (%)	68.9 ±13.5	69.6 ± 15.1	67.7 ± 10.0
Neuroelectric measurements			
Low working memory load			
P3 amplitude (µV)	10.1 ± 4.3	10.1 ± 4.6	10.0 ± 3.9
P3 latency (ms)*	469.6 ± 76.7	417.6 ± 80.8	465.8 ± 69.4
High working memory load			
P3 amplitude (µV)	10.1 ± 4.2	10.0 ± 4.3	10.5 ± 4.0
P3 latency (ms)*	495.1 ± 81.5	503.5 ± 84.6	479.9 ± 74.5

PA=Physical activity; MVPA=Moderate-to-Vigorous Physical Activity. Values are expressed as means ± standard deviations, unless otherwise indicated. *Lower values indicate better performance. †Sedentary time and PA variables were obtained from the hip. Upper-limbs absolute strength was measured by the handgrip strength test. Lower-limbs relative strength was measured by the standing long jump test. Speed-agility was measured by the 4×10-meter shuttle run test. Cardiorespiratory fitness was measured by the 20-meter shuttle run test.

Table 3. Hierarchical stepwise regressions for the association of physical fitness, sedentary time and physical activity (hip) with working memory task (n=79).

	Low working memory load								High working memory load							
	Mean reaction time (ms)				Response accuracy (%)†				Mean reaction time (ms)				Response accuracy (%)			
	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*
Physical fitness																
Upper-limbs absolute strength (kg)	0.064	0.009	0.095	0.398	−0.103	−0.010	−0.101	0.356	0.006	0.006	0.079	0.488	0.327	0.049	−0.270	**0.023***
Lower-limbs relative strength (cm)	0.090	0.035	−0.189	0.089	0.093	0.000	−0.016	0.886	0.026	0.026	−0.162	0.155	0.299	0.021	−0.162	0.141
Speed-agility (sec)‡	0.107	0.053	−0.231	**0.037***	0.105	0.011	0.107	0.327	0.063	0.063	−0.251	**0.026***	0.278	0.000	−0.001	0.995
CRF (laps)	0.066	0.012	−0.110	0.332	0.115	0.022	0.150	0.177	0.059	0.059	−0.243	**0.031***	0.281	0.003	0.061	0.586
Sedentary time and PA (min/day)																
Sedentary time	0.059	0.005	−0.068	0.541	0.095	0.001	0.039	0.724	0.017	0.017	−0.131	0.249	0.294	0.016	0.127	0.200
Light PA	0.057	0.002	−0.046	0.679	0.093	0.000	−0.015	0.888	0.000	0.000	0.007	0.953	0.284	0.006	−0.081	0.420
Moderate PA	0.055	0.000	0.021	0.854	0.095	0.002	−0.041	0.708	0.000	0.000	0.022	0.845	0.280	0.002	0.050	0.620
Vigorous PA	0.057	0.002	−0.043	0.698	0.110	0.017	0.131	0.231	0.010	0.010	−0.098	0.389	0.323	0.046	0.219	**0.028**
MVPA	0.055	0.000	0.011	0.922	0.093	0.000	−0.015	0.893	0.000	0.000	0.004	0.975	0.284	0.006	0.080	0.429

CRF = Cardiorespiratory fitness; ENMO=Euclidian norm minus one; PA=Physical activity; MVPA=Moderate-to-vigorous physical activity. β values are standardized. The bold font is used to highlight significance level at p<0.05. *Statistically significant values after adjustment for multiple comparisons by independent variables using the Benjamini-Hochberg method (1995). Upper-limbs absolute strength was measured by the handgrip strength test. Lower-limbs relative strength was measured by the standing long jump test. Speed-agility was measured by the 4×10-meter shuttle run test. Cardiorespiratory fitness was measured by the 20-meter shuttle run test. Potential confounders (i.e., sex, age, peak height velocity, body mass index, wave of participation, parental educational level and IQ) were included into step 1 of the hierarchical stepwise regression to test their association to the outcomes. Hierarchical stepwise regression models for low working memory load: For mean reaction time and the response accuracy, the parental educational level was included as confounder β=−0.234 and β=0.305, respectively; both P<0.05). Hierarchical stepwise regression models for high working memory load: For mean reaction time, no confounder was included; for response accuracy, wave of participation, age, and intelligence quotient were included as confounders (β_{wave} = −0.354, β_{age} = 0.254, and β_{IQ} = 0.319; all *P* < 0.05). ‡This variable was inverted so that higher values indicate better performance. †Normalized values were used in the analysis.

0.031). No significant association was observed between lower-limbs relative or absolute strength and working memory (*P* ≥ 0.05).

Sedentary time and physical activity

Higher vigorous PA was associated with higher response accuracy in the high load (β = 0.219, *P* = 0.028; **Table 3**). However, the significance disappeared after correcting for multiple comparisons. No associations were observed for sedentary time and the rest of PA intensities (*P* ≥ 0.05). Furthermore, when performing sedentary time and PA analyses from the non-dominant wrist-placement, no associations were found with working memory (*P* ≥ 0.05; **Table 5**).

Neuroelectric activity: P3 amplitude

Paired sample t-test did not show a significant difference between low and high working memory loads with respect to P3 amplitude (10.07 ± 4.33 μV and 10.14 ± 4.16 μV, respectively; *P* = 0.822).

Physical fitness

Higher lower-limbs relative strength, speed-agility and cardiorespiratory fitness were associated with larger P3 amplitude in both low and high working memory loads with β ranging from 0.251 to 0.337 (all *P* < 0.05; **Table 4**). However, the associations found for lower-limbs relative strength disappeared when it was expressed in absolute terms (β = 0.102 and β = 0.115 for low and high P3 amplitude; *P* > 0.05). No significant

Table 4. Hierarchical stepwise regressions for the association of physical fitness, sedentary time and physical activity (hip) with P3 amplitude in the parieto-occipital region low and high working memory loads (n=79).

	Low working memory load				High working memory load			
	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*
Physical fitness								
Upper-limbs absolute strength (kg)	0.058	0.006	−0.076	0.495	0.000	0.000	−0.021	0.851
Lower-limbs relative strength (cm)	0.103	0.051	0.227	**0.040***	0.070	0.070	0.264	**0.019***
Speed-agility (sec)†	0.157	0.105	0.325	**0.003***	0.128	0.128	0.358	**0.001***
Cardiorespiratory fitness (laps)	0.165	0.113	0.337	**0.002***	0.063	0.063	0.251	**0.025***
Sedentary time and PA (min/day)								
Sedentary time	0.067	0.042	−0.122	0.279	0.003	0.003	−0.055	0.630
Light PA	0.088	0.036	0.189	0.089	0.019	0.019	0.140	0.220
Moderate PA	0.088	0.036	0.191	0.085	0.020	0.020	0.141	0.214
Vigorous PA	0.204	0.152	0.390	**<0.001***	0.075	0.075	0.274	**0.015**
MVPA	0.106	0.054	0.233	**0.035**	0.029	0.029	0.169	0.135

ENMO=Euclidian norm minus one; PA=Physical activity; MVPA=Moderate-to-vigorous physical activity. β values are standardized. The bold font is used to highlight significance level at p<0.05. *Statistically significant values after adjustment for multiple comparisons by independent variables using the Benjamini-Hochberg method (1995). †This variable was inverted so that higher values indicate better performance. Potential confounders (i.e., sex, age, peak height velocity, body mass index, wave of participation, parental educational level and IQ) were included into step 1 of the hierarchical stepwise regression to test their association to the outcomes. Hierarchical stepwise regression models for the P3 amplitude from the low working memory load was adjusted by wave of participation (β=0.228, P=0.043). Hierarchical stepwise regression models for the P3 amplitude from the high working memory load was not adjusted by any confounder. Upper-limbs absolute strength was measured by the handgrip strength test. Lower-limbs relative strength was measured by the standing long jump test. Speed-agility was measured by the 4×10-meter shuttle run test. Cardiorespiratory fitness was measured by the 20-meter shuttle run test.

associations were found between upper-limbs absolute or relative strength and P3 amplitude ($P \geq 0.05$). These relationships can be visually observed in **Figure 2**.

non-dominant wrist-placement (**Table 6**) and after correcting for multiple comparisons, only higher vigorous PA was associated with larger P3 amplitude in the low load ($\beta = 0.289$, $P = 0.008$).

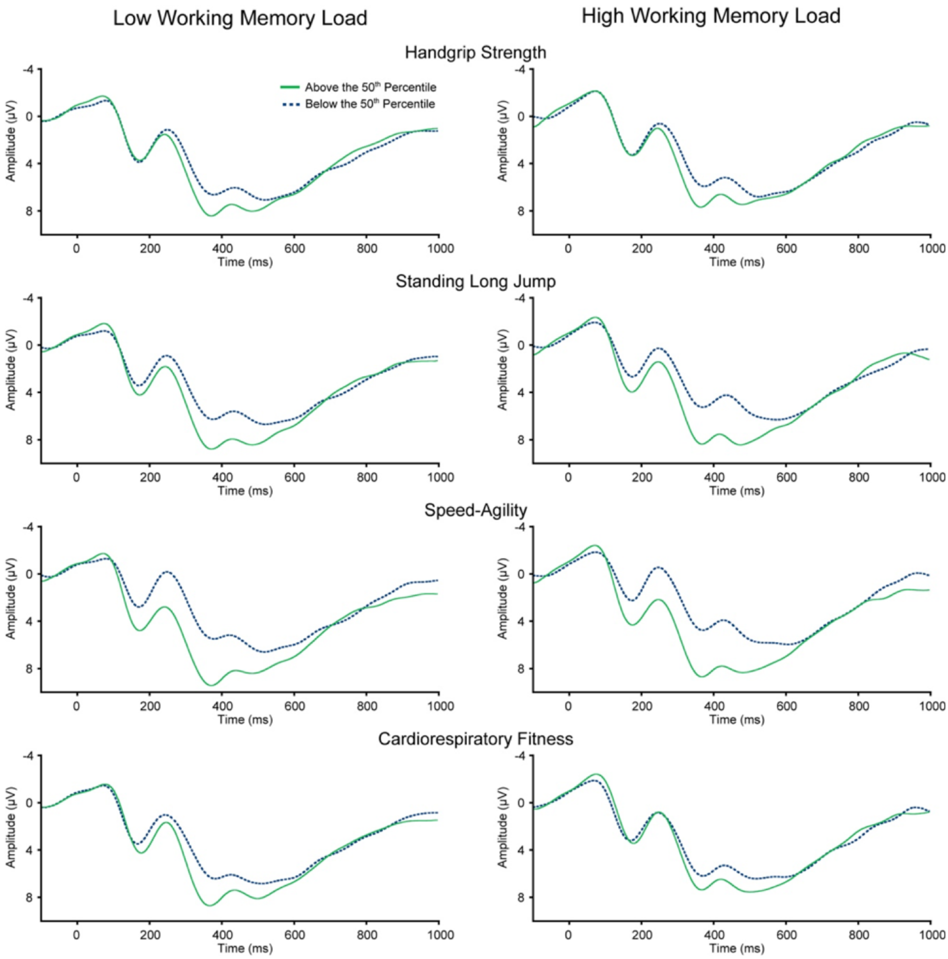

Figure 2. Median split waveforms time-locked to the onset of the choice stimulus for each domain of physical fitness.

Sedentary time and physical activity

After correcting for multiple comparisons, only higher vigorous PA from hip was associated with larger P3 amplitude in the low working memory load ($\beta = 0.390$; $P < 0.001$; **Table 4**). No associations were observed for sedentary time and the rest of PA intensities ($P \geq 0.05$). These relationships can be graphically observed in **Figure 3**. Furthermore, when using data from the

Neuroelectric activity: P3 latency

Paired sample t-test showed a significant difference between low and high working memory loads with respect to P3 latency (469.55 ± 76.55 ms and 495.09 ± 81.48 ms, respectively; $P = 0.008$).

Associations of physical fitness, sedentary time and PA with P3 latency can be observed in **Table**

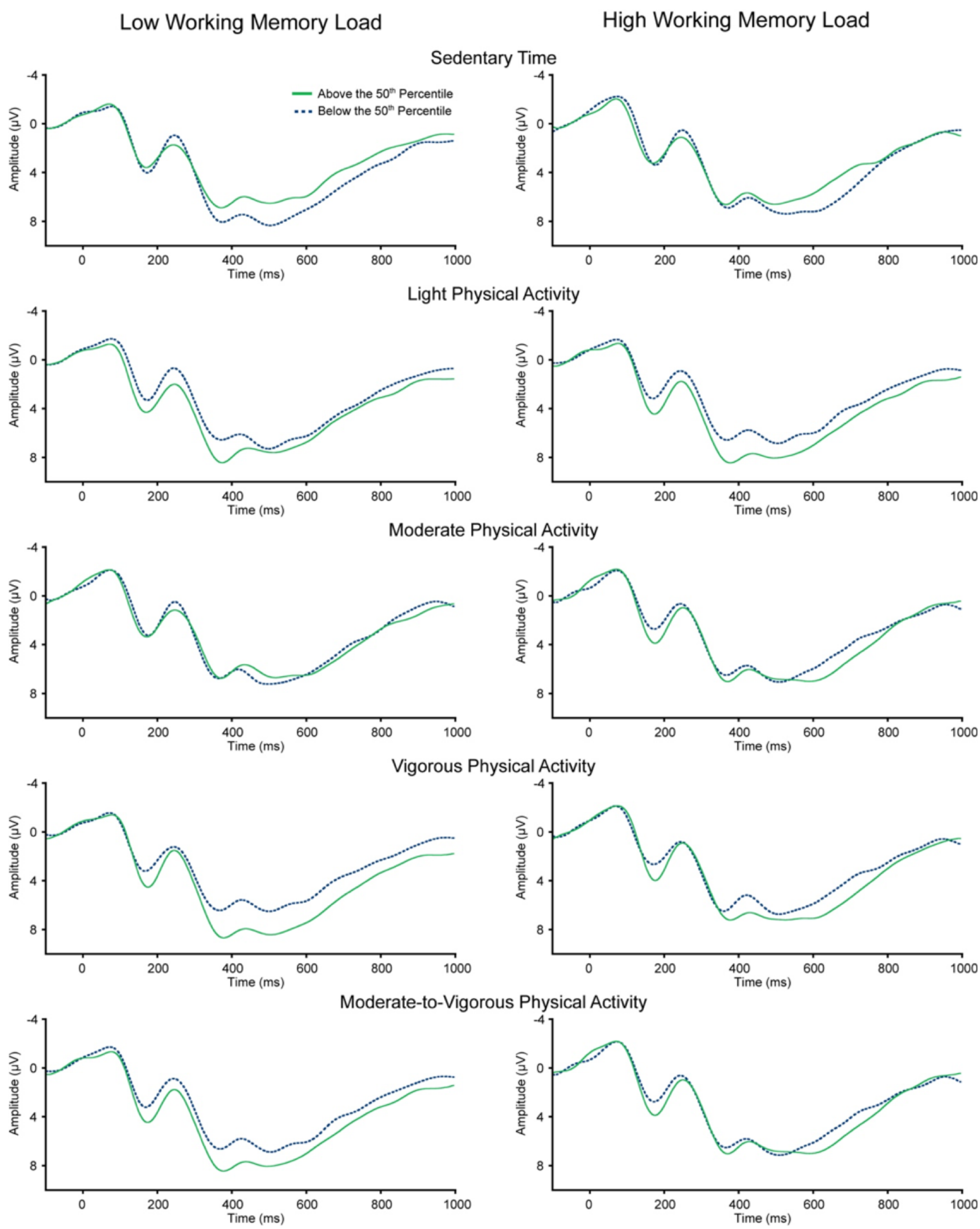

Figure 3. Median split waveforms time-locked to the onset of the choice stimulus for the relation of sedentary time and physical activity.

7, and in **Table 8** for the PA data from non-dominant wrist.

Physical fitness

Briefly, higher speed-agility was associated with shorter P3 latency in both low and high loads (β = –0.252 and β = –0.277, respectively; both *P* < 0.05; **Table 7**), as

Table 5. Hierarchical stepwise regressions for the association of sedentary time and physical activity (wrist) with working memory task (n=79).

	Low working memory load								High working memory load							
	Mean reaction time (ms)				Response accuracy (%)*				Mean reaction time (ms)				Response accuracy (%)			
	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*
Sedentary time	0.056	0.002	0.039	0.727	0.099	0.005	0.073	0.507	0.001	0.001	−0.038	0.738	0.310	0.032	0.181	0.067
Light PA	0.066	0.011	−0.108	0.339	0.100	0.007	−0.083	0.454	0.002	0.002	0.047	0.682	0.303	0.026	−0.161	0.104
Moderate PA	0.058	0.004	−0.060	0.593	0.113	0.000	−0.004	0.974	0.003	0.003	−0.057	0.618	0.281	0.004	0.062	0.541
Vigorous PA	0.055	0.000	0.015	0.897	0.114	0.001	0.036	0.743	0.003	0.003	−0.051	0.657	0.306	0.028	0.173	0.088
MVPA	0.057	0.002	−0.047	0.678	0.113	0.000	0.005	0.965	0.003	0.003	−0.058	0.610	0.285	0.008	0.090	0.381

ENMO=Euclidian norm minus one; PA=Physical activity; MVPA=Moderate-to-vigorous physical activity. β values are standardized. *Normalized values were used in the analysis. Potential confounders (i.e., sex, age, peak height velocity, body mass index, wave of participation, parental educational level and IQ) were included into step 1 of the hierarchical stepwise regression to test their association to the outcomes. Hierarchical stepwise regression models for low working memory load: for mean reaction time and the response accuracy, the parental educational level was included as confounder. Hierarchical stepwise regression models for high working memory load: for mean reaction time, no confounder was included; for response accuracy, age, wave of participation, and intelligence quotient were included as confounders.

Table 6. Hierarchical stepwise regressions for the association of sedentary time and physical activity (wrist) with P3 amplitude in the parieto-occipital region for low and high working memory load (n=79).

	Low working memory load				High working memory load			
	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*
Sedentary time	0.052	0.000	0.007	0.953	0.002	0.002	0.046	0.685
Light PA	0.063	0.011	−0.108	0.338	0.013	0.013	−0.115	0.315
Moderate PA	0.100	0.048	0.219	**0.048**	0.025	0.025	0.159	0.163
Vigorous PA	0.136	0.084	0.289	**0.008***	0.031	0.031	0.176	0.120
MVPA	0.111	0.059	0.244	**0.027**	0.029	0.029	0.170	0.135

ENMO=Euclidian norm minus one; PA=Physical activity; MVPA=Moderate-to-vigorous physical activity. β values are standardized. The bold font is used to highlight significance level at p<0.05. *Statistically significant values after adjustment for multiple comparisons by independent variables using the Benjamini-Hochberg method (1995). Potential confounders (i.e., sex, age, peak height velocity, body mass index, wave of participation, parental educational level and IQ) were included into step 1 of the hierarchical stepwise regression to test their association to the outcomes. Hierarchical stepwise regression models for the P3 amplitude from the low working memory load was adjusted by wave of participation. Hierarchical stepwise regression models for the P3 amplitude from the high working memory load was not adjusted by any confounder.

well as cardiorespiratory fitness but only in the low load (β = −0.314, *P* = 0.008).

Sedentary time and physical activity

No associations were found between sedentary time, PA, and P3 latency using either the hip or wrist data (*P* ≥ 0.05; **Table 7** and **8**, respectively).

Table 7. Hierarchical stepwise regressions for the association of physical fitness, sedentary time and physical activity (hip) with P3 latency in the parieto-occipital region for low and high working memory load (n=79).

	Low working memory load				High working memory load			
	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*
Physical fitness								
Upper-limbs absolute strength (kg)	0.104	0.000	−0.002	0.987	0.075	0.001	0.027	0.828
Lower-limbs relative strength (cm)	0.129	0.024	−0.172	0.148	0.100	0.026	−0.168	0.143
Speed-agility (sec)†	0.154	0.050	−0.252	**0.038***	0.146	0.071	−0.277	**0.014***
Cardiorespiratory fitness (laps)	0.184	0.080	−0.314	**0.008***	0.097	0.023	−0.156	0.168
Sedentary time PA (min/day)								
Sedentary time	0.109	0.005	0.069	0.527	0.078	0.003	0.059	0.601
Light PA	0.137	0.033	−0.181	0.093	0.075	0.001	−0.024	0.831
Moderate PA	0.106	0.001	−0.037	0.731	0.086	0.011	0.111	0.334
Vigorous PA	0.115	0.010	−0.103	0.347	0.074	0.000	−0.008	0.940
MVPA	0.107	0.002	−0.050	0.648	0.083	0.009	0.096	0.403

ENMO=Euclidian norm minus one; PA=Physical activity; MVPA=Moderate-to-vigorous physical activity. β values are standardized. The bold font is used to highlight significance level at p<0.05. *Statistically significant values after adjustment for multiple comparisons by independent variables using the Benjamini-Hochberg method (1995). †This variable was inverted so that higher values indicate better performance. Potential confounders (i.e., sex, age, peak height velocity, body mass index, wave of participation, parental educational level and IQ) were included into step 1 of the hierarchical stepwise regression to test their association to the outcomes. Hierarchical stepwise regression models for the P3 latency from the low working memory load was adjusted by age. Hierarchical stepwise regression models for the P3 latency from the high working memory load was adjusted by peak height velocity. Upper-limbs absolute strength was measured by the handgrip strength test. Lower-limbs relative strength was measured by the standing long jump test. Speed-agility was measured by the 4×10-meter shuttle run test. Cardiorespiratory fitness was measured by the 20-meter shuttle run test.

DISCUSSION

Main findings

Our findings contribute to the existent literature by suggesting that: (1) Not only cardiorespiratory fitness, as shown in previous research, but also speed-agility were consistently associated with working memory (i.e., shorter mean RT) and the P3 component (i.e., larger amplitude and shorter latency). However, inconsistent findings were observed for muscular strength, with upper-limbs absolute strength associated with lower response accuracy, and lower-limbs relative strength associated with larger P3 amplitude; (2) The relationship of PA with working memory and neuroelectric activity was intensity-dependent and seemed consistent across accelerometer locations and cut-points (i.e., hip and wrist). Thus, only vigorous PA related to a higher response accuracy and to a larger P3 amplitude regardless of the accelerometer location.

Table 8. Hierarchical stepwise regressions for the association of sedentary time and physical activity (wrist) with P3 latency in the parieto-occipital region for low and high working memory load (n=79).

	Low working memory				High working memory			
	R^2	R^2 change	β	P	R^2	R^2 change	β	P
Sedentary time	0.106	0.002	0.042	0.704	0.079	0.005	−0.069	0.539
Light PA	0.106	0.002	-0.040	0.716	0.074	0.000	−0001	0.993
Moderate PA	0.112	0.007	-0.087	0.427	0.079	0.004	0.071	0.551
Vigorous PA	0.109	0.005	-0.070	0.522	0.083	0.008	0.095	0.415
MVPA	0.112	0.007	-0.087	0.426	0.080	0.006	0.081	0.499

ENMO=Euclidian norm minus one; PA=Physical activity; MVPA=Moderate-to-vigorous physical activity. β values are standardized. Potential confounders (i.e., sex, age, peak height velocity, body mass index, wave of participation, parental educational level and IQ) were included into step 1 of the hierarchical stepwise regression to test their association to the outcomes. Hierarchical stepwise regression models for the P3 latency from the low working memory load was adjusted by age. Hierarchical stepwise regression models for the P3 latency from the high working memory load was adjusted by peak height velocity.

Physical fitness components and working memory

In the present study, speed-agility and cardiorespiratory fitness were associated with shorter RT observed in the high working memory load. Despite the lack of studies in children with overweight/obesity, our results regarding cardiorespiratory fitness are in line with several previous studies carried out in children with normal weight.[10,11] In these studies, higher-fit children had a higher response accuracy than their lower-fit peers during a working memory task. Similarly, in a randomized-controlled trial, increases in VO_2max resulting from a PA intervention consisting in 70 min of daily MVPA were associated with improvements in children's working memory.[40] A recent systematic review showed that complex motor skills (i.e., speed-agility) are strongly related to higher-order cognitive skills, which may include working memory.[15] Whereas our findings on fitness and working memory performance were observed for mean RT, the majority of previous research found these associations for response accuracy.[9,10] However, previous studies included a sample of children with normal weight, and since our study was carried out in children with overweight/obesity, it could be that in the present study, RT was a more sensitive index to detect fitness associations in this population. Thus, it may be that children in the present study carried out more proactive cognitive strategies (i.e., early selection, shorter RT) in order to achieve optimal completion of task goals via regulation of attentional engagement.[20] Collectively, as our results strengthen the existent evidence suggesting that different components of physical fitness may positively affects working memory,[3,40] future studies should focus on conducting intervention programs to gain causal evidence for the role of different fitness components in working memory in children with overweight/obesity.

In regards with muscular strength, we found inconsistencies in the associations between upper-limbs absolute strength and lower-limbs relative strength with respect to working memory. To our knowledge, only one study analyzed this relationship in children with normal weight, reporting contradictory findings with respect to ours with upper-limbs absolute strength in overweight/obese.[12] In that study, overall muscular strength was associated with higher response accuracy during high working memory load. Possible explanations for the discrepancies between studies include the use of different types of strength (i.e., absolute versus relative), different types of musculature involve (i.e., upper- versus lower-limbs versus overall muscular strength), or different types of working memory task (i.e., DNMS versus n-back). In particular, the reason for our negative association between upper-limbs absolute strength and response accuracy may be the overweight/obese status of our sample as it has been speculated previously,[41] as well as the higher levels of upper-limbs absolute strength of these individuals in our study (i.e., there was a significant correlation between body weight and upper-limbs absolute strength of r=0.542, P<0.001). In fact, this negative association remained significant after exploratory analysis using a relative-to-body weight measurement of upper-limbs strength. In this context, a recent systematic review reported shorter RT but more commission errors (i.e., less response accuracy) among children with higher BMI.[41] This fact may derive from a higher impulsivity of children with overweight/obesity[42] that are also the strongest ones, which may in turn explain the lower response accuracy observed in our study.

In general, the association found between cardiorespiratory fitness and mean RT was observed in the high working memory load. This indicates that higher-fit children were faster in responding and that the relationship of cardiorespiratory fitness may be selective for task loads engendering greater amounts of working memory. In fact, these findings are consistent with prior studies in children with normal weight and support the idea that cardiorespiratory fitness may be particularly beneficial for more cognitively demanding processes.[10,12] Further, when comparing RTs between high and low loads, higher RTs were observed in the high load compared with the low load, although this difference did not reach the significance. On the other hand, speed-agility was associated with shorter RT in both loads, indicating faster information processing speed regardless of the working memory demands. A recent study using the same sample as in the present research found that higher speed-agility was associated with better movement competency.[43] Proper movement competency is partly determined by a neuromuscular/motor control network,[44] which may also relate to shorter RTs. Considering that a prior study showed that children with overweight/obesity exhibited larger RT than their normal weight peers,[45] it may be that children with overweight/obesity have more room for improvement from speed-agility which may be reflected by shorter RTs. However, further studies comparing children with overweight/obesity and normal weight peers with respect to speed-agility and processing speed are needed to draw firm conclusions.

Physical fitness components and neuroelectric activity

To the best of our knowledge, this is the first study to investigate the association between multiple physical fitness components, sedentary time, PA, and ERPs (i.e., P3 amplitude and latency) during a working memory task in overweight/obese. To date, the majority of studies focused only on cardiorespiratory fitness and used an attention or inhibition task (mainly modified flanker tasks).[3] In our study, cardiorespiratory fitness was associated with higher P3 amplitude across memory loads. This concurs with previous findings showing that higher-fit children exhibited larger P3 amplitude or shorter P3 latency than their peers.[19,20] All these previous studies split the sample into two extreme fitness groups (i.e., high- and low-fit children) excluding those with middle level, what make possible that cardiorespiratory fitness group differences occurred via a physical fitness threshold.[3] However, in the present study we also obtained positive results with cardiorespiratory fitness as a continuous outcome by including the full range of this variable (i.e., as has been previously recommended).[3]

Apart from cardiorespiratory fitness, this study also provides novel data on the positive association of muscular strength and speed-agility with P3 amplitude and latency during a working memory task in children with overweight/obesity. Although direct comparisons cannot be made, our findings with lower-body muscular strength may be explained by the fact that muscular fitness is associated with a variety of health benefits in children[46] which have been associated with enhanced working memory.[47,48] However, this must be interpreted with caution since the associations for lower-limbs relative strength disappeared when it was expressed in absolute terms. Across all fitness components, speed-agility and cardiorespiratory fitness had the highest number of significant associations with working memory performance and P3 amplitude and latency, which may be explained by previous findings in the same sample showing that these two fitness components were also the main ones associated to brain volumes.[4] Specifically, these components were related to increased volume of cortical and subcortical brain structures[4] that have been shown to directly influence memory in children (e.g., hippocampus, premotor cortex, etc.).[49] These key neural structures subserving working memory processes are still developing throughout childhood,[50] suggesting that some brain structures might be highly susceptible to environmental factors such as engagement in aerobic exercise and motor tasks during development.[51] However, further randomized-controlled trials should confirm these findings.

The positive associations found for each fitness component with P3 amplitude were observed regardless of working memory load. This is supported by a prior study using an inhibitory control task, which showed that higher-fit children exhibited larger P3 amplitude than their lower-fit peers across task's conditions.[19] However, the majority of literature has declared that the association between physical fitness and P3 amplitude appears when task demands increase.[3,20] Despite this, the associations found across conditions in the present study may be due to different arguments. For instance, the characteristics and design of the DNMS task may be a limitation itself to detect selective associations of fitness with attentional resources allocation during high working memory processes.

Sedentary time, physical activity, working memory and neuroelectric activity

In regards to the relationship between sedentary time, PA, and working memory performance, we found that vigorous PA (from hip data) was associated with higher response accuracy in the high memory load. However, this finding should be interpreted with caution since the significant association disappeared after controlling for multiple comparisons. Two previous cross-sectional studies investigated this relationship in children with normal weight.[13,14] They showed non-significant associations of objectively-measured sedentary time, total PA, MVPA, with the Visual Memory Span task,[14] and the Spatial Span task.[13] None of these studies assessed other PA intensities (i.e., light, moderate or vigorous PA). Vigorous PA was also related to a larger P3 amplitude and it was consistent across accelerometer locations (i.e., hip or non-dominant wrist). Despite no previous study has analyzed the relation between PA and neuroelectric activity during working memory task, our association between vigorous PA and P3 amplitude may be due to an intensity-dependent relation of PA with working memory. This is supported by an intervention study where two different types of PA intensity-based physical education classes were delivered to an intervention and a control group.[52] In this study, a significant PA intensity effect was found since the children from the intervention group (i.e., higher PA intensity classes) had better performance after the program on a working memory test battery in comparison with their peers in the control group (i.e., regular physical education lessons). Another important finding from our study was the consistency between accelerometer's locations (i.e., hip and wrist) with respect to the relation of sedentary time and PA with working memory and neuroelectric activity. However, the lack of evidence in this respect indicate that these findings are preliminary and call for more studies investigating the association of different PA intensities and accelerometer-locations with the neuroelectric system in children.

Limitations and strengths

Several limitations of the present study must be highlighted. First, the cross-sectional design does not allow us to draw causal interpretations. Second, PA such as bicycling and swimming cannot be captured by the accelerometers, and our identification of sedentary time was not sensitive to postures, so we cannot differ between different sedentary behaviors and, therefore, some standing activity could be classified as sedentary time. On the other hand, the main strength of this study was that, to the best of our knowledge, this was the first study to investigate the relationship between different physical fitness components, not only cardiorespiratory fitness, and objectively sedentary time and PA with working memory and the neuroelectric activity underlying it in a sample of children with overweight/obesity. Other strengths were the objective and standardized assessment of physical fitness, PA and the cognitive variables; the analysis of different intensities of PA; the use of all predictors as continuous variables; and the availability of sedentary time and PA data from two different accelerometer locations (i.e., hip and non-dominant wrist).

CONCLUSION

Our results add to the literature on physical fitness and cognition by providing support not only for cardiorespiratory fitness relation, but also muscular strength and speed agility relation to working memory and the neuroelectric activity (i.e., P3 component) in children with overweight/obesity. Speed-agility and cardiorespiratory fitness were the fitness components more consistently related to both working memory performance and neuroelectric activity. The association of PA with working memory and neuroelectric activity was intensity-dependent, since only vigorous PA demonstrated a consistent (i.e., for both hip and wrist's locations) relationship. From a public health perspective, promoting physical activity that enhances speed-agility and cardiorespiratory fitness, and also reaches high intensity PA levels, may be important not only for the physical health, but also for working memory and underlying neuroelectric activity in children with overweight/obesity. However, our observational findings need to be supported with exercise-based randomized controlled trials inducing improvements in different fitness and PA components to test whether such improvements lead to better working memory in overweight/obese youth.

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Study 3

Fitness, physical activity, sedentary time, inhibitory control and neuroelectric activity in children with overweight/obesity

BACKGROUND

Childhood obesity has been catalogued as one of the most serious public health problems worldwide.[1] There is large body of evidence indicating that detrimental effects of childhood obesity may extend beyond metabolic and cardiovascular impairments[2] and may as well impact executive function and brain health.[3,4] Among the various aspects of top-down executive function, large negative associations have been observed for inhibitory control,[4,5] defined as the ability to suppress irrelevant information and focus on relevant aspects of the stimulus environment in order to activate appropriate and correct response schemas. In this context, higher levels of physical fitness and physical activity (PA), and reductions in sedentary time may attenuate the harmful effects of obesity on inhibitory control.[6] Given that preadolescent childhood may be a particularly sensitive period for both brain development[7] and health behaviors including physical fitness, PA and sedentary time,[8,9] it is of great public health importance to investigate the potential protective role of such health factors in brain functioning, especially in children with overweight/obesity.

A recent systematic review in children has evidenced a positive influence of cardiorespiratory fitness on inhibitory control,[10] including studies showing faster[11] and more accurate tasks' responses[12] among individuals with higher cardiorespiratory fitness levels. Findings for PA and sedentary time relative to inhibitory control are less consistent,[10] with positive and null associations reported for both sedentary time[13] and moderate-to-vigorous PA (MVPA).[13–15] Beyond the assessment of behavioral outcomes, prior studies have also assessed event-related brain potentials (ERP),[15–18] which provide an understanding of the underlying neuroelectric correlates of cognition that appear modifiable as a function of health factors such as physical fitness, PA and sedentary time.[19] In particular, the P3 is a positive-going ERP component whose amplitude is thought to be a neuroelectric correlate of inhibition and proportional to the amount of attentional resources allocated during stimulus engagement, and whose latency is believed to be proportional to the information processing speed.[20] Prior observational investigations showed that children with higher cardiorespiratory fitness levels exhibited larger P3 amplitude and shorter P3 latency compared to their lower-fit peers during tasks that tap aspects of inhibitory control.[16–18] However, this body of research has not considered the weight class of the children, and has solely focused on cardiorespiratory fitness, with a paucity of evidence for other components of fitness such as speed-agility or muscular strength.[10] Given that it has been shown that the main physical fitness components are differently associated with brain structure,[8,9] it is interesting to investigate this differential association using measures of brain function, such as neuroelectric activity.

With respect to PA and sedentary time, only one study has investigated the associations of moderate PA, vigorous PA, and MVPA with P3 amplitude and latency during an inhibitory control task.[15] However, this study sampled a combination of children with normal-weight with a small proportion of children with overweight/obesity, which limited the possibility of drawing conclusions for children with overweight/obesity as a risk group for cognitive impairments.[4] Furthermore, there is currently scarce information about the relationship of other health behaviors to neuroelectric and cognitive outcomes in obese individuals, including time in sedentary behaviors and time in light PA.

Accordingly, the aim of this study was to investigate the association of different physical fitness components (i.e., muscular strength, speed-agility, and cardiorespiratory fitness), various PA intensities, and sedentary time on behavioral and neuroelectric (i.e., P3 amplitude and latency) concomitants of inhibitory control in children with overweight/obesity. We hypothesized that higher levels of physical fitness and PA, and lower sedentary time would be associated to higher inhibitory control performance and the neuroelectric activity underlying it; specially for the task's condition requiring higher demands of inhibitory control.[17]

METHODS

Participants

This cross-sectional study was conducted under the umbrella of the ActiveBrains project (http://profith.ugr.es/activebrains).[21] The complete methodology, procedures and inclusion/exclusion criteria for participating in the project have been previously described.[21] An initial sample of 110 children aged 8–11 years with overweight/obesity were recruited from Granada, Spain. The present study used baseline data prior to randomization, collected from November 2014 to February 2016 in three different waves. A final sample of 84 children (10.1 ± 1.1 years old; 56% boys) with complete baseline data on selected outcomes was included in the present analysis. Description and characteristics of the study were given to parents or legal guardians, and a written informed consent was provided from both guardian and child. The ActiveBrains project was approved by the Ethics Committee on Human Research of the University of Granada, and was registered in ClinicalTrials.gov (identifier: NCT02295072).

Physical fitness components

The ALPHA health-related physical fitness test battery for children and adolescents was used to assess physical fitness components (i.e., muscular strength, speed-agility and cardiorespiratory fitness). A detailed description of the validity and reliability of the ALPHA battery has been provided elsewhere.[22] Upper-limb strength was assessed by the maximum handgrip strength test (TKK 5101 Grip D, Takei, Tokyo, Japan). This test was performed twice, and the maximum score of each hand was obtained and averaged as an absolute measurement of upper-limb strength (kg). Lower-limb strength was assessed using the standing long jump test. This test was performed three times and the longest jump was recorded in centimeters (cm) as a relative measurement of lower-limb strength. For exploratory analyses, we also computed an absolute strength measurement of lower-limbs (cm × body weight). Speed-agility was assessed using the 4×10-m shuttle-run test (4 × 10-m SRT). The test was performed twice and the fastest time was recorded in seconds. Since a longer completion time indicates poorer performance (i.e., slower and less agile) and for analysis purposes, we inverted this variable by multiplying test completion time (sec) by −1, so that higher scores indicated greater speed-agility levels. Cardiorespiratory fitness was assessed via a 20-meter shuttle-run test (20-m SRT). This test was performed once and always at the end of the fitness battery testing session. The total number of completed laps was registered.

Physical activity and sedentary time

PA and sedentary time were assessed with accelerometers (GT3X+, ActiGraph, Pensacola, FL, USA). Children simultaneously wore two accelerometers located on the right hip and non-dominant wrist during 7 consecutive days (24h/day). Participants were instructed to remove the accelerometers only for water activities (i.e. bathing or swimming). Raw data collected at a sampling frequency of 100 Hz were loaded in ActiLife (ActiGraph, Pensacola, FL, USA) and processed afterwards in R (v. 3.1.2, https://www.cran.r-project.org/) using the GGIR package (v. 1.6-0, https://cran.r-project.org/web/packages/GGIR/).[23] We calculated the Euclidean Norm Minus One G metric (ENMO, 1 G ~ 9.8 m/s^2) after auto-calibrating the acceleration signal.[23,24] The mean of ENMO with negative values rounded to zero was calculated over 5s epochs. Accelerometric information processing in GGIR consisted in: (1) Non-wear time detection;[25] (2) Detection of abnormally and sustained high acceleration values (i.e., clipped time); (3) Replacement of the non-wear and clipped time by the mean acceleration recorded within the same time frame for the rest of the measurement.[25] A replacement by 0 for all metrics was performed if no data were collected for a specific time frame for the rest of the days; (4) Identification of waking and sleeping hours based on an automatized algorithm guided by the diaries completed by the participants [26]. The inclusion criterion for a valid day was wearing the accelerometer with ≥16 h/day. A minimum of 4 valid days (3 weekdays and 1 weekend day) per week was required to be included in the analyses. The compliance wearing the accelerometer was high, with 98% of the sample wearing the accelerometers for ≥6 days. PA and sedentary time were classified into different intensities following hip- and non-dominant wrist-based cut-off points for the ENMO metric.[27,28] For the present study, the data are presented from the hip-mounted accelerometer, but analyses were replicated using data from non-dominant wrist. The variables included in this study were total minutes per day of light PA, moderate PA, vigorous PA, MVPA, and sedentary time.

Inhibitory control

All participants completed a modified picture-based version of the Eriksen flanker task to assess inhibitory control.[29] Trials from this task consisted of five cows that were focally presented (1.2° visual angle) on a computer screen using E-Prime software (Psychology Software Tools, Pittsburgh, PA). Participants were instructed to respond as quickly and accurately as possible with their dominant hand using an index finger press on a mouse to the direction (i.e., right or left) of the centrally presented target (i.e., 1.2-cm tall cow on a blue background). There were two different trials, congruent and incongruent, depending on the directions of the flanking nontarget stimuli (i.e., four identical cows) (**Figure 1**). Thus, for congruent trials, both the target and flanking stimuli were positioned in the same direction, either right or left. For incongruent trials, the target and flanker stimuli were positioned in opposite directions from each other, thus requiring the upregulation of inhibitory control to gate out perceptual interference and response schemas associated with the direction of the flanking

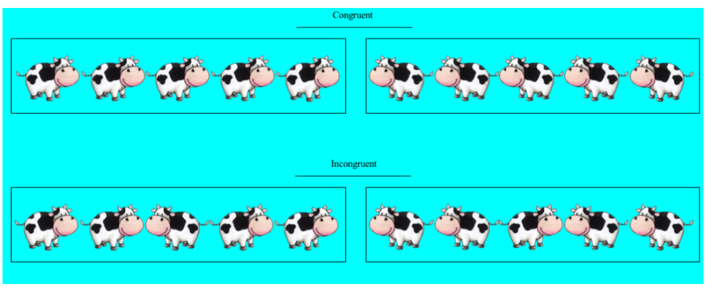

Figure 1. Congruent and incongruent trials from the picture-based version of the Eriksen flanker task.

stimuli. Participants were given a practice block consisting of 12 trials. Subsequently, a total of 144 experimental trials were presented randomly in three blocks of 48 trials each, with equal probability of appearance for congruent and incongruent trials. Measures of mean reaction time (RT) (s) and response accuracy (%) were collected.

Neuroelectric activity

Neuroelectric activity was recorded from 64 electrode sites arranged in an extended montage based on the International 10–10 system[30] using the ActiveTwo System of BioSemi (24-bit resolution, biopotential measurement system with Active Electrodes; Biosemi, Amsterdam, Netherlands). Data were digitized at a rate of 1024 Hz and a 100-Hz low-pass filter. Information regarding EEG data processing is provided in **Figure 2**. After processing, behavioral data were merged with EEG data and stimulus-locked epochs were created from a window of –199.0 prior to 1700 ms after stimulus onset and baseline corrected using the -100 to 0 ms pre-stimulus period. The P3 component was evaluated within each of the 64 electrode sites as the mean amplitude within a 50 ms interval surrounding the largest positive going peak within a 300 to 700 ms latency window following onset of the target stimulus. P3 latency was defined as the time point corresponding to the maximum P3 peak amplitude during this same latency window. Data for both amplitude and latency were then averaged across a 9-electrode site region of interest over the parietal and occipital regions (P1/Z/2, PO3/Z/4, O1/Z/2). This region of interest was selected as it corresponded to the topographic maxima of the P3 ERP component elicited in response to the picture-based flanker task used in the present investigation.

Potential confounders

Potential confounding variables selected for analyses were sex, peak height velocity (PHV; an indicator of maturity during childhood and adolescence computed from age and anthropometric variables following Moore's equations),[31] body mass index (BMI; kg/m^2), recruitment wave (i.e., 1, 2, or 3), self-reported parental educational level (i.e., neither had a university degree; one had a university degree; or both had a university degree), and intelligence quotient (IQ) assessed by the Spanish version of the Kaufman Brief Intelligence Test (K-BIT).[32]

Statistical analysis

The characteristics of the study sample are presented as means and standard deviations (SD) or percentages. After checking for normal distribution, response

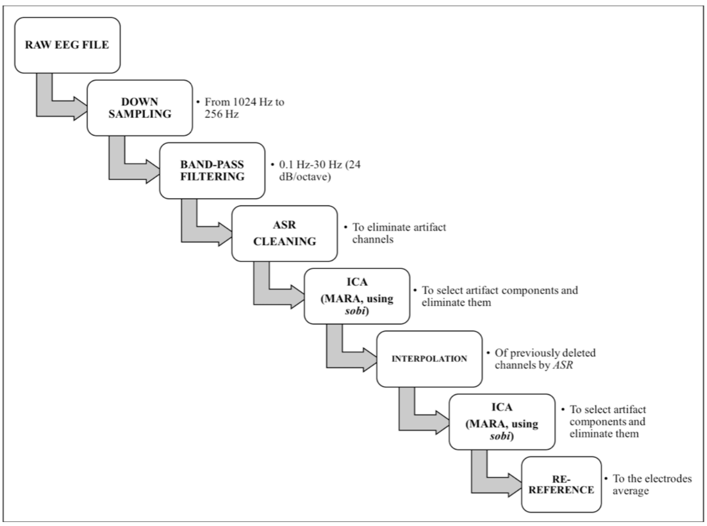

Figure 2. Electroencephalography (EEG) data processing steps. ASR = Artifact subspace reconstruction; ICA = Independent component analysis; MARA = Multiple artifact rejection algorithm. EEG data were processed using MATLAB (R2015b), EEGLab (13.5.4b), and ERPLAB (6.0) toolbox plug-ins.

accuracy from both the congruent and incongruent trials was normalized using Blom formula[33] because it showed skewed distribution. Interaction analyses were performed between sex and physical fitness, PA and sedentary time on the outcomes. No significant interactions between sex and the predictors on the outcomes were found ($P > 0.10$), hence analyses were performed for the whole sample. To examine the associations of physical fitness, PA and sedentary time with inhibitory control performance (i.e., RT and response accuracy) and neuroelectric activity (i.e., P3 amplitude and P3 latency), linear hierarchical regression analyses were performed. Potential confounders (i.e., sex, PHV, BMI, wave, parental educational level, and IQ) were included into step 1 of a stepwise method to test their association to the outcomes and, subsequently, each physical fitness, PA and sedentary time variable was entered individually into step 2, in separate regression analyses. Additionally, we computed the median for physical fitness, PA and sedentary time variables to visually represent their relationship with P3 amplitude and latency. An exploratory analysis was performed to test the associations when lower-limb strength was expressed in absolute terms. We corrected for multiple comparisons by defining statistical significance as a Benjamini-Hochberg false discovery rate $q < 0.05$.[34] All the statistical procedures were performed using SPSS software for Mac (version 22.0, IBM Corporation).

RESULTS

The descriptive characteristics for the study sample are presented in **Table 1**. No associations were observed for muscular strength variables with any of the behavioral (**Table 2**) or neuroelectric (**Table 3, Figure 3**) outcomes (*P's* > 0.05). Higher speed-agility was associated with shorter RT in both the congruent ($\beta = -0.276$) and incongruent ($\beta = -0.305$) conditions (*P's* ≤ 0.009; **Table 2**). Higher speed-agility was also associated with larger P3 amplitude only for incongruent trials ($\beta = 0.299$, $P < 0.01$; **Table 3, Figure 3**). Higher cardiorespiratory fitness was associated with shorter RT ($\beta = -0.278$, $P = 0.010$; **Table 2**) and larger P3 amplitude, only for incongruent trials ($\beta = 0.303$, $P < 0.01$; **Table 3**, **Figure 3**).

No associations were observed for light PA, and sedentary time with any of the behavioral (**Table 2**) or neuroelectric (**Table 3**, **Figure 4**), outcomes (*P's* > 0.05), regardless of the location of the accelerometer (**Table 4 and Table 5**). Higher moderate PA and MVPA were associated with longer P3 latency in both the congruent and incongruent trials (β ranging from 0.309 to 0.327, all $P \leq 0.004$), only for hip-worn PA data (**Table 3, Figure 3**). Higher vigorous PA was associated with larger P3 amplitude in the incongruent condition ($\beta = 0.274$, $P = 0.010$), only for hip-worn PA data (**Table 3, Figure 3**).

DISCUSSION

To the best of our knowledge, this is the first study to comprehensively assess the relationship of various components of physical fitness as well as objectively measured PA and sedentary time with behavioral and neuroelectric indices of inhibitory control. The findings replicate prior research with cardiorespiratory fitness, and extended the field by revealing a relationship of speed-agility with behavioral and neuroelectric outcomes of inhibitory control. However, such an association was not observed for muscular strength, suggesting selectivity in the relationship of the various components of fitness with cognition in children with overweight/obesity. In addition, sedentary time was not associated with inhibitory control outcomes,

Table 1. Descriptive characteristics of the study sample.

	All (n=84)	Boys (n=47)	Girls (n=37)
Sociodemographic characteristics			
Age (years)	10.1 ± 1.1	10.2 ± 1.1	9.9 ± 1.1
Peak height velocity (years)	−2.2 ± 1.0	−2.6 ± 0.7	−1.7 ± 1.0
Weight (kg)	56.1 ± 10.4	56.5 ± 10.0	55.7 ± 11.0
Height (cm)	144.3 ± 8.0	144.9 ± 7.0	143.7 ± 9.1
Body mass index (kg/m^2)	26.8 ± 3.5	26.8 ± 3.7	26.8 ± 3.4
Intelligence quotient (total score)	99.3 ± 11.7	98.1 ± 11.3	100.8 ± 12.2
Wave of participation 1/2/3 (%)	17.9/36.9/45.2	12.8/44.7/42.6	24.3/27.0/48.6
Parental university level (%)			
None of them	66.7	72.3	59.5
One of them	16.7	12.8	21.6
Both of them	16.7	14.9	18.9
Physical fitness components			
Upper-limb absolute strength (kg)	16.7 ± 3.6	17.5 ± 3.8	15.7 ± 3.1
Lower-limb relative strength (cm)	106.2 ± 18.3	109.5 ± 16.4	102.1 ± 19.9
Speed-agility (sec)*	15.0 ± 1.5	14.6 ± 1.5	15.5 ± 1.5
Cardiorespiratory fitness (laps)	16.1 ± 7.8	17.7 ± 7.9	14.2 ± 7.2
PA and sedentary time (min/day)†			
Light PA	64.9 ± 15.4	66.4 ± 15.0	62.9 ± 15.9
Moderate PA	32.6 ± 13.5	37.2 ± 14.6	26.7 ± 9.2
Vigorous PA	3.0 ± 2.0	3.7 ± 2.1	2.1 ± 1.4
MVPA	35.6 ± 14.9	40.9 ± 16.0	28.9 ± 10.0
Sedentary time	810.0 ± 59.5	799.2 ± 62.1	823.8 ± 53.6
Inhibitory control task			
Congruent			
Mean reaction time (ms)*	801.3 ± 119.5	801.6 ± 130.2	800.9 ± 106.2
Response accuracy (%)	94.5 ± 5.8	95.2 ± 4.9	93.5 ± 6.7
Incongruent			
Mean reaction time (ms)*	845.7 ± 132.2	844.2 ± 137.8	847.7 ± 126.7
Response accuracy (%)	90.5 ± 10.9	89.8 ± 12.3	91.5 ± 8.7
Neuroelectric measurements			
Congruent			
P3 amplitude (μV)	9.2 ± 4.5	9.2 ± 4.2	9.2 ± 4.9
P3 latency (ms)*	433.4 ± 52.1	441.1 ± 54.8	423.6 ± 47.3
Incongruent			
P3 amplitude (μV)	9.3 ± 4.7	9.6 ± 4.4	8.8 ± 5.1
P3 latency (ms)*	436.3 ± 53.0	441.7 ± 51.7	429.4 ± 54.6

PA=Physical activity; MVPA=Moderate-to-Vigorous Physical Activity. Values are expressed as mean ± standard deviation, unless otherwise indicated. *Lower values indicate better performance. †PA and sedentary time variables were obtained from the hip. Upper-limb absolute strength was measured by the handgrip strength test. Lower-limb relative strength was measured by the standing long jump test. Speed-agility was measured by the 4×10-meter shuttle run test. Cardiorespiratory fitness was measured by a 20-meter shuttle run test. Mean ± standard deviations values for neuroelectric measurements reflect the mean amplitude across a 9-electrode site region of interest (P1/Z/2, PO3/Z/4, O1/Z/2).

whereas associations for PA were observed using hip–worn accelerometer data. Collectively, speed-agility and cardiorespiratory fitness, and somehow PA, but not muscular strength and sedentary time, are related to inhibitory control and underlying brain activity.

The present study shows that higher speed-agility and cardiorespiratory fitness were associated with better inhibitory control during a modified flanker task. The associations observed for speed-agility are consonant with previous findings showing a relationship between other complex motor skills (e.g., jumping, sprinting, coordinating) and inhibitory control in children with normal weight.[35] With regards to cardiorespiratory fitness, the findings of the present study are in accordance with those of the only study including children with overweight/obesity and a flanker task.[11] In that study, higher cardiorespiratory

Table 2. Hierarchical regressions for the association of physical fitness, physical activity (PA), and sedentary time (hip) with inhibitory control for congruent and incongruent trials (n=84).

	Congruent (lower cognitive demand)								Incongruent (higher cognitive demand)							
	Mean reaction time (ms)				Response accuracy (%)*				Mean reaction time (ms)				Response accuracy (%)*			
	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*
Physical fitness																
Upper-limb absolute strength (kg)	0.120	0.014	−0.128	0.271	0.256	0.003	−0.076	0.560	0.024	0.024	−0.155	0.160	0.232	0.003	0.057	0.612
Lower-limb relative strength (cm)	0.145	0.038	−0.199	0.064	0.255	0.002	−0.053	0.660	0.066	0.066	−0.256	0.019	0.230	0.000	0.015	0.895
Speed-agility (sec)†	0.180	0.073	−0.276	**0.009**	0.288	0.035	0.255	0.053	0.093	0.093	−0.305	**0.005**	0.242	0.012	0.131	0.266
CRF (laps)	0.155	0.048	−0.223	0.036	0.254	0.001	0.039	0.772	0.077	0.077	−0.278	**0.010**	0.258	0.029	0.208	0.086
PA and sedentary time (min/day)																
Light PA	0.107	0.000	0.003	0.979	0.291	0.039	−0.204	0.043	0.008	0.008	0.091	0.410	0.244	0.014	−0.125	0.226
Moderate PA	0.112	0.005	0.077	0.484	0.270	0.017	−0.146	0.183	0.038	0.038	0.194	0.076	0.238	0.009	−0.101	0.344
Vigorous PA	0.120	0.013	−0.117	0.279	0.253	0.001	0.029	0.801	0.004	0.004	−0.059	0.592	0.231	0.002	−0.043	0.691
MVPA	0.110	0.003	0.054	0.623	0.266	0.014	−0.133	0.233	0.028	0.028	0.169	0.125	0.238	0.008	−0.098	0.360
Sedentary time	0.108	0.001	−0.033	0.758	0.257	0.004	0.067	0.528	0.009	0.009	−0.094	0.396	0.230	0.001	0.035	0.758

CRF = Cardiorespiratory fitness; MVPA=Moderate-to-vigorous physical activity. Bold font is used to highlight significant associations after adjustment for multiple comparisons using the Benjamini and Hochberg method ($q<0.05$). β values are standardized regression coefficients. *Normalized values were used in the analysis. †This variable was inverted so that higher values indicate better performance. Each physical fitness and PA/sedentary time variable was introduced in individual hierarchical regression models. Potential confounders (i.e., sex, peak height velocity, body mass index, wave of participation, parental educational level and intelligence quotient) were included into step 1 of the stepwise regression to test their association to the outcomes. Hierarchical regression models for physical fitness, PA and sedentary time with congruent condition parameters: for mean reaction time, peak height velocity, and intelligence quotient were included as confounders; for response accuracy, sex, peak height velocity, body mass index, and intelligence quotient were included as confounders. Hierarchical regression models for physical fitness, PA and sedentary time with incongruent trial parameters: for mean reaction time, no confounders were included; for response accuracy, peak height velocity, wave, body mass index, and intelligence quotient were included as confounders.

Table 3. Hierarchical regressions for the association of physical fitness, physical activity (PA), and sedentary time (hip) with P3 amplitude and latency for congruent and incongruent trials (n=84).

	P3 amplitude							
	Congruent (lower cognitive demand)				Incongruent (higher cognitive demand)			
	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*
Physical fitness								
Upper-limb absolute strength (kg)	0.060	0.014	−0.123	0.270	0.065	0.002	−0.051	0.649
Lower-limb relative strength (cm)	0.056	0.010	0.098	0.367	0.125	0.062	0.249	0.019
Speed-agility (sec)†	0.076	0.030	0.175	0.106	0.151	0.088	0.299	**0.005**
Cardiorespiratory fitness (laps)	0.074	0.028	0.169	0.119	0.154	0.092	0.303	**0.004**
PA and sedentary time (min/day)								
Light PA	0.063	0.017	0.129	0.235	0.067	0.004	0.065	0.547
Moderate PA	0.046	0.000	0.000	0.996	0.063	0.001	0.026	0.808
Vigorous PA	0.083	0.037	0.195	0.073	0.137	0.074	0.274	**0.010**
MVPA	0.047	0.001	0.025	0.820	0.066	0.004	0.059	0.582
Sedentary time	0.048	0.002	−0.046	0.675	0.081	0.019	−0.138	0.201
	P3 latency							
	Congruent (lower cognitive demand)				Incongruent (higher cognitive demand)			
	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*
Physical fitness								
Upper-limb absolute strength (kg)	0.013	0.013	0.116	0.294	0.007	0.007	0.082	0.459
Lower-limb relative strength (cm)	0.000	0.000	−0.006	0.954	0.010	0.010	−0.098	0.376
Speed-agility (sec)*	0.004	0.004	−0.064	0.560	0.017	0.017	−0.131	0.236
Cardiorespiratory fitness (laps)	0.008	0.008	−0.089	0.421	0.041	0.041	−0.202	0.066
PA and sedentary time (min/day)								
Light PA	0.009	0.009	0.095	0.389	0.015	0.015	0.122	0.268
Moderate PA	0.105	0.105	0.325	**0.003**	0.107	0.107	0.327	**0.002**
Vigorous PA	0.013	0.013	0.112	0.310	0.014	0.014	0.120	0.278
MVPA	0.096	0.096	0.309	**0.004**	0.098	0.098	0.312	**0.004**
Sedentary time	0.051	0.051	−0.226	0.038	0.015	0.015	−0.122	0.269

MVPA=Moderate-to-vigorous physical activity. β values are standardized regression coefficients. *This variable was inverted so that higher values indicate better performance. Bold font is used to highlight significant associations after adjustment for multiple comparisons using the Benjamini and Hochberg method ($q < 0.05$). Each predictor variable was introduced in individual hierarchical regression models. Potential confounders (i.e., sex, peak height velocity, body mass index, wave of participation, parental educational level and intelligence quotient) were included into step 1 of the stepwise regression to test their association to the outcomes. Hierarchical regression models for physical fitness, PA and sedentary time with P3 amplitude in both the congruent and incongruent trials were adjusted by intelligence quotient. Hierarchical regression models for physical fitness, PA and sedentary time with P3 latency in both the congruent and incongruent trials were not adjusted by any confounders. P3 amplitude and latency were computed from parieto-occipital region electrodes (i.e., P1, PZ, P2, PO3, POz, PO4, O1, Oz, O2).

fitness was associated with shorter mean RT.[11] Other investigations in children with normal weight reported similar results.[36,37] Future interventions should focus on studying the role of different physical fitness components in inhibitory control in children across the entire range of the BMI distribution.

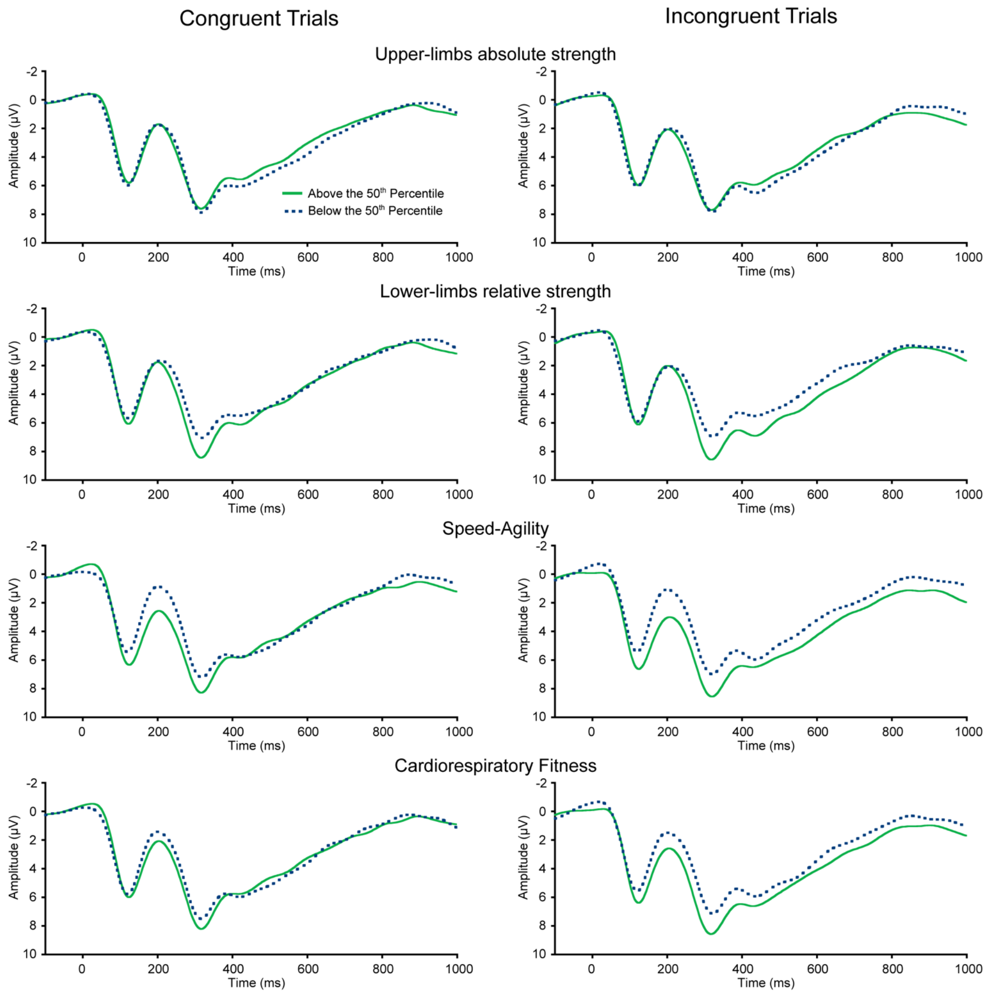

Figure 3. Median split waveforms time-locked to the onset of the choice stimulus for each domain of physical fitness.

Previous studies have focused on children with normal weight when studying the relationship between physical fitness and neuroelectric activity.[16–18] Overall, the findings of the present study support this body of research with cardiorespiratory fitness showing that higher-fit children exhibited larger P3 amplitude than their lower-fit peers during a flanker task.[16–18] Further, the non-association observed between cardiorespiratory fitness and P3 latency concurs with a recent study also showing null association for P3 latency in children.[18] Collectively, the associations found in the present study were generally observed in the incongruent condition, indicating that higher-fit children were more effective in allocating attentional resources and that the fitness (i.e., cardiorespiratory fitness and speed-agility) relationship may be selective for flanker conditions that engender greater amounts of inhibitory control, as has been previously reported.[17] Thus, the P3 may serve to reflect alterations in proactive cognitive strategies (i.e., early selection, shorter RT) in order to achieve an optimal completion of the task goal via adjustments in attentional engagement.[17] Although speculative, given the increased metabolic demands

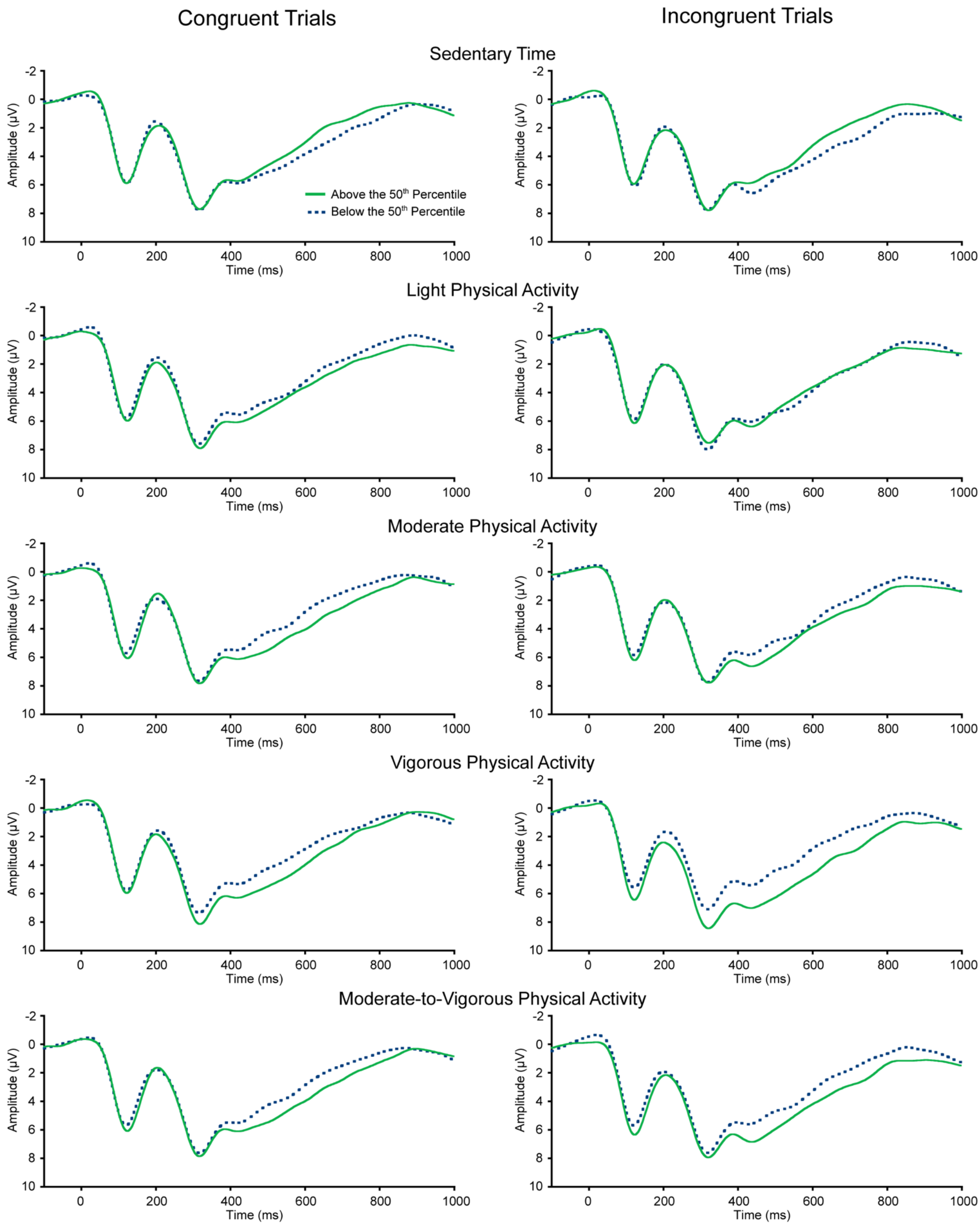

Figure 4. Median split waveforms time-locked to the onset of the choice stimulus for the relation of physical activity and sedentary time.

required for these proactive processes,[38] higher fitness level may favor the generation of necessary neuroelectric resources to engage proactive control mechanisms and therefore exhibit larger P3 amplitude.

Overall, observational studies in children covering the relationship of PA and sedentary time with inhibition have reported inconsistent findings.[10] For example, a prior study found seemingly different results in that higher MVPA was associated with shorter RT during a task involving attention but

Table 4. Hierarchical regressions for the association of sedentary time and physical activity (PA) non-dominant wrist) with inhibitory control for congruent and incongruent trials (n=84).

	Congruent (lower cognitive demand)								Incongruent (higher cognitive demand)							
		Mean reaction time (ms)				Response accuracy (%)*				Mean reaction time (ms)				Response accuracy (%)*		
	R^2	R^2 change	β	P	R^2	R^2 change	β	P	R^2	R^2 change	β	P	R^2	R^2 change	β	P
Light PA	0.107	0.000	0.022	0.839	0.263	0.010	−0.105	0.300	0.005	0.005	0.069	0.534	0.236	0.007	−0.085	0.418
Moderate PA	0.107	0.000	−0.013	0.912	0.260	0.008	−0.104	0.368	0.012	0.012	0.108	0.329	0.229	0.000	0.006	0.960
Vigorous PA	0.111	0.004	0.069	0.542	0.265	0.012	−0.133	0.253	0.007	0.007	0.085	0.443	0.231	0.002	−0.045	0.681
MVPA	0.107	0.000	0.003	0.976	0.262	0.010	−0.117	0.319	0.012	0.012	0.108	0.329	0.229	0.000	−0.005	0.966
Sedentary time	0.108	0.001	−0.036	0.741	0.258	0.005	0.077	0.466	0.008	0.008	−0.087	0.429	0.230	0.000	−0.017	0.877

MVPA=Moderate-to-vigorous physical activity. β values are standardized regression coefficients. *Normalized values were used in the analysis. Each PA/sedentary time/ variable was introduced in individual hierarchical regression models. Potential confounders (i.e., sex, peak height velocity, body mass index, wave of participation, parental educational level and intelligence quotient) were included into step 1 of the stepwise regression to test their association to the outcomes. Hierarchical regression models for PA and sedentary time with congruent trial parameters: for mean reaction time, peak height velocity, and intelligence quotient were included as confounders; for response accuracy, sex, peak height velocity, body mass index, and intelligence quotient were included as confounders. Hierarchical regression models for PA and sedentary time with incongruent trial parameters: for mean reaction time, no confounders were included; for response accuracy, peak height velocity, wave, body mass index, and intelligence quotient were included as confounders.

children who spent more time being sedentary achieved better scores on a sustained attention test.[13] In contrast, the non-significant associations found in the present study between PA and inhibitory control are in accordance with two recent studies, which also used modified flanker tasks.[14,15] In these studies, moderate PA, vigorous PA, and MVPA objectively measured from the waist were not associated with either mean RT or response accuracy in any of the task conditions in children.[14,15] It may be that PA is rarely intensive in children with overweight/obesity and of insufficient duration to enhance physical fitness and, in turn, inhibition. However, this hypothesis must be confirmed in future intervention studies in children with overweight/obesity. Another explanation for the null associations between PA and inhibitory control might be the fact that PA was measured only during one week and the PA behavioral pattern of the participants during that week may not be representative of their total PA. Despite the controversial findings, emergent evidence from randomized controlled trials have suggested a positive effect of PA interventions on executive function in children.[10,39] Thus, further research is needed to gain insights into the intensity-response effect of PA on cognition, specifically in the context of overweight/obesity during childhood.

Importantly, only one study previously investigated ERPs to gain a better understanding of the role of PA in

Table 5. Hierarchical regressions for the association of physical activity (PA) and sedentary time (wrist) with P3 amplitude and latency for congruent and incongruent trials (n=84).

	P3 amplitude							
	Congruent (lower cognitive demand)				Incongruent (higher cognitive demand)			
	R^2	R^2 change	β	*P*	R^2	R^2 change	β	P
Light PA	0.047	0.001	0.027	0.803	0.066	0.003	−0.059	0.588
Moderate PA	0.049	0.003	0.057	0.601	0.067	0.005	0.069	0.522
Vigorous PA	0.046	0.000	0.018	0.868	0.069	0.007	0.084	0.443
MVPA	0.049	0.003	0.052	0.635	0.068	0.006	0.075	0.489
Sedentary time	0.047	0.001	−0.031	0.776	0.074	0.011	−0.106	0.324
	P3 latency							
	Congruent (lower cognitive demand)				Incongruent (higher cognitive demand)			
	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*
Light PA	0.002	0.002	−0.040	0.719	0.016	0.016	−0.125	0.258
Moderate PA	0.040	0.040	0.200	0.068	0.028	0.028	0.169	0.125
Vigorous PA	0.032	0.032	0.178	0.105	0.032	0.032	0.178	0.105
MVPA	0.042	0.042	0.204	0.062	0.031	0.031	0.177	0.107
Sedentary time	0.030	0.030	−0.174	0.114	0.001	0.001	−0.037	0.737

MVPA=Moderate-to-vigorous physical activity. β values are standardized regression coefficients. Each PA/sedentary time variable was introduced in individual hierarchical regression models. Potential confounders (i.e., sex, peak height velocity, body mass index, wave of participation, parental educational level and intelligence quotient) were included into step 1 of the stepwise regression to test their association to the outcomes. Hierarchical regression models for PA and sedentary time with P3 amplitude in both the congruent and incongruent trials were adjusted by intelligence quotient. Hierarchical regression models for PA and sedentary time with P3 latency in both the congruent and incongruent trials were not adjusted by any confounders. P3 amplitude and latency were computed from parieto-occipital region electrodes average (i.e., P1, PZ, P2, PO3, POz, PO4, O1, Oz, O2).

underlying processes of inhibitory control.[15] In that study, although a significant pattern was only found for the relationship between bouts of vigorous PA and P3 latency, this association disappeared upon correction for multiple comparisons. In contrast, the present study found that only higher amounts of vigorous PA were associated with larger P3 amplitude even after multiple comparisons correction. We also found that higher moderate PA and MVPA were associated with longer P3 latency; i.e., children with higher levels of moderate PA and MVPA demonstrated slower cognitive processing speed. However, these findings were obtained only when using PA data from the hip accelerometer-location, but not with the wrist-based monitor. Notably, different methodological considerations (i.e., accelerometer-location, cut-points, etc.) may influence analyses between sedentary time, PA and various health outcomes, which may account, in part, for controversies between studies.[40] Therefore, the inconsistent evidenced across studies investigating the association of PA and sedentary time with the neuroelectric system in children using different methodological aspects (i.e., accelerometer-location, cut-points, etc.) to process accelerometer data should be considered when formulating such comparisons in the literature. Furthermore, although there is still not clear evidence on which accelerometer location better register PA levels, the present study shows that, at least from a cognitive-neuroelectric perspective, PA data from hip accelerometer-location is more strongly related to inhibitory control and neuroelectric indices than the wrist-based PA data. Further studies must confirm or contrast the present results.

With respect to sedentary time, no significant associations were observed with any of the behavioral or neuroelectric outcomes. Other studies have reported that only select sedentary behaviors (e.g., watching TV, playing videogames), but not total sedentary time may be associated to cognition.[41] However, little information is available in children on the relationship for sedentary variables with neuroelectric activity underlying cognitive processes. Thus, it seems important for further studies to determine how different sedentary behaviors (i.e., screen-time, reading) could influence inhibition and neuroelectric activity in children.

The main limitation of the present study is the cross-sectional design, which does not allow us to draw causal interpretations. Furthermore, there are some physical activities (e.g., bicycling, swimming) that cannot be well-measured via accelerometry, and further such measurement cannot distinguish between types of sedentary behaviors. The main strength of this study was that, to the best of our knowledge, it is the first to investigate the relationship between different physical fitness components, objectively-measured sedentary time, and PA intensities with inhibitory control and neuroelectric outcomes in a sample of children with overweight/obesity. Other strengths include the objective and standardized assessments of all variables as well as the use of data from different accelerometer locations (i.e., hip and non-dominant wrist).

CONCLUSION

Our results provide initial evidence to suggest that not only cardiorespiratory fitness, but also speed-agility, are associated with inhibitory control and P3 amplitude in children with overweight/obesity. There were no significant associations between upper- and lower-limbs measurements of muscular strength, PA, or sedentary time and inhibitory control. The associations observed for vigorous PA with P3 amplitude, and for moderate PA with P3 latency were accelerometer location-dependent, since they disappeared when considering the findings from the non-dominant wrist-based measure. Since physical fitness components may be differentially associated with inhibitory control and the neuroelectric activity subserving this aspect of cognition, promoting PA programs that enhances speed-agility and cardiorespiratory fitness, may be important for better understanding whether such health improvements benefit inhibitory control in youth with overweight/obesity.

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Study 4

Physical fitness and brain source analysis during a working memory task in children with overweight/obesity

BACKGROUND

There is emerging evidence indicating the potential benefits of physical fitness on cognitive and brain health.[1] Likewise, substantial research also suggests that being more fit is beneficial to the harmful effects of obesity such as metabolic syndrome or hypertension.[2,3] Given the high prevalence of childhood obesity and in a context where being obese may also negatively influence the structure and function of several brain regions,[4,5] physical fitness emerges as a factor that may counteract this negative influence. Specifically, when studying the role of physical fitness in brain in youth, its different components (i.e., cardiorespiratory fitness, speed-agility and muscular fitness) may have differential effects.[3,6] Therefore, when investigating physical fitness and brain health in children, all of the fitness components should be considered.

Improving physical fitness could be of help for the development of brain and executive function domains related to learning and academic achievement, such as working memory.[1,7] Working memory refers to a brain system that provides temporary storage and manipulation of the information necessary for such complex cognitive tasks as language comprehension, learning, and reasoning.[8] It is commonly divided into three phases: encoding, maintenance, and retrieval. Overall, previous studies have shown that cardiorespiratory fitness and muscular fitness are related to better working memory performance in children with normal-weight.[9,10] To date, only a recent study carried out in the same sample as in the present study (i.e., children with overweight/obesity) used electroencephalography (EEG), and more specifically, an event-related brain potential (ERP) approach, to study the relationship between physical fitness components and neuroelectric indices of working memory.[11] Findings from this study showed that higher levels of cardiorespiratory fitness and speed-agility were associated with higher amplitude (i.e., increased attentional capacity) and shorter latency (i.e., faster processing speed) of the P3 component during a working memory task, therefore reflecting better retrieval.[11] However, the specific role of physical fitness components in other working memory processes assessed during an EEG measurement, such as encoding or maintenance, as well as on the activation of specific brain areas during these working memory processes have not yet been studied in children.

Both EEG and functional magnetic resonance imaging (fMRI) are methods used to explore aspects of the brain's functional system that underlie cognitive and mental functions.[12,13] Overall, studies of physical fitness and EEG during cognitive tasks have mainly used ERP, which provide a time-sensitive record of the neuroelectric response during cognitive engagement.[14] Although ERPs are temporally quite sensitive, a major limitation of this approach is the lack of spatial resolution, as they are unable to calculate the three-dimensional spatial origin of the brain current source density.[15] In contrast, fMRI can provide comparatively precise spatial resolution, although this measure lacks the high temporal resolution observed with EEG/ERPs. In fact, fMRI is inherently slow (i.e., on the scale of seconds) and the low temporal resolution of this measurement may mask temporally separated activations into a single, more spread activity.[16] In an effort to temper these limitations, brain source analysis emerges as an innovative and validated analysis (e.g., standardized low-resolution brain electromagnetic tomography, sLORETA) derived from EEG recordings to provide both the localization of three-dimensional brain current source density in specific areas as well as high temporal resolution.[17,18] In sum, this analysis provides an estimation of the scalp current source density, which has been shown to provide a much richer, and more accurate, view of the spatial-temporal dynamics of brain activity.[16] For instance, while the scalp potential spatial resolution is usually considered to be around 6–9 cm,[19] current source density estimation allows to reach a spatial resolution of 2–3 cm, close to the size of brain areas. This approach has not been previously addressed under a sport sciences perspective and may therefore enhance the understanding of the relationship between the various physical fitness components and brain function during working memory operations.

Accordingly, there remains a clear need for examining the association of the various physical fitness components on spatial-temporal current source density of specific brain areas while performing working memory operations (i.e., encoding, maintenance, and retrieval) in children with overweight/obesity. Therefore, this study aimed to: (i) investigate the association of the various physical fitness components (i.e., cardiorespiratory fitness, speed-agility, and muscular fitness) with brain current source density during a working memory task; and (ii) to examine whether fitness-related associations in current source density are related to working memory performance and academic achievement in a sample of children with overweight/obesity. Although the evidence on fitness components and current source density using brain source analysis during cognitive tasks is nonexistent, we examined previous research using other neuroimaging approaches (e.g., ERPs)[1] to formulate our hypothesis that the various physical fitness components would be positively associated with changes in regional current source density underlying a working memory task. Further, we infer that fitness-related current source density would be related to working memory performance and academic achievement.

METHODS

Participants

The present study takes place under the umbrella of the ActiveBrains project (http://profith.ugr.es/activebrains). The complete methodology, procedures and inclusion/exclusion criteria have been described elsewhere.[20] An initial sample of 110 Spanish children aged 8-11 years were recruited from Granada, Spain. For the present cross-sectional analysis, data prior to randomization were used and a final sample of 85 children with overweight/obesity (10.1 ± 1.1 years old; 62% boys) were included with complete baseline data for physical fitness components, brain current density, working memory task performance and academic achievement. The baseline data collection occurred from November 2014 to February 2016 in three waves.

Description and characteristics of the study were given to parents or legal guardians and a written informed consent was obtained from both guardian and child. The ActiveBrains project was approved by the Ethics Committee on Human Research of the University of Granada, and was registered in ClinicalTrials.gov (identifier: NCT02295072).

Physical fitness components

The different components of physical fitness (i.e., cardiorespiratory fitness, speed-agility and muscular fitness) were assessed following the ALPHA (Assessing Levels of Physical fitness and Health in Adolescents) health-related physical fitness test battery for children and adolescents which is valid and reliable.[21,22] Details of the battery have been described elsewhere.[23] In brief, cardiorespiratory fitness was assessed by the 20-meter shuttle-run test (20-m SRT).[24] The test was performed once at the end of the fitness testing session. The total number of completed laps was registered.

Speed-agility was assessed using the 4 × 10-meter shuttle-run test (4 × 10-m SRT). The test was performed twice and the fastest time was recorded in seconds. In this test, a longer time indicates poorer performance, so this variable was inverted by multiplying test completion time (sec) by −1, so that higher scores indicated better performance.

Muscular fitness was assessed using maximum handgrip strength and the standing long jump tests. A digital hand dynamometer with an adjustable grip (TKK 5101 Grip D, Takei, Tokyo, Japan) was used to assess the handgrip strength. Each child performed the test twice, and the maximum score of each hand was obtained and averaged, and subsequently divided by body weight (kg) to obtain a relative-to-body weight measurement of upper body muscular fitness. The standing long jump test was performed three times. Children jumped as far forward as possible, and the longest distance was recorded in centimeters (cm). The individual score of each test was standardized as follows: z-standardized value = (value – the sample mean) / standard deviation (SD). A muscular fitness score was then calculated as the mean of the two standardized scores. For exploratory analyses, we also computed an absolute measurement of muscular fitness from standardized scores of upper (kg) and lower muscular fitness (cm × kg).

EEG register during working memory task

EEG recording

Neuroelectric activity was recorded using a 64-channel Active Two BioSemi EEG system (24-bit resolution, BioSemi; Amsterdam, Holland) arranged in an extended montage based on the International 10–10 system.[25] Data were digitized at a rate of 1024 Hz and a 100-Hz low-pass filter. Details of the online BioSemi reference method can be found at http://www.biosemi.com/faq/cms&drl.htm. EEG data were pre-processed using EEGLab version 13.5.4b,[26] and ERPLAB toolbox version 6.0,[27] which consisted of: (i) down-sampling to 256Hz, (ii) band-pass filtering between 0.1 and 30Hz (24 dB/octave), (iii) re-referenced to all electrodes average, (iv) application of artifact subspace reconstruction (ASR) to eliminate artifact channels, (v) removing eye-blink-related components by using an independent component analysis (ICA) with Multiple Artifact Rejection Algorithm (MARA),[26,28] and (vi) interpolation of the previously deleted channels by ASR. After the pre-processing, behavioral data were merged with the EEG data. Epochs were created from −10,000 to 2,000 ms surrounding the onset of the choice trial. Trials with artifact exceeding ±100 µV were rejected. For the present study, the overall amplitude (µV) of the neuroelectric activity was collected.

Brain source analysis (sLORETA)

sLORETA is a method for localizing the neuroelectric activity in the brain based on scalp potentials from multiple-channel EEG recordings and can be understood as an EEG-based neuro-imaging technique.[18] This technique is used to estimate the brain areas that potentially underlie the observed brain current source density.[18] For each participant, the overall averaged amplitude (μV) for each working memory load (i.e., low and high) was submitted to the sLORETA transform to estimate the time course and brain areas of current density for each of the 6,239 voxels of the sLORETA brain map. sLORETA is based on standardized EEG recordings, which are modeled onto a probabilistic head model provided by the Montreal Neurological Institute (MNI). Thus, active brain areas were then identified by allocating the raw sLORETA values of individual voxels to their corresponding Brodmann areas (BAs) on the basis of the coordinates of the MNI. sLORETA images represent the electrical activity at each voxel as the squared standardized magnitude of the estimated current source density ($\mu A/mm^2$). A dependent variable that indicates the difference in current source density between high and low working memory loads (hereafter referred to as H – L current density difference) was obtained. We focused on the H – L difference since working memory load has been shown to modulate the brain activation dynamics serving working memory,[29] as well as physical fitness has been associated with differences in the flexible modulation of executive function depending on the task demands.[30] Thus, larger H – L differences describe higher neuroelectric activation during the high memory load compared with the low load.

Working memory task

All participants completed a modified version of the Delayed Non-Match-to-Sample computerized task to assess working memory.[31] A trial consisted of three phases: Stimuli presentation (encoding), pre-target awaiting (maintenance), and target choice (retrieval) (**Figure 1**). The encoding phase (i.e., 5,000 ms) included a memory set of four sequential stimuli, which participants were later asked to recall. After the fourth stimulus, a maintenance phase was undertaken for 4,000 ms. Finally, a target consisting of two different Pokemon cartoons was presented during the retrieval phase (i.e., 1,800 ms) and participants were asked to select the cartoon that had not been shown in the four previous stimuli that comprised the memory set. A total of 16 practice trials plus 140 experimental trials were presented. There were two experimental conditions, for the high working memory load (i.e., 100 trials) four different stimuli were presented during the encoding phase, whereas for the low working memory load (i.e., 40 trials) the four stimuli were identical. The associations between physical fitness components and working memory performance (i.e., response accuracy and reaction time) can be found elsewhere.[11] For the present study, response accuracy (%) in the high working memory load was used as an indicator of working memory performance in the analyses.

Academic achievement

The total standardized achievement score obtained from the Spanish version of the validated Woodcock-Johnson III test battery[32] was used as a measurement of academic achievement. This score is based on sub-scores of reading, mathematics and writing. The battery was individually administered by a trained evaluator in a 100 to 120 min session. Data from the Woodcock-Johnson III test battery were processed in the Compuscore and profile software version 3.1 (Riverside Publishing Company, Itasca, IL, USA).

Potential confounders

Sex, age, peak height velocity, fat mass index, intelligence quotient, wave of participation, and parental educational level were used as potential confounders in the analyses. Peak height velocity is a commonly used indicator of maturity in childhood and

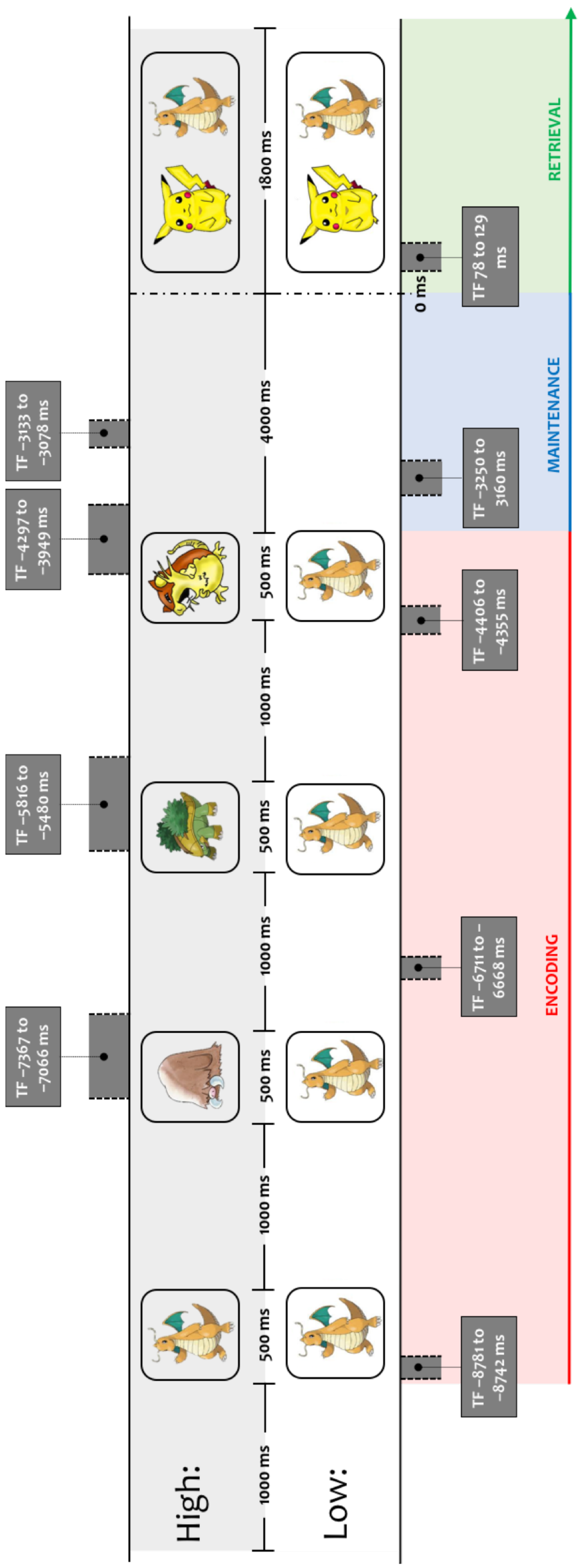

Figure 1. Example of high and low working memory trials and selected time frames with significant neuroelectric activity (i.e., overall amplitude) differences between high and low working loads across working memory phases (i.e., encoding, maintenance, retrieval). TF=Time Frame

adolescence, and we used age and anthropometric variables to calculate it following Moore's equations.[33] Fat mass was assessed by dual-energy x-ray absorptiometry (DXA, Discovery densitometer from Hologic) and then we calculate fat mass index (fat mass in kg / height in m^2) as an indicator of adiposity. The total composite intelligence quotient was assessed by the Spanish version of the valid and reliable Kaufman Brief Intelligence Test (K-BIT).[34] Wave of participation was a categorical variable according to the first moment of participation in the study (wave 1, 2, or 3). Parental educational level was assessed by a self-reported questionnaire completed by parents, and we combined responses of both parents as: Neither had a university degree; one had a university degree; or both had a university degree.[35]

Statistical analysis

Characteristics of the study sample are presented as means and SD or percentages for categorical variables. Interaction analyses were performed between sex and physical fitness on the outcome (i.e., H – L current density difference) and no significant interactions were found (all $P \geq 0.10$). Thus, analyses were performed for the whole sample.

Analyses for the present study are structured in four steps: First, overall amplitude epochs from high and low working memory loads were submitted to a nonparametric mass-univariate randomization *t* test in sLORETA.[36] Note that this test controls for multiple-comparisons by using the empirical single-threshold of the t-max statistic.[37] The time frames in which the H – L amplitude difference (μV) exceeded this t-max threshold were identified within the whole epoch window (i.e., –10,000 to 2,000 ms) and each of the three phases (**Figure 1**). Second, sLORETA estimations of brain current source densities in both high and low loads were also submitted to a mass-univariate *t* test (5,000 random samples) separately for each previously-identified significant time frame. The mass-univariate *t* test provided the number of voxels and their BAs' location exceeding the t-max threshold. Third, linear stepwise regression analyses were performed to examine the associations of each physical fitness component (i.e., cardiorespiratory, speed-agility and muscular fitness) with the H – L current density difference. Potential confounders were included in step 1 to test their association to each outcome. Subsequently, each physical fitness component was entered into step 2 in individual regression models. The analyses presented are those showing a significant association with at least one physical fitness component. Fourth, linear stepwise regressions were carried out to analyze the association of fitness-related H – L current density differences with working memory and academic achievement indicators. Potential confounders were included into step 1 and fitness-related H – L difference in each BA was individually entered into step 2 as predictor. Exploratory analyses were also performed to test the associations when muscular fitness was expressed in absolute terms (i.e., not accounting for body weight). A significance level of $P < 0.05$ was set and multiple comparisons correction in the regression models was performed using the Benjamini-Hochberg method ($q < 0.05$).[38] All the regression analyses were performed using SPSS software for Mac (version 20.0, IBM Corporation).

RESULTS

Brain current source density differences between high and low working memory loads

Table 1 shows the characteristics of the study sample. The mass-univariate *t* test yielded significant

Table 1. Descriptive characteristics of the study sample

	All (n=85)	Boys (n=53)	Girls (n=32)
Sociodemographic characteristics			
Age (years)	10.1 ± 1.1	10.2 ± 1.1	9.8 ± 1.1
Weight (kg)	56.9 ± 10.7	57.8 ± 10.2	55.3 ± 11.6
Height (cm)	144.8 ± 8.1	145.6 ± 7.6	143.6 ± 8.9
Body mass index (kg/m²)	26.9 ± 3.5	27.1 ± 3.6	26.5 ± 3.5
Peak height velocity offset (years)	−1.8 ± 0.9	−2.3 ± 0.7	−1.0 ± 0.7
Fat mass index (kg/m²)	11.7 ± 2.8	11.6 ± 2.8	12.0 ± 3.0
Intelligence quotient (standardized score)	100.6 ± 19.1	98.4 ± 18.9	104.3 ± 19.0
Wave of participation, (%)			
First	14	10	22
Second	40	45	31
Third	46	45	47
Parental university level, (%)			
None of them	65	72	53
One of them	22	17	31
Both of them	13	11	16
Physical fitness components			
Cardiorespiratory fitness (laps)*	16.4 ± 7.9	17.4 ± 8.2	14.8 ± 7.2
Speed-agility (sec)†	14.9 ± 1.5	14.8 ± 1.6	15.1 ± 1.5
Muscular fitness (z-score)‡	0.0 ± 0.9	0.0 ± 0.9	0.0 ± 0.9
High load working memory			
Response accuracy (high load)	67.4 ± 14.8	68.6 ± 15.8	65.4 ± 12.9
Academic achievement			
Total achievement standard score**	109.6 ± 11.0	108.5 ± 10.2	111.5 ± 12.2

Values are expressed as means ± standard deviations, or percentages. *Measured by the 20-meter shuttle run test. †Measured by the 4 x 10-m shuttle run test; values were multiplied by −1 before analyses so that higher values indicate better performance. ‡z-score computed from handgrip strength (kg/kg) and standing long jump (cm) tests. **Computed from the Woodcock Johnson III test battery

differences for the overall amplitude (µV) comparison between high and low working memory loads at 10 different time frames (t-max = 5.101, *P* < 0.001; **Figure 2** and **Table 2**). Therefore, **Figure 2** and **Table 2** depicts the sLORETA estimations of brain current densities at each of these time frames submitted to mass-univariate *t* test for each hemisphere (left, L and right, R) and for high and low working memory loads. The t-max for the H – L current density differences (µA/mm²) across selected time frames ranged from 3.138 to 3.407 (all *P* ≤ 0.002). In brief, in the encoding phase, the time frame from −7,367 to −7,066 ms (i.e., presentation of 2nd stimulus) showed the highest number of voxels presenting significant H – L current density differences (*P* < 0.001) in a total of 22 BAs (t-max = 3.293). During the maintenance phase, the time frame from −4,297 to −3,949 ms (i.e., right after the presentation of 4th stimulus) showed the highest number of voxels with significant differences in a total of 21 BAs (t-max = 3.367). For the retrieval phase, only the time frame from 78 to 129 ms showed significant H – L current density differences in a total of 27 BAs (t-max = 3.144, *P* < 0.001).

Association of physical fitness components with H – L current source density differences

After correcting for multiple comparisons, higher cardiorespiratory fitness was associated with higher current source density in the high load in comparison with the low load in a total of 4 BAs with β ranging from 0.283 to 0.297 (all *P* ≤ 0.009) during the encoding phase (**Table 3, Figure 3**). Of these 4 BAs, RBA10 included the superior/medial/middle frontal gyri and

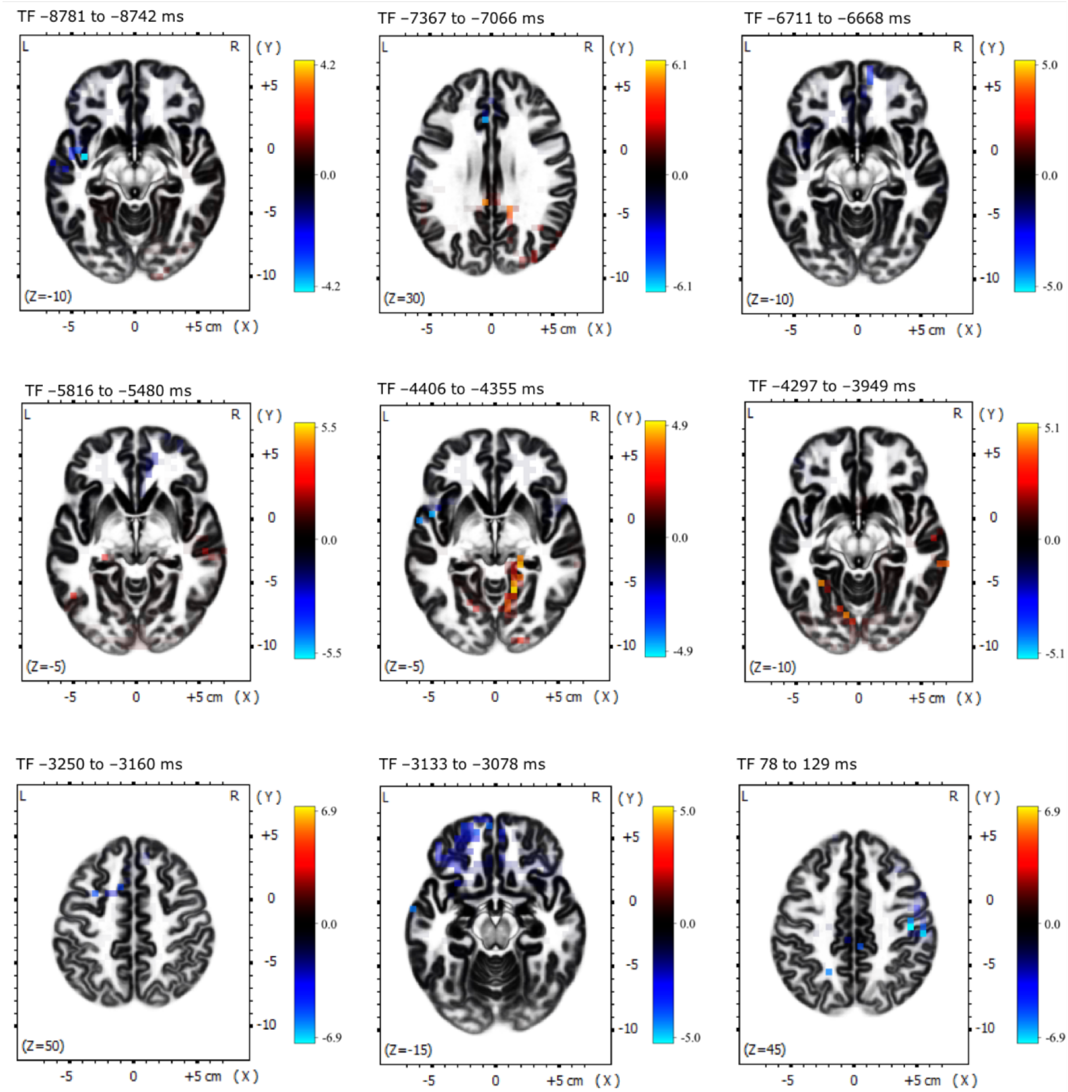

Figure 2. Brains' slices showing significant current source density differences between high working memory load and low working memory load by time frames (TF) (n=85). Multiple comparisons correction was performed using an approximation to the permutation analysis based on t-max statistic. t-max for −8781 to −8742 ms = 3.407 (P = 0.002); t-max for −7367 to −7066 ms = 3.293 ($P <$ 0.001); t-max for −6711 to −6668 ms = 3.138 ($P <$ 0.001); t-max for −5816 to −5480 ms = 3.305 ($P <$ 0.001); t-max for −4406 to −4355 ms = 3.313 ($P <$ 0.001); t-max for −4297 to −3949 ms = 3.367 ($P <$ 0.001); t-max for −3250 to −3160 ms = 3.019 ($P <$ 0.001); t-max for −3133 to −3078 ms = 3.181 ($P <$ 0.001); t-max for 78 to 129 ms = 3.144 ($P <$ 0.001).

the anterior cingulate gyrus (P = 0.009), and the association with cardiorespiratory fitness occurred between the 2st and 3rd stimuli. Other positive associations of cardiorespiratory fitness with H – L current density difference were observed in LBA30, including posterior cingulate and parahippocampal gyri (P = 0.006); and in RBA17 and RBA18, including cuneus, and middle occipital and lingual gyri (P = 0.006), and they all occurred during the presentation of the 4th stimuli previous to the target. Cardiorespiratory fitness was also positively associated with H – L current density difference in

Table 2. Number of voxels by time frames with significant current source density differences between high and low working memory loads (n=85).

BA / H	−8781 to −8742 ms		−7367 to −7066 ms		−6711 to −6668 ms		−5816 to −5480 ms		−4406 to −4355 ms		−4297 to −3949 ms		−3250 to −3160 ms		−3133 to −3078 ms		78 to 129 ms	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	27
46	-	-	-	-	-	1	-	-	3	-	-	-	1	-	-	-	-	8
45	-	-	-	-	-	-	-	-	2	-	-	-	2	-	2	2	-	4
44	2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	3	-	-
43	-	-	-	-	1	-	-	-	-	-	-	-	-	-	2	-	-	2
42	-	-	-	-	-	-	-	3	-	-	-	1	-	-	-	-	-	1
41	-	-	-	4	-	-	2	1	1	-	2	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	1	-	-	-	5	-	-	-	-	5	9
39	-	-	-	2	-	-	-	-	-	-	-	3	-	-	-	-	-	-
38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	9
37	-	-	-	2	-	-	3	-	-	3	1	-	-	-	-	-	18	-
36	-	-	-	3	-	-	-	-	-	2	-	-	-	-	-	-	5	6
35	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
32	-	-	3	-	-	1	-	2	-	-	2	-	5	-	-	-	4	1
31	-	-	4	8	-	-	1	3	-	-	-	1	-	-	-	-	6	1
30	-	-	-	-	-	-	-	-	4	3	-	2	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
27	-	-	1	-	-	-	2	-	-	5	-	-	-	-	-	-	-	-
24	-	-	1	-	-	-	1	4	-	-	1	-	1	-	-	-	-	-
23	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-
22	5	-	-	-	1	-	-	-	1	-	-	1	-	-	-	-	-	6
21	2	-	-	2	-	-	-	3	1	-	-	6	-	-	-	-	1	10
20	4	-	-	1	-	-	-	3	-	2	-	1	-	-	-	-	12	12
19	-	-	-	4	-	-	3	7	-	13	6	13	-	-	-	-	-	-
18	-	-	1	11	-	-	-	1	1	7	7	3	-	-	-	-	-	-
17	-	-	5	9	-	-	-	-	-	3	-	-	-	-	-	-	-	-
13	-	-	-	1	-	-	2	-	3	-	-	-	-	-	5	-	3	11
11	-	-	-	-	-	2	-	-	-	-	-	-	-	-	13	6	-	2
10	-	-	-	2	-	6	-	8	2	-	-	-	4	-	4	-	2	31
9	-	-	1	-	3	-	-	9	-	-	7	-	4	-	5	-	3	5
8	2	1	-	1	-	2	-	-	-	-	5	-	7	1	4	1	-	5
7	-	-	-	2	-	-	1	4	-	-	5	4	-	-	-	-	4	2
6	1	1	6	1	2	-	5	2	-	4	5	-	13	-	4	-	3	16
5	-	-	-	1	-	-	-	-	-	-	3	1	-	-	-	-	1	1
4	-	-	-	-	1	-	-	-	-	-	-	-	-	-	2	-	6	10
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7

5	-	-	-	1	-	-	-	-	-	-	3	1	-	-	-	-	1	1
4	-	-	-	-	1	-	-	-	-	-	-	-	-	-	2	-	6	10
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7

BA = Brodmann areas; L = Left; R = Right. Multiple comparisons correction was performed using an approximation to the permutation analysis based on t-max statistic. t-max for −8781 to −8742 ms = 3.407 (*P* = 0.002); t-max for −7367 to −7066 ms = 3.293 (*P* < 0.001); t-max for −6711 to −6668 ms = 3.138 (*P* < 0.001); t-max for −5816 to −5480 ms = 3.305 (*P* < 0.001); t-max for −4406 to −4355 ms = 3.313 (*P* < 0.001); t-max for −4297 to −3949 ms = 3.367 (*P* < 0.001); t-max for −3250 to −3160 ms = 3.019 (*P* < 0.001); t-max for −3133 to −3078 ms = 3.181 (*P* < 0.001); t-max for 78 to 129 ms = 3.144 (*P* < 0.001).

LBA45 and LBA44, including the inferior frontal gyrus (*P* ≤ 0.019), and these associations occurred during the maintenance phase. During the retrieval phase, cardiorespiratory fitness was positively associated with higher current source density in the high load in comparison with the low load in RBA36, including the fusiform and parahippocampal gyri (*P* = 0.013).

After correcting for multiple comparisons, higher muscular fitness was associated with lower current source density in the high load in comparison with the low load only in LBA6, including the precentral gyrus (β = −0.261, *P* = 0.016), during the retrieval phase. After exploratory analysis with absolute muscular fitness, overall all associations remained significant (data not shown). Speed-agility fitness was not significantly associated with H – L current density difference after correcting for multiple comparisons.

Associations of physical fitness-related H – L current density differences with working memory performance and academic achievement

Cardiorespiratory fitness-related H – L current density differences in LBA30 observed during the encoding phase were positively associated with better response accuracy in the high working memory load (β = 0.268, *P* = 0.005) (**Table 4**). No significant associations were observed for academic achievement nor for muscular fitness-related BAs.

DISCUSSION

The present study indicated that cardiorespiratory fitness was the only physical fitness component positively associated with brain current source density during all processes of working memory (i.e.,

Table 3. Brodmann areas (BA) showing individually significant associations of physical fitness components with current source density difference between high and low working memory loads by time frame (TF) (n=85).

		Cardiorespiratory fitness (laps)				Speed-agility (sec^{-1})*				Muscular fitness (z-score)†			
BA	Hem	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*
Encoding phase													
TF −8781 to −8742 ms													
6	R	0.010	0.010	−0.098	0.372	0.032	0.032	−0.180	0.099	0.059	0.059	−0.242	0.026
8	R	0.016	0.016	−0.128	0.242	0.068	0.068	−0.260	0.016	0.049	0.049	−0.221	0.042
TF −7367 to −7066 ms													
32	L	0.001	0.001	−0.025	0.821	0.012	0.012	−0.111	0.311	0.046	0.046	−0.214	0.049
TF −6711 to −6668 ms													
10	R	0.080	0.080	0.283	**0.009**	0.027	0.027	0.165	0.132	0.004	0.004	0.063	0.565
TF −5816 to −5480 ms													
-	-	-	-	-	-	-	-	-	-	-	-	-	-
TF −4406 to −4355 ms													
30	L	0.088	0.088	0.297	**0.006**	0.012	0.012	0.107	0.328	0.005	0.005	0.072	0.510
17‡	R	0.189	0.079	0.291	**0.006**	0.120	0.011	0.109	0.313	0.120	0.011	0.110	0.307
18‡	R	0.195	0.077	0.284	**0.006**	0.126	0.008	0.091	0.396	0.129	0.010	0.104	0.331
37‡	R	0.133	0.046	0.236	0.040	0.091	0.003	−0.063	0.583	0.090	0.002	0.048	0.667
TF −4297 to −3949 ms													
22‡	R	0.083	0.008	0.090	0.411	0.088	0.013	0.118	0.281	0.121	0.046	0.221	0.041
Maintenance phase													
TF −3250 to −3160 ms													
45	L	0.070	0.070	0.264	**0.015**	0.012	0.012	0.109	0.321	0.004	0.004	−0.066	0.548
44	L	0.064	0.064	0.254	**0.019**	0.031	0.031	0.177	0.105	0.000	0.000	0.011	0.921
TF −3133 to −3078 ms													
8	L	0.000	0.000	0.013	0.907	0.011	0.011	−0.106	0.334	0.050	0.050	−0.225	0.039
Retrieval phase													
TF 78 to 129 ms													
6	L	0.033	0.033	−0.182	0.095	0.044	0.044	−0.211	0.053	0.068	0.068	−0.261	**0.016**
5	L	0.002	0.002	−0.045	0.679	0.029	0.029	−0.171	0.118	0.059	0.059	−0.243	0.025
3	L	0.001	0.001	−0.025	0.818	0.035	0.035	−0.186	0.088	0.048	0.048	−0.218	0.045
2	R	0.003	0.003	0.051	0.645	0.000	0.000	0.004	0.970	0.047	0.047	−0.218	0.045

22	R	0.000	0.000	−0.002	0.983	0.004	0.004	−0.060	0.585	0.061	0.061	−0.247	0.023
36‡	R	0.149	0.067	0.265	**0.013**	0.101	0.030	−0.178	0.104	0.085	0.002	0.049	0.652

Hem = Hemisphere; L = Left; R = Right. β values are standardized. ‡Adjusted by age. *Speed-agility was reverted so that higher values indicate better results. †z-score computed from handgrip strength (kg/kg) and standing long jump (cm) tests. ‡Adjusted by peak height velocity. Statistically significant values (P<0.05) controlled for multiple comparisons using the Benjamini and Hochberg method (q<0.5) are shown in bold. Each physical fitness component was introduced in individual stepwise regression models. Potential confounders (i.e., sex, age, peak height velocity, fat mass index, intelligence quotient, wave of participation, and parental educational level) were included into step 1 of the stepwise regression to test their association to the outcomes.

encoding, maintenance, and retrieval) among children with overweight/obesity. No such associations were observed for speed-agility nor muscular fitness. Overall, the positive associations between cardiorespiratory fitness and H – L current density differences were observed during pre-target phases of the working memory task (i.e., encoding and maintenance) and were localized in BAs of the frontal, limbic, and occipital regions. There was also a positive association between cardiorespiratory fitness and a BA of the temporal lobe during the retrieval phase. On the other hand, muscular fitness was inversely associated with current source density in only one BA of the frontal lobe during retrieval phase of the working memory task. In addition, the cardiorespiratory fitness-related brain current source density observed in limbic regions was further associated with higher response accuracy in the high working memory load.

In our study, cardiorespiratory fitness was positively associated with higher current density during the high load in comparison with the low load in frontal, limbic, and occipital BAs during the encoding phase of the working memory task. In particular, BA17 and BA18 (i.e., cuneus, and middle occipital and lingual gyri) are areas responsible for processing visual information,[39] and thus are determinants for the processing and later storage of the memory set allowing for a correct answer once the target is later presented. Furthermore, BA30 (i.e., posterior cingulate and parahippocampal gyri) play a crucial role in the storage of visual objects during a delayed period.[40] This may explain that the cardiorespiratory fitness-related association with current density in BA30 was therefore associated with a better response accuracy in the high load. Taking into account the activation of these areas during the memory set displays, particularly during the encoding

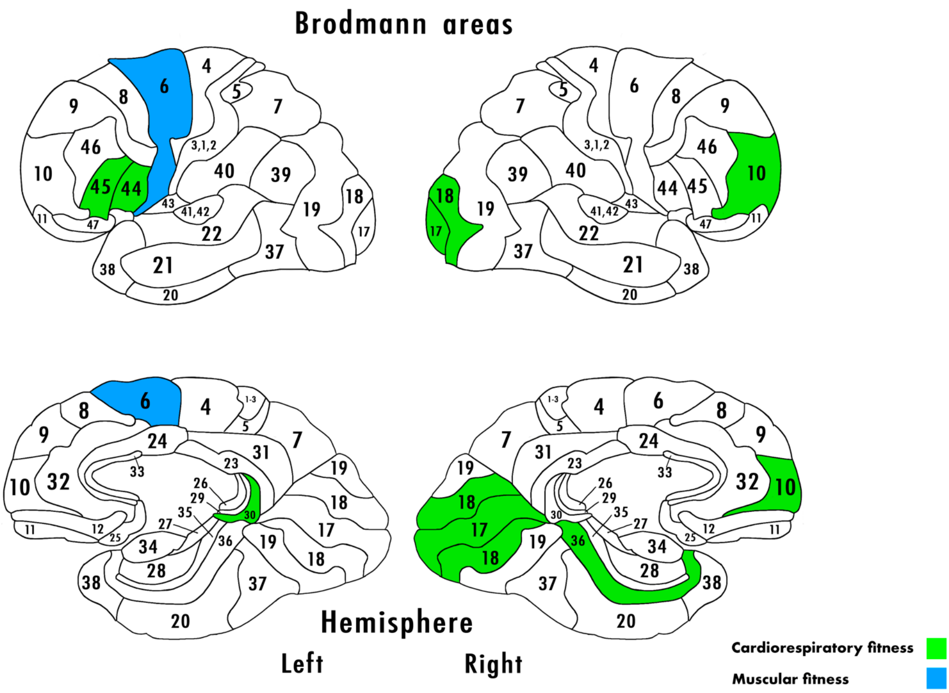

Figure 3. Brodmann areas showing significant associations of the physical fitness components with current source density difference between high and low working memory loads (n=85).

phase (i.e., during the appearance of the last stimulus to recall pre-target), it seems that cardiorespiratory fitness is associated with an enhanced capacity to visually process and store information during the memory set-target interval in children with overweight/obesity.

Other associations were observed between cardiorespiratory and the current density localized within frontal areas (i.e., BA45 and BA44) during the maintenance phase of the working memory task. These prefrontal areas have been shown to activate during working memory processes and particularly when participants are accessing information and evaluating it previously to the presentation of the target.[41] Furthermore, these areas are known to be involved in organization, regulation, sustaining attention and working memory.[41,42] Taking this into account, cardiorespiratory fitness may not play an important role only for encoding processes but also for the information maintenance during working memory as well as for the activation of prefrontal areas.

Our findings are supported by a prior study carried out in the same sample as in the present study.[11] This prior study addressed the relationship of physical fitness components and the neuroelectric activation during working memory using an ERP approach. They found that cardiorespiratory fitness was positively associated with working memory performance (i.e., shorter mean reaction time) and the amplitude of P3 component.[11] Other investigations addressing long-term effects of exercise also support our findings.[43,44] Specifically, a previous randomized controlled trial showed that increases in VO_2max (i.e., oxygen uptake attained during maximal exercise intensity, indicator of cardiorespiratory fitness) resulting from an intervention of 70 min of daily MVPA were associated with improvements in children's working memory and the contingent negative variation.[44] Another study also demonstrated that a physical activity intervention benefits inhibitory control processes reflected in the P3 component,[43] which has been associated with the allocation of attentional resources during the updating of working memory.[45]

Regarding the two other components of physical fitness, while speed-agility was not associated with brain current source density, higher levels of muscular fitness were associated with lower current source density in the high load in comparison with the low load solely in the LBA6 located in the frontal lobe. This association was observed during the retrieval phase of the working memory task. Previous literature regarding both speed-agility and muscular fitness in relation to brain current source density is nonexistent, which hampers direct comparisons with other studies. Interestingly, LBA6 belong to the left inferior frontal gyrus, located in the prefrontal gyrus, a region that also showed a deactivation (i.e., low working memory brain activity > high working

Table 4. Associations between physical fitness-related current source density difference between high and low working memory loads and working memory performance and academic achievement by time frame (TF) (n=85).

		High load working memory performance (Response accuracy)*				Academic achievement (Total achievement standard score)†			
BA	Hem	R^2	R^2 change	β	*P*	R^2	R^2 change	β	*P*
Cardiorespiratory fitness-related BAs									
Encoding phase									
TF −6711 to −6668 ms									
10	R	0.254	0.007	−0.087	0.374	0.505	0.013	0.113	0.156
TF −4406 to −4355 ms									
30	L	0.317	0.070	0.268	**0.005**	0.496	0.004	−0.061	0.449
17	R	0.284	0.038	0.203	0.042	0.496	0.004	−0.062	0.433
18	R	0.274	0.027	0.174	0.085	0.500	0.007	−0.087	0.276
37	R	0.262	0.015	0.129	0.202	0.500	0.007	−0.087	0.277
Maintenance phase									
TF −3250 to −3160 ms (M)									
45	L	0.268	0.022	0.148	0.124	0.498	0.006	0.079	0.319
44	L	0.261	0.014	0.120	0.214	0.500	0.008	0.088	0.267
Retrieval phase									
TF 78 to 129 ms									
36	R	0.248	0.001	0.033	0.739	0.495	0.002	−0.051	0.531
Muscular fitness-related BAs									
Maintenance phase									
TF −3250 to −3160 ms (M)									
45	L	0.268	0.022	0.148	0.124	0.498	0.006	0.079	0.319
TF −3133 to −3078 ms (M)									
8	L	0.251	0.004	0.064	0.514	0.493	0.001	−0.024	0.763
Retrieval phase									
TF 78 to 129 ms (R)									
2	R	0.255	0.008	0.092	0.349	0.495	0.003	−0.055	0.491
22	R	0.267	0.020	0.142	0.140	0.493	0.001	0.026	0.744

Hem = Hemisphere; L = Left; R = Right. β values are standardized. Statistically significant values ($P < 0.05$) controlled for multiple comparisons using the Benjamini and Hochberg method (q<0.5) are shown in bold. *Adjusted by age and intelligence quotient. †Adjusted by intelligence quotient and parental educational level. Stepwise regression models were performed individually for the association of high–low current density difference with high response accuracy and academic achievement. Potential confounders (i.e., sex, age, peak height velocity, fat mass index, intelligence quotient, wave of participation, and parental educational level) were included into step 1 of the stepwise regression to test their association to the outcomes. Total achievement standard score was computed from the Woodcock Johnson III test battery.

memory brain activity) after acute exercise.[46] Given that prefrontal gyrus is thought to play an executive role in semantic processing, including encoding and retrieval processing,[47] the results of the present study possibly indicate that decreased activation in the left hemisphere might represent a reduction of semantic processing or may refer to functional compensation for the increased activation of the contralateral hemisphere. Further the reduction of activity in this region is in line with the default mode hypothesis.[48] This hypothesis suggests that the deactivation of some prefrontal regions generally occurs during goal-directed cognitive demands,[49] such as in the high working memory load of the present study. In light of this, the inverse associations found in the present study could suggest that muscular fitness may be related to a deactivation of several BAs during highly demanding tasks. However, these hypotheses are speculative and must be further examined in additional investigations.

There are several mechanisms that could explain the positive findings of the present study from a neurophysiological perspective. In particular, the posterior hippocampus experiences a developmental expansion during childhood and adolescence,[50] which is consistent with rapid improvements in memory performance during this period.[51] This depends upon reciprocal connectivity between the posterior two-thirds of the hippocampus and a broader posterior medial network.[50] This network includes many brain regions whose current source density is positively associated with physical fitness in the present study such as the cuneus, the posterior cingulate cortex and the parahippocampal cortex. In this context, key neural structures supporting working memory processes such as the prefrontal cortex (i.e., BA10, 45, 44) or the hippocampus might not be fully developed during childhood,[52] suggesting that some brain areas might be highly susceptible to environmental factors such as engagement in aerobic and muscular strength activities during childhood.[53] Taken together, the association between physical fitness and brain current source density during working memory processes in children with overweight/obesity seems biologically plausible.

Another finding of the present study showed that a higher activation of the posterior cingulate and parahippocampal gyri (i.e., BA30) during high load relative to low working memory load, which was related to cardiorespiratory fitness in early models, was associated with better working memory performance. Despite this, the neuroimaging literature has identified a broad network of brain areas (i.e., frontoparietal, motor and cingulate networks) underlying working memory function, which emphasizes the need to consider a broad range of areas and their functional interaction, rather than a single system.[54] Accordingly, whereas excess body mass may impair brain function,[55] physical fitness may counteract this negative effect in obese samples.[56]

The main limitation of the present study is its cross-sectional nature which does not allow us to draw causal inferences. Further, although we explored the role of potential confounders in the analyses, it is not possible to guarantee that other confounders not measured in the present study could explain the observed associations. On the other hand, the main strength of the present study is that, to the best of our knowledge, this is the first study using brain source analysis (i.e., sLORETA) to investigate the relationship between physical fitness and differences in source localization across differential working memory demands in children. Additional strengths include: i) The examination of the main health-related physical fitness components (i.e., cardiorespiratory, speed-agility and muscular fitness),[3] as previous studies have solely focused on cardiorespiratory fitness;[1] ii) the use of the whole cardiorespiratory fitness spectrum of the targeted population, whereas previous studies split the sample into two extreme fitness groups (i.e., high- and low-fit children), excluding those in the mid-range. In fact, this was highlighted by a recent Position Stand as a limitation of the existent evidence on fitness and brain function;[1] iii) a modest sample of 85 children with overweight/obesity when compared with previous literature using similar methods.[57]

CONCLUSION

In conclusion, our results suggest that cardiorespiratory fitness, but not speed-agility nor muscular fitness, is positively associated with brain current source density during a working memory task in children with overweight/obesity. In particular, cardiorespiratory fitness is associated with regions of the frontal, limbic and occipital lobes (e.g., parahippocampal gyrus or lingual gyrus) during the phase where higher amounts of working memory are required (i.e., encoding and maintenance), suggesting that cardiorespiratory fitness may be associated with an enhancement of the capacity to visually process and store information. Further, cardiorespiratory fitness was found associated to the current source density in regions of the temporal lobes (e.g., fusiform and parahippocampal gyri) during the retrieval phase of working memory, suggesting that this fitness component may be associated with brain areas that recall of stored information. The fitness-related association with brain current density of the posterior cingulate and parahippocampal gyri was further related to better working memory performance. Therefore, physical exercises that improve cardiorespiratory fitness may be an effective approach to stimulate brain activity related to working memory in children with overweight/obesity. However, further exercise-based randomized controlled trials inducing improvements in the various fitness components are needed to test whether such improvements lead to better brain health in this population.

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Study 5

Sedentarism, physical activity, steps, and neurotrophic factors in children with overweight/obesity

BACKGROUND

Childhood obesity has shown to be negatively related to cognitive functions and detectable structural abnormalities in the brain.[1,2] Likewise, obesity may also influence stored and circulating neurotrophic factors such as brain-derived neurotrophic factor (BDNF) in humans,[3] although literature to this respect is inconsistent.[4] It has been observed that BDNF missense mutations in its receptor, TrkB, have been associated with weight gain in both humans and mice.[4] Further, evidence have shown a significant reduction of circulating BDNF levels in children with obesity compared to normal-weight peers.[5,6] Importantly, BDNF plays a key role in synaptic plasticity, neuronal transmission, and cell growth and survival throughout the cortex.[7] This factor is produced in the brain and in selected peripheral tissues such as platelets.[8] Platelets are the major non-neural source of BDNF from which it reaches plasma and is able to pass the blood-brain barrier, only when it is not bound to platelets.[9,10] Interestingly, the positive correlation between BDNF in the brain and circulating BDNF suggests that circulating BDNF levels may reflect the levels in the central nervous system.[11] Furthermore, BDNF may be released from the brain to the periphery during the practice of physical activity (PA).[12] Apart from BDNF, other neurotrophic factors such as vascular endothelial growth factor (VEGF) or insulin growth factor-1 (IGF-1) are important for neural growth and neuron survival.[13] Hence, it seems of relevance to examine how protective environmental factors, such as lifestyle behaviors (e.g., sedentarism or PA), may influence neurotrophic factors in a particularly vulnerable population such as children with overweight/obesity.

Emerging evidence suggest that PA has a beneficial effect on the brain and cognitive processes in children.[14] Neurotrophic factors have been suggested as potential mechanisms underlying this relationship.[15] From all these factors, BDNF may be the most important one that has been suggested to be upregulated by PA.[13] Indeed, BDNF may play a crucial role in the PA's influence on brain structure and as an underlying factor of the PA-induced cognitive improvement. However, in humans, there is inconsistent evidence on the role of PA in neurotrophic factors.[16–19] PA may increment serum BDNF concentrations in adolescents[16,19] and adults,[18] although there are other studies showing a negative association between PA and BDNF.[17] In children, to the best of our knowledge, there are only two observational studies and they did not find significant associations.[20,21] However, no previous cross-sectional studies have focused on children with obesity nor have analyzed the role of step-related behaviors in neurotrophic factors. In addition, the BDNF plays a key role in the energy homeostasis and the appetite regulation,[22] which highlights even more the importance of examining the potential relationship of sedentary time and PA with brain in the context of obesity during childhood. Particularly, walking (hereinafter step-related behaviors) is the most popular PA behavior, as well as the ideal PA intervention to improve health across sedentary populations, such as the obese ones.[23,24] Thus, the aim of the present study was to analyze the association of sedentary time, PA and step-related behaviors with BDNF and other neurotrophic factors (i.e., VEGF and IGF-1) in children with overweight/obesity.

METHODS

Participants

The present cross-sectional study was developed under the framework of the ActiveBrains project (http://profith.ugr.es/activebrains).[25] A total of 110 children with overweight/obesity aged 8–11 years were recruited from Granada (Spain) after meeting the defined inclusion criteria, which have been described elsewhere.[25] The study was conducted in three waves. The present cross-sectional analyses used baseline data from 97 children with overweight/obesity (10.0 ± 1.2 years old; 58% boys) with complete baseline data on sedentary time, time-based PA, steps-related behaviors, and neurotrophic factors. For VEGF analyses, a sample of 88 participants was used after excluding those children with lower VEGF levels than the kit could detect. The baseline data collection took part from November 2014 to February 2016.

A description of the purpose and characteristics of the study was given to the parents or legal guardian and written informed consent was provided by them allowing the child to participate. The ActiveBrains project was approved by the Ethics Committee on Human Research of the University of Granada, and was registered in ClinicalTrials.gov (identifier: NCT02295072).

Sedentary time, physical activity, and steps metrics

Sedentary time, PA, and step-related behaviors were assessed by accelerometer (GT3X+, ActiGraph, Pensacola, FL, USA) taking into account the latest advances in data processing.[26] Children wore simultaneously two accelerometers located on the right hip and non-dominant wrist for 7 consecutive days (24h/day). They were instructed to remove them only for water activities (i.e., bathing or swimming) and to record waking-up and sleep onset times during the 7 days on a diary. Raw data were collected at a sampling frequency of 100 Hz were loaded in ActiLife (ActiGraph, Pensacola, FL, USA) and processed then in R (v. 3.1.2, https:// www.cran.r-project.org/) using the GGIR package (v. 1.6-0, https://cran.r-project.org/web/packages/GGIR/).[27] We calculated the Euclidean Norm Minus One G metric (ENMO, 1 G ~ 9.8 m/s2) after auto-calibrating the acceleration signal.[27,28] The mean of ENMO with negative values rounded to zero was calculated over 5 s epochs. Simultaneously, we derived the number of steps/minute (step cadence) from the hip-worn accelerometer using the ActiLife software. Then, we imported steps information to R for further analyses in the GGIR package.

Accelerometric information processing in GGIR consisted in: a) Non-wear time detection by the Van Hees et al. approach.[29] b) Detection of abnormally and sustained high acceleration values (i.e., clipped time). c) Replacement of the non-wear and clipped time by the mean acceleration recorded within the same time frame for the rest of the measurement.[29] A replacement by 0 for all metrics was performed if no data were collected for a specific time frame for the rest of the days. d) Identification of waking and sleeping hours based on an automatized algorithm guided by the diaries completed by the participants.[30] The inclusion criterion for a valid day was wearing the accelerometer ≥16 h/day. A minimum of 4 valid days (3 weekdays and 1 weekend day) per week was required to be included in the analyses. The compliance wearing the accelerometer was high, with 98% of the sample wearing the accelerometers for ≥6 days.

Sedentary time and PA were classified into different intensities following Hildebrand et al. hip- and wrist-based cut-off points for the ENMO metric.[31,32] Since ActiGraph's step detection algorithm is adapted to the hip location, the main analyses of the present study were performed using hip data, although analyses for sedentary time and PA were replicated using estimates from the non-dominant wrist-worn accelerometer and presented as supplementary material.

The PA variables included in this study were total minutes per day at light, moderate, vigorous, and MVPA for hip and wrist. With regards to steps, the volume of steps/day and the peak 60-, 30-, and 1-min cadences were computed following previously published procedures.[33] We also derived time spent in the following cadence bands intensities (i.e., steps/min): 0 (Non-movement), 1–19 (Incidental movement), 20–39 (Sporadic movement), 40–59 (Purposeful movement), 60–79 (Slow walking), 80–99 (Medium walking), 100–119 (Brisk walking), and 120+ steps/min (Faster locomotor movements, e.g., running).[33]

Neurotrophic factors

Blood samples were obtained for biochemical and hematological screening tests between 08.30 AM and 10.30 AM after a minimum of 8 hours overnight fasting condition at the San Cecilio University Hospital and the Virgen de las Nieves Maternity Hospital (Granada, Spain). All participants had up to 11 ml of blood drawn from the antecubital vein. The blood for plasma samples was drawn into tubes containing ethylenediaminetetraacetic acid (EDTA) and kept on ice for around 60 min. After collection and transportation of the samples, they were centrifuged (10 min at 4°C, 1000xg), aliquoted under cold conditions by ice, and immediately stored at -80°C in the Center of Biomedical Research (Granada, Spain) until analysis.

The analysis of mature BDNF, VEGF, and IGF-1 levels in plasma was performed using the Luminex IS 100/200 system (Luminex Corporation, Austin, TX, USA) with the XMap technology and using human monoclonal anti-bodies (Milliplex Map Kit, Millipore, Billerica, MA, USA). For mature BDNF, we used the Human Neurodegenerative Disease Magnetic Bead Panel 3 (Catalog #HNDG3MAG-36K; EMD Millipore Corporation, Billerica, MA, USA); for VEGF, we used the Human Angiogenesis/Growth Factor Magnetic Bead Panel (Catalog #HAGP1MAG-12K; EMD Millipore), and for IGF-1, we used the Human IGF-1, II Magnetic Bead Panel (Catalog #HIGFMAG-52K; EMD Millipore). In the Luminex IS 100/200 system, assay sensitivities or minimum detectable concentrations for BDNF, VEGF-A, and IGF-1 assays were 0.23 ng/ml, 8.1 pg/ml, and 15 ng/ml, respectively. Those samples not reaching the minimum detectable were excluded from the analyses. The intra-assay % coefficient of variation for BDNF, VEGF-A, and IGF-1 was estimated to be <5.4, 3.5 and 10, respectively, and inter-assay at <5.3, 10, 15, respectively.

Potential confounders

After testing with correlation analyses which of the variables could be a potential confounder, sex, peak height velocity (PHV), fat mass index, wave of participation, and the parental educational level were used as potential confounders in the analyses. PHV is an indicator of maturity offset during childhood and adolescence.[34] We used age and anthropometric variables (height –girls- and seated height -boys-) to calculate PHV following Moore's equations.[34] The difference in years between PHV and chronological age was defined as a value of maturity offset. Fat mass index (kg/m^2) was assessed by Dual-energy X-ray absorptiometry (DXA, Discovery densitometer from Hologic). Wave of participation was a categorical variable according to the first moment of participation of each child in the study (wave 1, 2, or 3). Parental educational level was assessed by a self-reported questionnaire completed by parents, and we combined responses of both of them as: neither had a university degree; one had a university degree; or both had a university degree.[35]

Statistical analysis

The characteristics of the study sample are presented as means and standard deviations (SD) or percentages. Non-normally distributed outcomes are presented as median and interquartile range (IQR). Prior to all analyses, the extreme values were winsorized to limit their influence; this was done by replacing raw scores with less than the 1st percentile of the cohort-wide distribution with the value of the 1st percentile and replacing scores greater than the 99th percentile with the 99th percentile value.[36] Furthermore, all outcomes were checked for normal distribution and BDNF, VEGF, and IGF-1 were normalized since they showed skewed distributions. Interaction analyses were performed between sex and sedentary time, PA and steps-related behaviors on the neurotrophic factors. No significant interactions with sex were found ($P \geq 0.10$); therefore, analyses were performed for all the participants together.

Linear regression analyses were performed to examine the association of estimations from hip-worn accelerometers of sedentary time, PA, and step-related behavior with neurotrophic factors (i.e., BDNF, VEGF and IGF-1) adjusting by potential confounders. Sedentary time and PA analyses were replicated for the non-dominant wrist-placement data. We also performed linear regression analyses to examine the association between time accumulated (min/day) in different cadence bands of 0, 1–19, 20–39, 40–59, 60–79, 80–99, 100–119, and 120+ steps/min and the BDNF, adjusting by potential confounders. We performed collinearity diagnosis between physical activity intensities and between step cadences. No multi-collinearity was observed among any of the independent variables (variance inflation factor, VIF < 10). A significance level of $P < 0.05$ was used. All the statistical procedures were performed using the SPSS software for Mac (version 22.0, IBM Corporation).

RESULTS

Descriptive characteristics of the sample are shown in **Table 1**. Times accumulated at different cadence bands are shown in **Table 2**. A significant association was found between light PA, moderate PA, MVPA, and peak 60-min steps cadence with BDNF (β ranging from 0.195 to 0.242, all $P < 0.037$) (**Table 3**). An association was also found between light PA and VEGF (β = 0.207, $P = 0.048$). No significant associations were found for the relationship of sedentary time with any of the neurotrophic factors nor for the relationship between PA, step-related behaviors, and IGF-1 ($P > 0.05$). When performing analyses with non-dominant wrist-placement data (**Table 4**), the associations of light PA with BDNF and VEGF disappeared (all $P > 0.05$). However, moderate PA and MVPA remained significantly associated with BDNF (β = 0.220, $P = 0.041$ and β = 0.246, $P = 0.027$, respectively). An association was also observed between vigorous PA and BDNF (β = 0.244, $P = 0.032$).

Figure 1 shows the relationship between time accumulated at different steps cadence bands and BDNF, adjusting for potential confounders. Among all the cadence bands, a significant association was found for the time spent in the 40-59 steps/min cadence band (i.e., Purposeful movement) and the time spent in 60-79 steps/min cadence band (i.e., Slow walking) with BDNF (β = 0.198, $P = 0.044$, and β = 0.205, $P = 0.040$, respectively).

Table 1. Descriptive characteristics of the study sample.

	All (n=97)	Boys (n=56)	Girls (n=41)
Physical characteristics			
Age (years)	10.1 ± 1.2	10.2 ± 1.2	9.9 ± 1.1
Peak height velocity offset (years)	-2.3 ± 1.0	-2.7 ± 0.8	-1.7 ± 1.0
Weight (kg)	56.0 ± 10.7	56.6 ± 10.3	55.2 ± 11.2
Height (cm)	144.1 ± 8.4	144.6 ± 7.9	143.5 ± 9.1
BMI (kg/m^2)	26.8 ± 3.5	26.9 ± 3.6	26.6 ± 3.4
BMI categories (%)*			
Overweight	24	23	24
Obesity grade I	46	50	42
Obesity grade II	21	16	27
Obesity grade III	9	11	7
Fat mass index (kg/m^2)	11.8 ± 2.8	11.5 ± 2.8	12.1 ± 2.8
Wave of participation (%)			
First	16.5	10.7	24.4
Second	42.3	50.0	31.7
Third	41.2	39.3	43.9
Parental university level (%)			
None of them	64.9	71.4	56.1
One of them	17.5	14.3	22.0
Both of them	17.5	14,3	22.0
Sedentary time (min/day)	818.3 ± 45.6	812.2 ± 44.1	826.7 ± 46.9
Physical activity (min/day)			
Light PA	65.3 ± 15.5	67.6 ± 15.1	62.1 ± 15.7
Moderate PA	32.4 ± 13.6	36.8 ± 14.5	26.4 ± 9.6
Vigorous PA	3.0 ± 2.1	3.7 ± 2.2	2.1 ± 1.4
MVPA	35.4 ± 14.1	40.5 ± 16.0	28.5 ± 10.6
Steps			
Volume (steps/day)	8588.6 ± 2176.7	9163.9 ± 2416.0	7802.9 ± 1499.5
Peak 60-min cadence (steps/min)	63.2 ± 13.1	66.1 ± 14.3	59.2 ± 10.2
Peak 30-min cadence (steps/min)	77.5 ± 14.0	79.5 ± 15.0	74.7 ± 12.1
Peak 1-min cadence (steps/min)	111.0 ± 13.0	111.2 ± 13.5	110.9 ± 12.5
Neurotrophic factors (median (IQR))			
BDNF (ng/ml)	3.0 (4.4)	3.0 (4.1)	2.9 (6.1)
VEGF (pg/ml)†	35.4 (35.9)	37.7 (34.8)	34.5 (51.1)
IGF-1 (ng/ml)	86.6 (38.8)	80.2 (39.4)	90.8 (52.7)

PA=Physical activity; MVPA=Moderate-to-Vigorous Physical Activity; BDNF=Brain-derived neurotrophic factor; VEGF=Vascular endothelial growth factor A; IGF-1=Insulin-like growth factor-1; BMI=body mass index. Values are means ± standard deviations, unless otherwise indicated. *BMI categories were defined (i.e. overweight, obesity grade I, II, III) according to Cole and Lobstein (2012). †Sample for VEGF was n=88 (n=55 boys; n=33 girls). Sedentary time, physical activity and step-related behaviors were obtained from the Euclidian norm minus one metric in hip.

Table 2. Time (min/day) accumulated at different cadence band of steps.

	All (n=97)	Boys (n=56)	Girls (n=41)
Cadence bands (steps/min)			
Non-movement (0)	346.4 ± 79.7	342.5 ± 82.7	351.9 ± 76.2
Incidental movement (1-19)	440.3 ± 64.6	437.0 ± 63.9	444.8 ± 65.9
Sporadic movement (20-39)	71.1 ± 17.8	71.8 ± 18.8	70.1 ± 16.6
Purposeful movement (40-59)	27.0 ± 8.6	29.4 ± 9.2	23.7 ± 6.6
Slow walking (60-79)	15.7 ± 7.5	18.2 ± 8.1	12.2 ± 4.7
Medium walking (80-99)	10.4 ± 6.2	12.0 ± 7.1	8.1 ± 3.8
Brisk walking (100-119)	6.4 ± 5.4	7.4 ± 6.5	5.1 ± 3.2
Faster locomotion (120+)	1.4 ± 1.9	1.6 ± 2.1	1.1 ± 1.6

Values are means ± standard deviations.

Table 3. Associations of sedentary time, physical activity and steps (measured with Euclidian norm minus one metric in hip) with neurotrophic factors (n=97).

	BDNF (ng/ml)		VEGF (pg/ml)*		IGF-1 (ng/ml)	
	β	*P*	β	*P*	β	*P*
Sedentary time (min/day)	-0.147	0.107	-0.078	0.479	0.130	0.192
Physical activity (min/day)						
Light PA	0.242	**0.006**	0.207	**0.048**	-0.187	0.055
Moderate PA	0.237	**0.016**	0.148	0.203	-0.095	0.385
Vigorous PA	0.098	0.343	0.156	0.195	-0.018	0.875
MVPA	0.234	**0.019**	0.159	0.180	-0.091	0.413
Steps						
Total number of steps/day	0.182	0.055	0.110	0.331	-0.105	0.314
Peak 60-min cadence (steps/min)	0.195	**0.037**	0.055	0.621	-0.061	0.556
Peak 30-min cadence (steps/min)	0.171	0.064	0.039	0.724	-0.036	0.724
Peak 1-min cadence (steps/min)	0.034	0.720	-0.041	0.712	-0.046	0.658

PA=Physical activity; MVPA=Moderate-to-vigorous physical activity; BDNF=Brain-derived neurotrophic factor; VEGF=Vascular endothelial growth factor A; IGF-1=Insulin-like growth factor-1. β values are standardized. *Sample for VEGF was n=88 (n=53 boys; n=35 girls). These analyses were adjusted for the following covariates: sex, peak height velocity, fat mass index, wave of participation and parental educational level. The bold font is used to highlight significance level at *P* < 0.05. Normalized values were used in the analyses.

DISCUSSION

The main finding of the present study was that objectively-measured PA and step-related behaviors, but not sedentary time, were positively associated with BDNF in children. Particularly, light PA, moderate PA, MVPA, and peak 60-min steps cadency were related to BDNF, being the associations of moderate PA and MVPA consistent from either hip or wrist accelerometer data. No significant associations were found between PA and steps with VEGF and IGF-1, apart from the borderline association observed between light PA and VEGF. No association was found between sedentary time and the neurotrophic factors. In addition, the time spent in purposeful

Table 4. Associations between sedentary time, physical activity, measured with Euclidian norm minus one metric in non-dominant wrist, and neurotrophic factors (n=97).

	BDNF (ng/ml)		VEGF (pg/ml)†		IGF-1(ng/ml)	
	β	*P*	β	*P*	β	*P*
Sedentary time (min/day)	-0.097	0.288	-0.055	0.609	0.074	0.460
Physical activity (min/day)						
Light PA	0.022	0.801	0.073	0.478	-0.028	0.775
Moderate PA	0.220	**0.041**	0.071	0.577	-0.078	0.516
Vigorous PA	0.244	**0.032**	0.229	0.088	-0.054	0.666
MVPA	0.246	**0.027**	0.110	0.406	-0.080	0.516

β values are standardized. These analyses were adjusted for the following covariates: sex, peak height velocity, fat mass index, wave of participation and parental educational level. The bold font is used to highlight significance level at P<0.05. PA=Physical activity; MVPA=Moderate-to-vigorous physical activity; BDNF=Brain-derived neurotrophic factor; VEGF=Vascular endothelial growth factor A; IGF-1=Insulin-like growth factor-1. †Sample for VEGF was n=88 (n=53 boys; n=35 girls). Normalized values were used in the analyses.

movements (i.e., 40–59 steps/min) and slow walking (i.e., 60–79 steps/min) was associated with BDNF. Our findings suggest that different intensities and types of PA, mainly moderate and MVPA and walking at slow-medium cadences may increase plasma BDNF levels in children with overweight/obesity. However, these findings must be interpreted with caution due to the methodological limitations when measuring neurotrophic factors,[37] as well as to the complexity of PA analyses and the emerging variety of methods to analyze it.[26]

To the best of our knowledge, this is the first study that analyzes the association between objectively-measured sedentary time, PA and step-related behaviors with neurotrophic factors (i.e., BDNF, VEGF and IGF-1) in a sample of children with overweight/obesity. Only two observational studies in healthy normal-weight children have previously analyzed this relationship. In line with our results, Gabel et al. did not find any association between sedentary time and plasma BDNF levels in 7–10-year-old children.[21] In contrast to our cross-sectional results, a recent 2-year longitudinal study did not find a relationship between objectively-measured PA and serum BDNF in children aged 8–11 years.[20] When analyzing steps, our positive results between the peak 60-min cadence and BDNF are in contrast to the negative associations found by another study in adults.[38] The inconsistency and contradictory findings regarding the relationship between PA, steps and BDNF might be due to differences between studies with respect to the sample's characteristics (i.e., overweight/obese versus normal weight peers); the age group analyzed (i.e., children versus adolescents or adults); the study design (i.e., cross-sectional versus longitudinal); and the methodology followed for

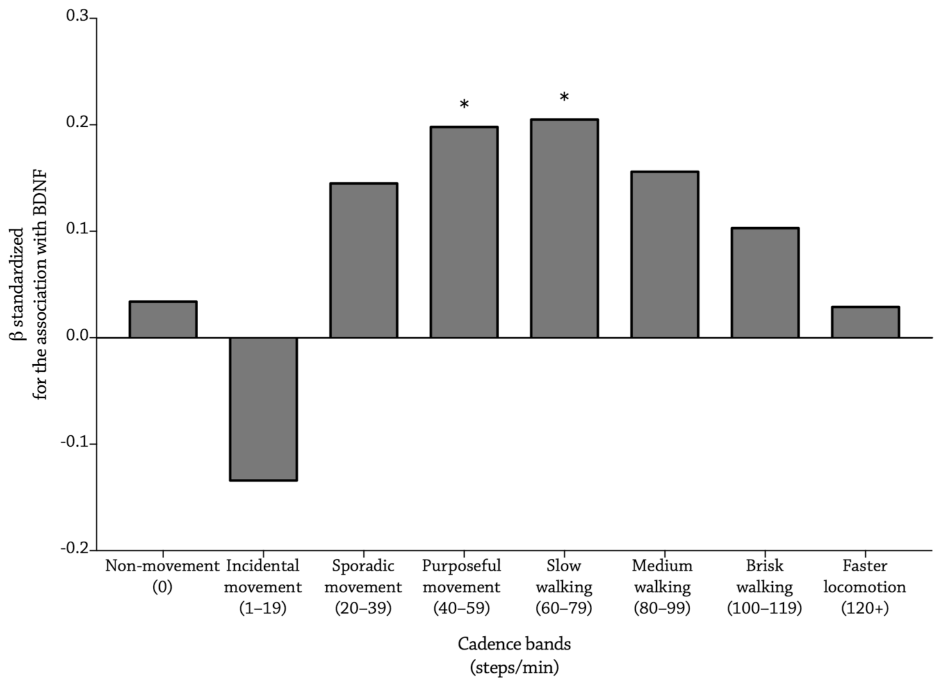

Figure 1. Relationship between time (min/day) accumulated at different cadence bands and levels of brain-derived neurotrophic factor (BDNF). These analyses were adjusted for the following covariates: sex, peak height velocity, fat mass index, wave of participation and parental educational level. The asterisk (*) is used to highlight significance level at P<0.05.

assessing and processing PA (i.e., objective versus subjective methods) or for analyzing the neurotrophic factors levels (i.e., differences regarding kits used, pre-storage treatments of blood samples – clotting/icing time, centrifugation strategy– or the way BDNF is measured in peripheral blood –plasma BDNF versus serum BDNF). With respect to the differences in BDNF measurements, much higher concentrations of BDNF has been observed in serum in comparison to plasma.[8,39] On one hand, the clotting time methodology chosen can be critical for serum BDNF levels.[8,40] On the other hand, plasma is obtained from blood samples drawn into tubes containing anti-coagulants, preventing coagulation and thereby activation of platelets and BDNF release. Due to the smaller amount of platelet-associated BDNF in plasma, BDNF measured in plasma may, to a higher extent than serum BDNF, reflect the concentration of free BDNF. However, there is still a need to better understand how much it reflects brain levels and how it relates to PA.

Despite findings from most observational studies suggest an inverse relationship between PA and peripheral BDNF levels,[18] the positive associations found in our study are supported by previous literature focusing on the effects of physical exercise on BDNF in humans.[18,41] Particularly, two studies analyzed the changes in children's BDNF level after a lifestyle intervention which included an exercise component. Corripio et al.[42] observed that BDNF in plasma was increased in prepubertal obese children after a 2-year lifestyle intervention which included 30 to 45 min of moderate exercise 3 times per week. On the contrary, another study did not find any significant change in serum BDNF in children of different weight loss after one-year exercise therapy (i.e., physical games) once per week.[6] In adults, a recent meta-analysis showed that both acute and regular programmed exercise had a significant impact on BDNF concentrations, reflecting a moderate and small effect size (Hedges'g=0.46, P<0.001; and Hedges'g=0.28, P=0.005, respectively for acute and regular exercise intervention studies).[41] Another study found that the impact on adult's BDNF levels might be exercise intensity-dependent.[19] In fact, we observed a significant association between vigorous PA and BDNF when the wrist-location data was used. No information is yet available regarding which accelerometer-location is more valid and reliable in children,[26] what highlight the need of reporting both hip and wrist data whenever this is feasible. In our study, moderate PA and MVPA intensities were consistently associated with BDNF when using either hip or wrist PA data. This fact suggest that a moderate intensity of PA could be a higher stimulus for children with overweight/obesity to increase BDNF levels. However, further investigations are needed in order to clarify the effects of different PA intensities accelerometer-locations on neurotrophic factors.

Another interesting finding of this study was the consistently (with both hip and wrist data) no significant associations between sedentary time, PA and steps with VEGF and IGF-1 (only a borderline association was found between light PA and VEGF). Although BDNF, VEGF and IGF-1 are all considered neurotrophic factors and have several characteristics in common, each of them has a different functionality. Whereas BDNF is an important nerve growth factor that facilitates the growth and survival of various neurons and regulates synaptic plasticity,[7] both VEGF and IGF-1 contribute to the stimulation of angiogenesis and hippocampal neurogenesis.[13] Thus, the influence of PA may be different depending on the factor, what could explain the significant associations found for BDNF and the non-associations for VEGF and IGF-1.

When analyzing which of the steps cadence bands were associated to BDNF, we observed a significant association of the time accumulated in purposeful movement (i.e., 40–59 steps/min) and in slow walking (60–79 steps/min) with BDNF. In this regard, walking is the most popular PA behavior, as well as the ideal PA intervention to be recommended to improve health across sedentary populations, such as the one of the present study.[23,24] Additionally, the fact that our sample only accumulate an average of 7.8 min/day in bands over 100 steps/min limits the possibility to detect any significant relation between these high cadences and BDNF. To the best of our knowledge, no previous studies have analyzed the relation between time in different cadence bands and neurotrophic factors. The cadence bands appearing significantly associated to BDNF in children with overweight/obesity could be considered as bands of light PA. This, together with the fact that we also found an association between light PA and BDNF, may suggest that light activities such as walking may be enough to increase levels of the BDNF in children with overweight/obesity. In fact, children with overweight/obesity have shown a higher metabolic cost when walking at same speeds in comparison with normal-weight peers.[43] This fact may indicate that children with overweight/obesity could be more sensible to neurophysiological changes at lower absolute intensities, yet the relative intensity (e.g., % of maximal heart rate) might be similar to higher cadences conducted by leaner children. Additionally, obese children do not achieve cadences that are as high as those reached by either overweight or normal-weight children, and therefore it may be difficult to investigate whether high cadences are associated with neurotrophic factors.[33,43] Taking into account the difficulties to perform physical activities of higher intensity for this type of population, walking may be of help to increase total PA levels and health,[24] and therefore have neurotrophic benefits.[44]

Several explanations have been suggested in order to physiologically explain our associations between PA and BDNF.[18] First, BDNF can pass through the blood-brain barrier in both directions,[10] and it may be speculated whether peripheral BDNF circulating in blood is more efficiently uptaken or released by the brain or platelets in physically active individuals.[12] However, this must be interpreted with caution since platelets cannot pass the blood-brain barrier, and at least 80% of the BDNF in plasma comes from platelets.[8] Second, exercise may have beneficial effects on platelet function, being platelets a main storage for peripheral BDNF.[45] Third, aerobic exercise increases hippocampal levels of BDNF in animals.[13] Animal models have also shown that BDNF can pass the blood-brain barrier from the brain to the plasma,[10] and it is likely that exercise cause a production of BDNF in human brain. All these neurobiological mechanisms may explain the association of PA and steps with BDNF in the present study. However, further studies are needed to elucidate the underlying mechanisms on the association between PA and BDNF.

Caution must be applied when interpreting our findings due to several limitations. Firstly, the cross-sectional design does not allow inferences about causality to any of the associated outcomes. Secondly, plasma BDNF bound to platelets cannot cross the blood-brain barrier[46] and therefore the BDNF level in the brain may be rather reflected by the amount of free BDNF in plasma (not bounded to platelets).[39] Further, normal plasma still contains a large number of platelets after centrifugation, and since BDNF is released from platelets due to activation (e.g. when a blood vessel is punctured), this fact may highly affect the level of BDNF in plasma measured in vitro.[8] Thirdly, in our study we used a statistical approach to analyze PA that has been previously used in the literature focusing on neurotrophic factors and that allows us to make direct comparisons with previous studies. However, nowadays it is complex to choose a way to analyze PA, what is reflected in the wide variety of statistical approaches to analyze PA in the literature. Many of these ways to analyze PA should be performed when a large sample size is available, as they require all predictors (i.e., sedentary time, light physical activity, moderate physical activity and vigorous physical activity) coexisting in the same model, therefore decreasing the degrees of freedom and, also, the statistical power. Our relatively small sample (N = 97) discourage any attempt of applying statistical models requiring larger sample sizes to answer these questions. Thus, in order to find a consensus and clarify which is the best method to analyze PA, future studies using larger sample sizes should address different type of PA analysis when analyzing its association with neurotrophic factors. On the other hand, the main strength of this study was its novelty, being the first study to investigate the relationship between sedentary time, PA and steps with neurotrophic factors in a sample of children with overweight/obesity. Additional strengths include the objective measurements of sedentary time, PA and steps using raw accelerations in two different locations (hip and non-dominant wrist) and the use of the most advanced technology to analyze neurotrophic factors (i.e., Luminex 200).

CONCLUSION

The results of the present study suggest that light to moderate PA intensity and step-related behaviors, but not sedentary time, are positively associated to BDNF in children with overweight/obesity. Moderate PA and MVPA seem to be consistent in the association with BDNF regardless of the accelerometer location. Particularly, the time spent in walking at slow cadences may be stimulus enough to influence the levels of BDNF in children with overweight/obesity. No associations were found between PA, sedentary time, and VEGF and IGF. Importantly, we revealed for the first time that light PA, moderate PA, MVPA and time spent in walking at slow cadences, but not sedentary time, were associated with BDNF in children with overweight/obesity. These findings shed light on that children in an overweight/obesity status may have more room for BDNF increments induced by physical activity. Further, walking at slow cadences may be stimulus enough for this population to influence levels of BDNF. Result from the present study must be interpreted with caution taking into account the limitations and variety of methods used to measure neurotrophic factors and to analyze PA. Thus, further studies using other methods must confirm or contrast our results.

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SECTION 2

Intervention studies: Effects of exercise on brain activity during task performance, cognitive and academic performance, and brain structure

Study 6

Effects of a 4.5-month exercise program on brain activity underlying working memory and inhibitory control in children with overweight/obesity

BACKGROUND

Childhood is considered a critical period for cognitive and neural development and maturation.[1,2] At this stage, children are sensitive to experience biological and behavioral changes in their brain's structure and function.[1–3] In this context, an aspect that has shown high prevalence and that may negatively alter brain health is obesity, since it has been associated with detectable structural brain abnormalities underlying executive function operations.[4,5] In this scenario, there exist a fundamental neurobiological principle that affirms that biological events in the brain, although small, are amenable to modification by environmental factors.[6] This fact rises the importance of understanding how environmental factors such as physical activity (PA) influence brain health and subsequently executive function during childhood's neurodevelopment.[6]

The rapid development of neuroelectric and neuroimaging techniques in the present century has favored the advance of research to the understanding of the neural foundations of physical activity-induced benefits to brain health.[7] Brain activity changes derived from the use of functional magnetic resonance imaging (fMRI) and resulting from exercise interventions have been observed in children with normal-weight[8] and with overweight/obesity.[9,10] Derive from the neuroelectric system, electroencephalography (EEG) and, more specifically, the event-related brain potential (ERP) (e.g., P3 component) has been the most prominent approach used in studies with PA.[11] In general, this body of evidence has shown that children participating in exercise programs exhibited larger amplitude[12–14] and shorter latency[12] of the P3 ERP component. Collectively, subsequent substantial effects of exercise occurred on tasks that measured higher-order executive functions and that are considered vital to success in school,[15] such as working memory (i.e., ability to temporary storage and manipulate information)[16] or inhibitory control (i.e., ability to suppress irrelevant information and maintain focus),[8,12,13] although null effects have been also observed.[9] Particularly, these two key aspects of executive function (i.e., working memory and inhibitory control) have been identified by a recent meta-analysis as the ones for which children with overweight/obesity show more deficits.[17]

As commented before, the vast majority of research addressing exercise effects, including EEG measurements has used an ERP approach.[11,18] Despite the excellent milliseconds temporal resolution of this measurement, a fundamental limitation of this approach is the inverse solution, that is, the challenge in determining activated brain regions by scalp-recorded EEG activity.[19] fMRI yet has a far better spatial resolution than EEG, is inherently slower (i.e., on the scale of seconds) and the low temporal resolution of this measurement may mask temporally separated activations into a single, more spread activity.[20] In the last decade, EEG source localization algorithms have emerged as an alternative approach that inherit the high temporal resolution of EEG and generate an estimation (i.e., current source density) of the brain activity.[21] Specifically, standardized low-resolution brain electromagnetic tomography (sLORETA) has become an accepted EEG-based tool for the reliable detection of temporal-spatial brain activity together with a simple, an economical, and a noninvasive use.[22] Thus, in order to better understand brain activity changes induced by exercise, we used an EEG-based brain source analysis to take advantage of its unique characteristic of spatially estimating brain activation involved in higher cognitive function (e.g., working memory or inhibitory control) in the specific time frame in which these processes occur (i.e., the millisecond range).

To the best of our knowledge, there is only one study in children with normal-weight that has used EEG-based sLORETA brain source localization to investigate the acute effects of moderate exercise on current source density.[23] However, no previous study investigated the long-term effects of exercise on current source density underlying working memory and inhibitory control operations in children with overweight/obesity. Furthermore, aerobic exercise has been the most predominant type of exercise in the studies addressing the effects of exercise on brain activity,[8,9,12,13] with no information to date about the effects of other types of exercise (e.g., muscular exercise). Therefore, the aim of the present study was to investigate whether a 4.5-month physical exercise program induces significant changes from pre- to post-intervention in spatial-temporal brain current source density while performing tasks of working memory and inhibitory control in children with overweight/obesity. Based upon previous research, we hypothesized that a 4.5-month physical exercise program would improve tasks' performance and alter brain activation (i.e., current source density) during working memory and inhibitory control tasks in children with overweight/obesity.

METHODS

Design and Participants

The ActiveBrains project was a randomized controlled trial (RCT) aimed to examine the effects of a 4.5-month exercise program on brain structure and function, cognitive and academic performance, and physical and mental health in children with overweight/obesity (http://profith.ugr.es/activebrains).[24] Details of the ActiveBrains project have been previously described.[24] Inclusion criteria to participate were: 1) to be 8 to 11.9 years-old; 2) to be classified as overweight or obese;[25,26] 3) to not suffer from physical disabilities or neurological disorders; 4) in the case of girls, to have not started menstruation; 5) to report no use of medications that influenced central nervous system function; 6) to be right-handed (i.e., measured by the Edinburgh inventory);[27] and 7) to not report an attention-deficit hyperactivity disorder (ADHD).[28]

Recruitment process predominantly consisted in contacting families with children with overweight/obesity from databases at the Unit of Pediatrics of the University Hospitals San Cecilio and Virgen de las Nieves (Granada, Spain). Additional strategies included contacting the head teacher of both public and private schools from Granada to spread informative pamphlets. Furthermore, advertising related to the project was broadcasted in the local media throughout newspaper, radio, and television. All data were collected at pre- and post-intervention from November 2014 through June 2016.

A total of 115 children were assessed for eligibility and finally 112 were accepted in the study after meeting inclusion/exclusion criteria (**Figure 1**). Of them, 3 children chose not continue and therefore 109 were randomly allocated to an exercise group, which participated in the exercise program, or to a wait-list control group. The wait-list control group strategy has been used in previous researches,[8,29] and implied that the participants belonging to this group also received the exercise program once all the assessments of the project had been completed. Random allocation of the participants into exercise or control group was done using a computer random number generator in SPSS software for Windows (version 20.0; Armonk, NY, USA), allowing for the equal probability of being

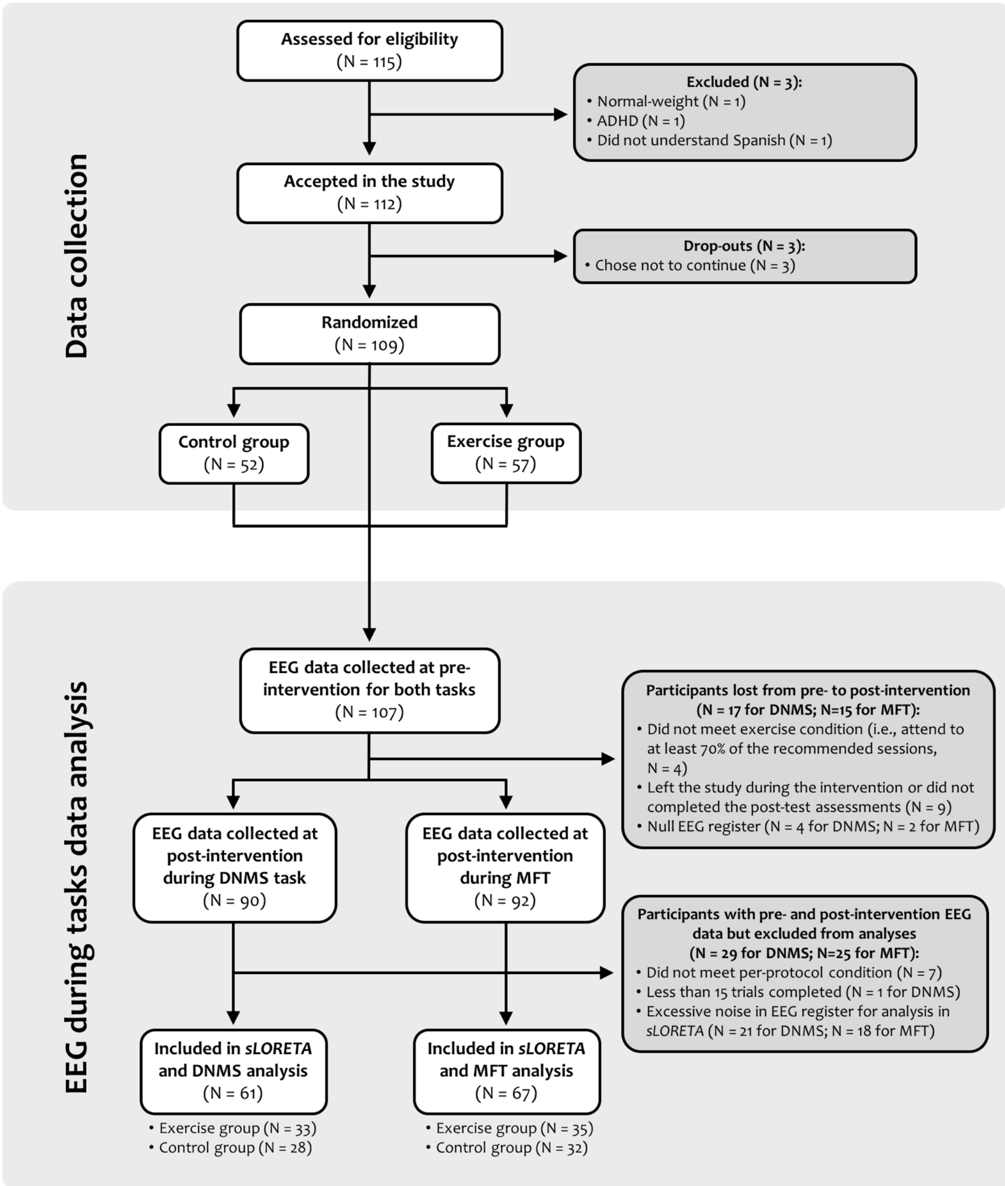

Figure 1. Flow chart describing the study sample selection for the analysis. ADHD = Attention-Deficit Hyperactivity Disorder; EEG = Electroencephalography; DNMS = Delayed-non-matched to sample task; MFT = Modified flanker task; sLORETA = Standardized low-resolution brain electromagnetic tomography

allocated to any of the two groups. Several protocols were followed in order to reduce the risk of bias: (1) The computer random generation was conducted by a person not involved in the assessments; (2) randomization was done immediately after the pre-intervention evaluation; and (3) the physical trainers in charge of running the program were not involved in the assessments. However, due to practical reasons (i.e., limited number of staff due to budget restrictions), some of the researches involved in the post-intervention evaluations were not fully blinded to the participants' group allocation.

For the present study, EEG data were collected during a Delayed non-match-to-sample (DNMS) task (i.e., working memory) and a Modified flanker task (MFT) (i.e., inhibitory control). Of the 109 randomized children, 107 successfully completed the EEG and task performance assessments at pre-intervention (**Figure 1**). At post-intervention, EEG data during DNMS task were collected for 90 children, and EEG data during MFT for 92 children. The reasons derived of having 17 participants (in the case of DNMS) and 15 participants (in the case of MFT) lost from pre- to post-intervention assessments and, therefore, not presenting post-intervention EEG data were: 9 dropped-out after randomization, 4 did not meet exercise program condition (i.e., to attend to at least 70% of recommended sessions), and 4 (in the case of DNMS) and 2 (in the case of MFT) had a null EEG recording at post-intervention. Participants were included in analyses only if they had valid EEG data (i.e., good quality of EEG register) and valid cognitive tasks data (i.e., more than 15 trials completed) for both pre- and post-intervention assessments, as well as they met the per-protocol criteria (see next section). Therefore, of the 90 and 92 children with pre- and post-intervention EEG data, 7 were excluded from analyses due to not meeting the per-protocol criteria, 1 (in the case of DNMS) was excluded due to having less than 15 trials completed for this task, and 21 (in the case of DNMS) and 18 (in the case of MFT) were excluded due to having an excessive noise of pre- or post-intervention EEG register after visual inspection. In result, a total of 61 participants (exercise group, n = 33; control group, n = 28), and 67 participants (exercise group, n = 35; control group, n = 32) were included in analyses for working memory (i.e. DNMS task) and inhibitory control (i.e., MFT), respectively.

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

Physical exercise program

The physical exercise program was based on meeting the international PA guidelines (http://www.health.gov/paguidelines/) and had a duration of 4.5-month. Participants were offered 5 sessions/week from Monday to Friday with a session's duration of 90 min. They were recommended to attend to the program at least 3 times/week, yet we advised the families that "the more, the better" (i.e., up to the 5 sessions/week). For the present study, the final sample of 67 children with overweight/obesity (10.00 ± 1.10 years; 61.2% boys) included in analyses met the per-protocol criteria of: (i) having completed the pre- and post-intervention EEG and task assessments, (ii) having attended to at least to a 70% of the recommended 3 sessions/week (i.e., exercise group); and (iii) having kept their usual lifestyle (i.e., control group).

The exercise program was based on physical multi-games, with a noticeable emphasis on the playful component in order to increase the adherence to the program. Each session was structured in three parts: 1st) a 5-10 min physical games-based warm-up; 2nd) a 60-min aerobic part consisting of around four to five physical multi-games demanding moderate-to-vigorous intensities, with special emphasis on high intensity; 3rd) a 20-min strength training consisting of muscle- and bone-strengthening game-based activities. The strength part included around 6-7 large-muscle-groups strength exercises in sets of 10-12 repetitions using therabands, fitballs and/or own bodyweight; and 4th) a 5-10 min cool-down part consisting of stretching and relaxation exercises.

The intensity of the program was controlled in all the children and in all the sessions. Every child wore always the same heart rate monitor (POLAR RS300X, Polar Electro Oy Inc., Kempele, Finland) individually programmed based on the maximum heart rate achieved by each child in a maximal incremental test described elsewhere.[24] The intensity progress was weekly checked by trained personnel mainly to identify whether any child was training at a lower intensity than intended. This allowed the physical trainers to rise the motivation and adapt the intensity of the games when needed.

Control group

Children allocated into the control group were advised to continue with their usual life, yet we provide them with a pamphlet including nutrition and PA recommendations. Based on the wait-list strategy explained before, all the children belonging to the control group received the exercise program once the trial had been completed.

Measurements

A full measurement session consisted in an EEG recording during two different executive function tasks (i.e., DNMS and MFT) of around 85–90 min total duration. The protocol for each child undergoing this measurement was divided in four phases. First, the EEG head cap was fit to child's head and electrodes filled with Electrode-Gel for around 20 min. Second, the first executive function task (e.g., DNMS) was initialized. Third, after finishing the first task, evaluators run an EEG check out at the same time that participant had a resting time of around 10 min. Fourth, the second task (e.g., MFT) was initialized. The order of the tasks performance was counterbalance between participants. Every measurement was carried out always by same trained evaluators. Both tasks were presented focally on a blue background on a computer screen using E-Prime software (Psychology Software Tools, Pittsburgh, PA).

Delayed non-matched-to-sample task

A modified version of the DNMS computerized task was used during EEG recordings to assess working memory.[30] A full description of the task and its protocol has been provided elsewhere.[31] A trial consisted of three phases: stimuli presentation (i.e., encoding, from −9,000 to −4,000 ms), pre-target awaiting (i.e., maintenance, from −4,000 to 0 ms), and target choice (retrieval, from 0 to 1,800 ms). The encoding phase (i.e., 5,000 ms duration) included a memory set of four sequential stimuli, which participants were later asked to recall. Stimuli were adapted for children, and thus, *Pokemon* cartoons were presented. After the fourth stimulus, a maintenance phase was undertaken for 4,000 ms. Finally, a target consisting of two different *Pokemon* was presented during the retrieval phase (i.e., 1,800 ms duration). During this phase, participants were asked to select the *Pokemon* that had not been shown in the four previous stimuli that comprised the memory set. A total of 16 practice trials plus 140 experimental trials were presented. There were two experimental conditions, for the high working memory load condition (i.e., 100 trials), four different stimuli were presented during the encoding phase, whereas for the low working memory load condition (i.e., 40 trials) the four stimuli were identical. Duration of the task ranged from 35 to 45 min. For the present study, mean reaction time (s; RT) and response accuracy (%) were registered.

Modified flanker task

All participants completed a modified picture-based version of the Eriksen flanker task to assess inhibitory control.[32] Trials from this task consisted of five cows focally presented at a 1.2° visual angle. Participants were instructed to respond as quickly and accurately as possible with their dominant hand to the direction (i.e., right or left) of the centrally presented target (i.e., 1.2 cm tall cow). There were two different trials (i.e., congruent and incongruent), depending on the directions of the flanking nontarget stimuli (i.e., four identical cows). For congruent trials, both the target and flanking stimuli were positioned in the same direction, either right or left (). For incongruent trials, the target and flanker stimuli were positioned in opposite directions from each other, thus requiring the upregulation of inhibitory control (). Participants were given a practice consisting of 12 trials, and a total of 144 trials, with equal probability of appearance for congruent and incongruent trials. Measures of mean RT (s) and response accuracy (%) were collected.

Neuroelectric activity recording

Neuroelectric activity was recorded using a 64-channel Active Two BioSemi EEG system (24-bit resolution, BioSemi; Amsterdam, Holland) arranged in an extended montage based on the International 10–10 system.[33] Further details of this recording can be found in previous research.[31] In brief, data were digitized at a rate of 1024 Hz and a 100-Hz low-pass filter. EEG data were pre-processed using EEGLab version 13.5.4b,[34] and ERPLAB toolbox version 6.0,[35] which consisted of: (i) Down-sampling to 256Hz, (ii) band-pass filtering between 0.1 and 30Hz (24 dB/octave), (iii) re-referenced to all electrodes average, (iv) application of artifact subspace reconstruction to eliminate artifact channels, (v) removing eye-blink-related components by using an independent component analysis (ICA) with Multiple Artifact Rejection Algorithm (MARA),[34,36] and (vi) interpolation of the previously deleted channels by artifact subspace reconstruction. After the pre-processing, data from each task were merged with EEG data. Stimulus-locked epochs were created from −10,000 to 2,000 ms surrounding the onset of the choice trial for the DNMS task and from a window of −100.0 prior to 1,700 ms after stimulus onset for MFT. Baseline correction was applied using the −100.0 to 0 ms pre-stimulus period.

Brain source analysis

sLORETA is a method for localizing the neuroelectric activity in the brain based on scalp potentials from multiple-channel EEG recordings and can be understood as an EEG-based neuro-imaging technique.[22] This technique is used to estimate the brain areas that potentially underlie the observed brain current source density and has been validated against fMRI.[22,37] Current source density has been previously shown to provide a rich and accurate view of the spatial-temporal dynamics of brain activity.[20] The overall averaged amplitude (μV) for each working memory load condition (i.e., low and high) and each inhibitory control condition (i.e., congruent and incongruent) for every participant at pre- and post-intervention were submitted to the sLORETA transform to estimate the current density time course of each of the 6,239 voxels of the sLORETA brain map. sLORETA is based on standardized EEG recordings, which are modeled onto a probabilistic head model provided by the Montreal Neurological Institute (MNI). Thus, active brain areas were then identified by allocating the raw sLORETA values of individual voxels to their corresponding Brodmann areas (BAs) on the basis of the coordinates of the MNI. sLORETA images represent the activity at each voxel as the squared standardized magnitude of the estimated current source density (μA/mm^2).

Statistical analysis

Characteristics of the study sample are presented as means and standard deviations (SD), or percentages as appropriate. Two analyses were performed in the present study to test the effects of the ActiveBrains program (i) on working memory and inhibitory control performance, and (ii) on the current source density estimated during these tasks.

Effects of the ActiveBrains program on working memory and inhibitory control performance

The effects of the ActiveBrains exercise program on the outcomes (i.e., RT and response accuracy) for each task (i.e., DNMS and MFT) and each condition of the task (i.e., high and low working memory, and incongruent and congruent inhibitory control) were tested with analysis of covariance (ANCOVA) using post-intervention data as dependent variables, group (i.e., exercise vs. control) as fixed factor, and pre-intervention data as covariates. When needed, raw scores from each outcome were first winsorized to limit the influence of extreme values; this method allows replacing extreme high/low values for the closest (highest/lowest) valid value.[38] The z-scores for each cognitive outcome at post-intervention were also formed by dividing the difference of the raw score of each participant from the pre-intervention mean by the pre-intervention standard deviation, SD (i.e., (post-intervention individual value – pre-intervention mean) / pre-intervention SD), as in previous RCT.[38] This can be interpreted as an effect size indicator, since this z-score variable means how many SD the outcome has changed from pre-intervention. All the statistical procedures were performed using the SPSS software for Mac (version 20.0, IBM Corporation). A significance difference level of $P < 0.05$ was set.

Effects of the ActiveBrains program on current source density

The effects of the ActiveBrains program on current source density during DNMS and MFT tasks were tested with an independent groups analysis using sLORETA software. Through this test, the post and the pre-intervention current source densities in the exercise group were compared to those of the control group. Therefore, this test allowed to analyze which group had a higher change of the current source density from pre- to post-intervention. For this purpose, sLORETA estimations of current source densities of every participants in the exercise and control groups were submitted individually for each task condition to a mass-univariate *t* test (5,000 random samples). This test controls for multiple comparisons correction by using an approximation to the permutation analysis based on the empirical single-threshold of the t-max statistic.[39,40]

RESULTS

Effects of the ActiveBrains program on working memory and inhibitory control performance

Table 1 shows the pre-intervention characteristics of the study sample. **Table 2** presents the effects of the ActiveBrains program on raw and z-score post-intervention working memory and inhibitory control outcomes after adjustment for pre-intervention values. Overall, both the exercise and control groups significantly presented shorter RTs at post-intervention either in the low and high task conditions

Table 1. Pre-intervention characteristics of study sample.

	All		Exercise group		Control group	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Sex						
Girls (n %)	26	38.8%	11	31.4%	15	46.9%
Boys (n %)	41	61.2%	24	68.6%	17	53.1%
Age (years)	67	10.00 ± 1.10	35	9.95 ± 1.14	32	10.05 ± 1.08
Body mass index (kg/m^2)	67	26.58 ± 3.59	35	27.05 ± 4.10	32	26.06 ± 2.88
Wave of participation						
First (n %)	9	13.4%	6	17.1%	3	9.4%
Second (n %)	22	32.8%	11	31.4%	11	34.4%
Third (n %)	36	53.7%	18	51.4%	18	56.3%
Working memory	61		33		28	
Low working memory load						
Mean reaction time (ms)*		897.17 ± 153.52		890.56 ± 150.83		904.95 ± 159.05
Response accuracy (%)		81.35 ± 13.65		82.88 ± 12.63		79.55 ± 14.80
High working memory load						
Mean reaction time (ms)*		885.86 ± 153.54		873.44 ± 150.36		900.49 ± 158.70
Response accuracy (%)		68.22 ± 13.55		70.35 ± 13.07		65.71 ± 13.90
Inhibitory control	67		35		32	
Congruent condition						
Mean reaction time (ms)*		803.13 ± 122.39		810.56 ± 120.24		795.00 ± 126.12
Response accuracy (%)		93.82 ± 6.85		94.68 ± 6.13		92.88 ± 7.55
Incongruent condition						
Mean reaction time (ms)*		852.26 ± 144.49		852.25 ± 149.68		852.26 ± 140.98
Response accuracy (%)		85.92 ± 19.15		86.90 ± 18.20		84.85 ± 20.37

Values are expressed as means ± standard deviations (SD), unless otherwise indicated. *Higher values indicate lower performance. Working memory was measured by the Delayed non-match-to-sample task. Inhibitory control was measured by the Modified flanker task.

of the working memory task (i.e., DNMS) and either in the congruent and incongruent conditions of the inhibitory control task (i.e, MFT), although the post- – pre-intervention change between groups was non-significant for any of these tasks ($P \geq 0.484$).

Effects of ActiveBrains program on current source density

Table 3, **Figure 2,** and **Figure 3** present the brain regions for each hemisphere (left, L and right, R) and time frames (TFs) showing the post- – pre-intervention change in current source density between exercise and control groups during the performance of the working memory task. In the high working memory load condition, the exercise group, with respect to the control group, showed a higher increment of the current source density from pre- to post-intervention in 4 different TFs of the encoding phase of the task. In the TF from *−6,547 to −6,516 ms* (i.e., the time after the presentation of the 2nd stimuli), the current source density change was observed in 5 BAs (i.e., RBA13, 22, 31, 40 and 41) with peak t values ranging from 3.4 to 3.8 and cluster size (*k*) ranging from 11 to 39. In the TF from *−6,027 to −6,008 ms* (i.e., the time before the presentation of the 3rd stimuli), the current source density change was observed in 3 BAs, particularly in bilateral BA9 (peak t = 3.9, *k* = 23 for LBA9; peak t = 3.7, *k* = 15 for RBA9) and in LBA8 (peak t = 3.8, *k* = 26). In the TF from *−4,090 to −4,074 ms* (i.e., the time during the presentation of the last working memory stimuli), the current source density change between groups was observed in 4 BAs (i.e., LBA18, 22, 30 and 37) with peak t values ranging from 3.5 to 4.3 and cluster size ranging from 13 to 18. In the TF from *−4,027 to −3,988 ms* (i.e., time between the presentation of the last stimuli and the preparation for the maintenance phase), the current source density

Table 2. Effects of the ActiveBrains exercise program on raw and z-score post-intervention working memory and inhibitory control performance.

			Mean (95% CI)				
	N_{all}	N	Intervention group	N	Control group	Difference between groups	P
Working memory*	61	33		28			
Low load							
Mean reaction time†							
Raw score			845.96 (805.08 to 886.84)		824.91 (781.21 to 868.61)	21.05 (−38.81 to 80.90)	0.484
z-score			−0.33 (−0.60 to −0.07)		−0.47 (−0.76 to −0.19)	0.14 (−0.25 to 0.53)	
Response accuracy							
Raw score			82.72 (78.74 to 86.71)		83.81 (79.55 to 88.07)	−1.09 (−6.95 to 4.77)	0.712
z-score			0.10 (−0.19 to 0.39)		0.18 (−0.13 to 0.49)	−0.08 (−0.51 to 0.35)	
High load							
Mean reaction time†							
Raw score			827.59 (779.85 to 875.33)		829.92 (778.88 to 880.97)	−2.33 (−72.321 to 67.66)	0.947
z-score			−0.38 (−0.69 to −0.07)		−0.36 (−0.70 to −0.03)	−0.02 (−0.47 to 0.44)	
Response accuracy							
Raw score			68.05 (64.20 to 71.90)		68.80 (64.68 to 72.93)	−0.76 (−6.45 to 4.93)	0.791
z-score			−0.01 (−0.30 to 0.27)		0.04 (−0.26 to 0.35)	−0.06 (−0.48 to 0.36)	
Inhibitory control‡	67	35		32			
Congruent condition							
Mean reaction time†							
Raw score			757.62 (722.02 to 793.23)		757.70 (720.47 to 794.94)	−0.08 (−51.65 to 51.49)	0.998
z-score			−0.37 (−0.66 to −0.08)		−0.37 (−0.68 to −0.07)	−0.00 (−0.42 to 0.42)	
Response accuracy							
Raw score			95.11 (93.38 to 96.84)		94.49 (92.69 to 96.30)	0.612 (−1.90 to 3.13)	0.625
z-score			0.19 (−0.06 to 0.44)		0.10 (−0.17 to 0.36)	0.09 (−0.28 to 0.46)	
Incongruent condition							
Mean reaction time†							
Raw score			794.73 (757.91 to 831.54)		782.45 (743.95 to 820.96)	12.27 (−41.00 to 65.54)	0.647
z-score			−0.40 (−0.65 to −0.14)		−0.48 (−0.75 to −0.22)	0.08 (−0.28 to 0.45)	
Response accuracy							
Raw score			92.21 (89.58 to 94.83)		90.90 (88.16 to 93.65)	1.30 (−2.50 to 5.11)	0.496
z-score			0.33 (0.19 to 0.47)		0.26 (0.12 to 0.40)	0.07 (−0.13 to 0.27)	

z-score values indicate how many standard deviations have the post-intervention values changed with respect to the pre-intervention mean and standard deviation. E.g., a 0.14 z-score means that the mean value at post-intervention is 0.14 standard deviations higher than the mean value at pre-intervention, indicating a positive change, with negative values indicating the opposite. All data presented are adjusted for pre-intervention values. *Working memory was measured by the Delayed non-match-to-sample task. †Higher values indicate lower performance. ‡Inhibitory control was measured by the Modified flanker task.

Table 3. Brain regions and time frames (TF) showing post- – pre-intervention differential change in current source density (μA/mm²) between exercise and control groups during working memory and inhibitory control tasks.

BA	Hem	Brain region	X	Y	Z	Peak t	Cluster size
		Working memory*					
High load							
Encoding phase							
TF −6,547 to −6,516 ms							
13	R	Insula	35	−35	20	3.8	39
22	R	Superior temporal gyrus	50	−35	5	3.6	16
31	R	Cingulate gyrus	20	−45	25	3.5	11
40	R	Supramarginal gyrus	50	−50	20	3.4	19
41	R	Superior temporal gyrus	35	−35	15	3.8	22
TF −6,027 to −6,008 ms							
9	L	Medial frontal gyrus	−5	40	35	3.9	23
8	L	Superior frontal gyrus	−15	30	55	3.8	26
9	R	Medial frontal gyrus	0	40	35	3.7	15
TF −4,090 to −4,074 ms							
37	L	Fusiform gyrus	−55	−60	−20	3.5	17
30	L	Posterior cingulate	−10	−65	10	4.3	18
22	L	Superior temporal gyrus	−60	−45	5	3.7	13
18	L	Cuneus	−15	70	15	4.1	14
TF −4,027 to −3,988 ms							
11	R	Middle frontal gyrus	35	40	−20	2.7	24
13	R	Insula	35	15	15	3.2	101
20	R	Temporal Sub-gyral	40	−10	−25	2.7	18
22	R	Superior temporal gyrus	45	−20	0	2.9	14
38	R	Superior temporal gyrus	35	5	−15	2.8	32
41	R	Transverse temporal gyrus	40	−25	10	3.1	22
47	R	Inferior frontal gyrus	40	25	0	2.8	49
Maintenance phase							
No significant effects of exercise program							
Retrieval phase							
No significant effects of exercise program							
Low load							
No significant effects of exercise program							
		Inhibitory control†					
Incongruent condition							
TF 508 to 528 ms							
10	L	Middle frontal gyrus	−35	50	15	−4.1	5
Congruent condition							
No significant effects of exercise program							

BA = Brodmann areas; Hem = Hemisphere; L = Left; R = Right. *Working memory was measured by the Delayed non-match-to-sample task. †Inhibitory control was measured by the Modified flanker task. Independent groups analysis was performed using standardized low-resolution brain electromagnetic tomography (sLORETA) software to test post- – pre-intervention differential change between exercise and control groups (i.e., exercise group, post- – pre-current source density = control group, post- – pre-current source density). Multiple comparisons correction was performed using permutation analysis based on t-max threshold statistic. Anatomical coordinates (X, Y, Z) are given in Montreal Neurological Institute (MNI) Atlas space.

change between groups was observed in 7 BAs (RBA11, 13, 20, 22, 38, 41 and 47) with peak t values ranging from 2.7 to 3.2 and cluster size ranging from 14 to 101. There was not a significant current source density differential change between groups in TFs of the maintenance and retrieval phases of the high load condition and neither in the low working memory load condition.

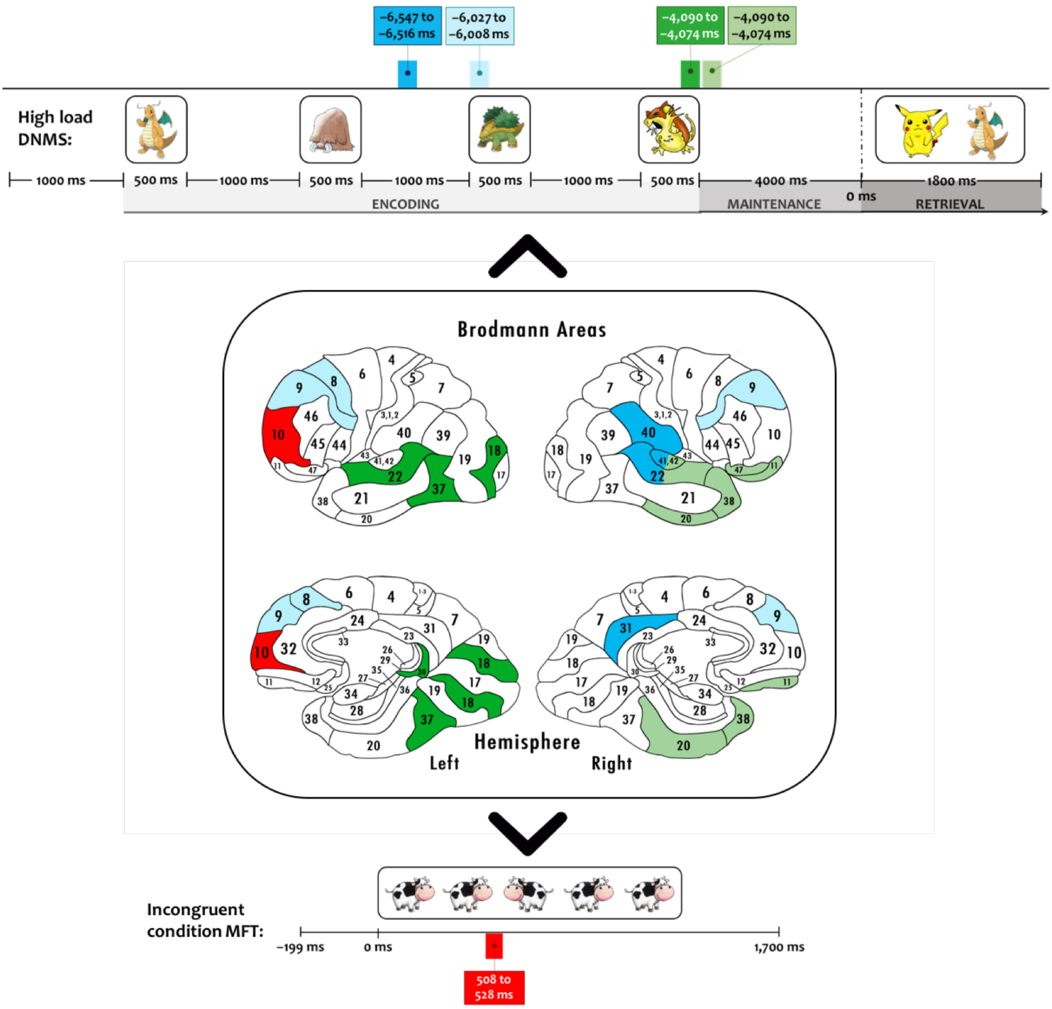

Figure 2. Graphical representation of Brodmann areas showing exercise-induced effects and times frames in which those effects occurred for working memory (i.e., Delayed non-matched-to-sample task, DNMS) and inhibitory control (i.e., Modified flanker task, MFT) tasks. Colors of Brodmann areas link to the colors of the times frames in which the effects of exercise were found.

Table 3, **Figure 2,** and **Figure 4** present the brain regions for each hemisphere and TFs showing the post- – pre-intervention differential change in current source density between exercise and control groups during the performance of an inhibitory control task. In the incongruent condition, only in the TF from *508 to 528 ms* (i.e., approximately the moment of information processing previous to the answer), the LBA10 showed a lower post- – pre-intervention current source density incremental change in the exercise group with respect to the control group (peak t = −4.1, *k* = 5). No significant current source density change was observed in the congruent condition of the task.

DISCUSSION

The present study, carried out in a sample of children with overweight/obesity, shows the effects of a long-term exercise program on the brain activity underlying

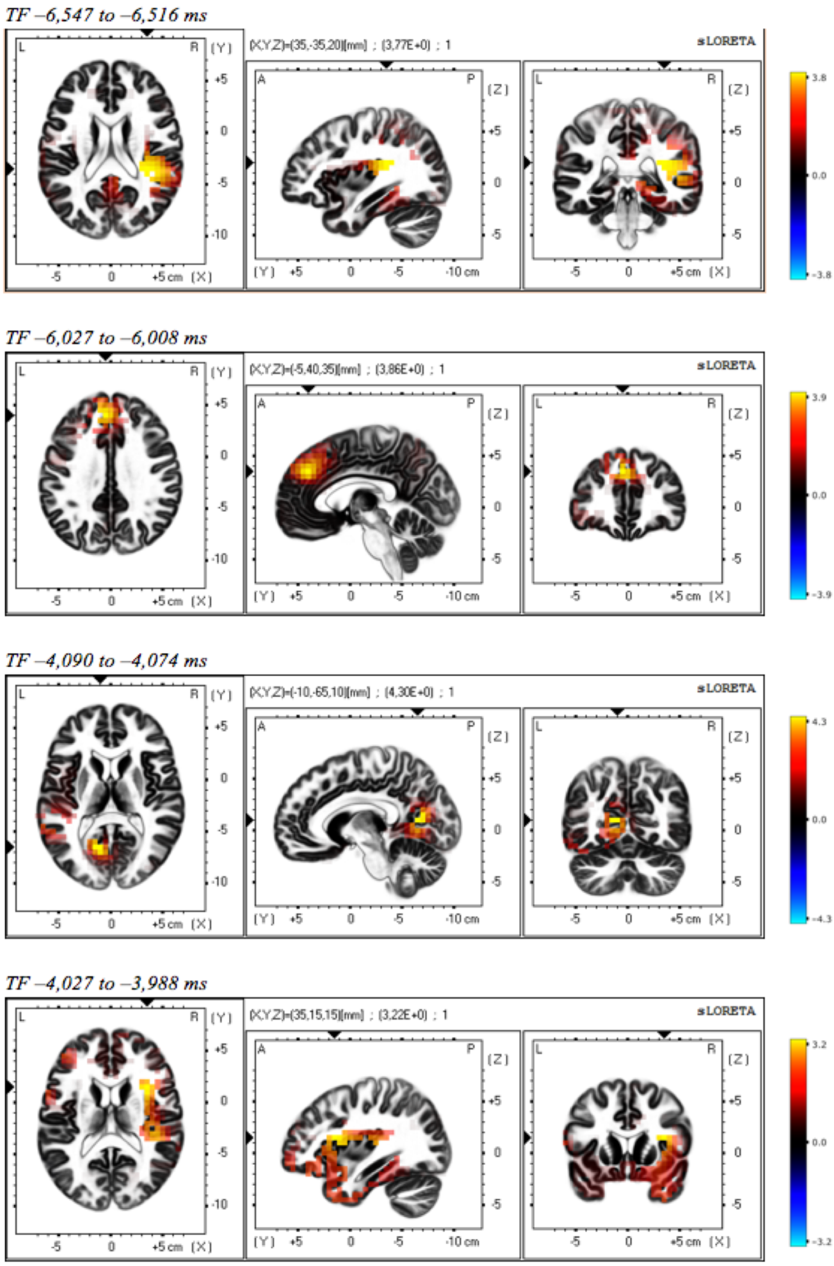

Figure 3. Brain areas and time frames (TFs) showing post- – pre-intervention differential change in current source density (µA/mm2) between exercise and control groups in the high load of the working memory task. L = Left; R = Right; A = Anterior; P = Posterior. Working memory was measured by the Delayed non-match-to sample task. Independent groups analysis was performed using standardized low-resolution brain electromagnetic tomography (sLORETA) software to test post- – pre-intervention differential change between exercise and control groups (i.e., exercise group, post- – pre-current source density = control group, post- – pre-current source density). The color bar represents peak t values (also shown between parenthesis following the coordinates), with red and yellow colors indicating that post- – pre-current source density's change was higher for the exercise group than for the control group, and blue colors indicating that post- – pre-current source density's change was higher for the control group than for the exercise group. Anatomical coordinates (X, Y, Z) are given in Montreal Neurological Institute (MNI) Atlas space.

working memory and inhibitory control based, for the first time, on a brain source analysis that takes advantage of the high temporal resolution of the EEG at the same time that provides a spatial representation. We found that both the exercise and control group improved task performance on working memory and inhibitory control tasks, although no differences between groups were observed. Importantly, between-groups differences in changes of current source density from pre- to post-intervention were seen even in the absence of tasks' performance differences. Particularly, children with overweight/obesity randomly assigned to a 4.5-month exercise program had a significantly higher increase of brain activation from pre- to post-intervention mainly in areas of the frontal and temporal lobes, and, specifically, during the encoding phase of the high working memory load condition of the DNMS, in comparison with peers from the control group. On the other hand, participants from the exercise group presented a lower current source density change from pre- to post-intervention in a single brain area of the frontal lobe during the phase of information processing previous to the answer of the incongruent condition of the inhibitory control task (i.e., MFT), in comparison with the exercise group. All these findings together support previous findings reporting that exercise effects may be selective of the most cognitively demanding tasks.[13]

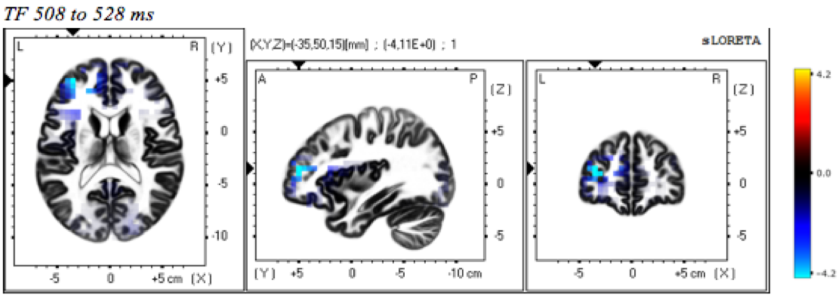

Figure 4. Brain areas and time frames (TFs) showing post- – pre-intervention differential change in current source density (µA/mm^2) between exercise and control groups in the incongruent condition of the modified flanker task. L = Left; R = Right; A = Anterior; P = Posterior. Independent groups analysis was performed using standardized low-resolution brain electromagnetic tomography (sLORETA) software to test post- – pre-intervention differential change between exercise and control groups (i.e., exercise group, post- – pre-current source density = control group, post- – pre-current source density). The color bar represents peak t values (also shown between parenthesis following the coordinates), with red and yellow colors indicating that post- – pre-current source density's change was higher for the exercise group than for the control group, and blue colors indicating that post- – pre-current source density's change was lower for the exercise group than for the control group. Anatomical coordinates (X, Y, Z) are given in Montreal Neurological Institute (MNI) Atlas space. Inhibitory control was measured by the Modified flanker task.

Our findings showing an equal improvement of tasks performance (i.e., shorter RTs in working memory and inhibitory control tasks) for both the exercise and control group and no differences between groups partially concur with those of a previous RCT (i.e., the SMART RCT) carried out also in children with overweight/obesity.[9] In that study, both the children participating in an 8-month exercise program and those from a control group showed an improvement of the response accuracy from pre- to post-intervention in a flanker task that measured inhibitory control.[9] They argued that the lack of differences between groups may be due to common factors, such as maturation or education.[9] In contrast to the before, a 9-month after-school PA program (i.e., the FITKids RCT), including at least 70 min of moderate-to-vigorous PA per day, resulted in improvements in working memory,[16] and inhibitory control,[8,13] only for those children belonging to the exercise group. However, other studies of a lower-quality design (i.e., no RCT), some also including a sample of children with overweight,[41] obtained similar findings to ours showing no effects of exercise on working memory[41,42] and inhibitory control.[42,43] The inconsistency of findings across studies might be due to differences with respect to the sample's characteristics (i.e., children with overweight/obesity versus normal weight peers), with respect to the design of the task used for assessing working memory (i.e., DNMS, Sternberg task,[16] Digit-span task,[42] or Random number generation task[41]), and inhibitory control (i.e., flanker task[8,9,13,43] or Stroop[42]), and with respect to different durations and design's characteristics of the interventions (i.e., 9 months,[8,13,16] 8 months,[9] 6 months,[41] 2 years[42] or 1.5 months[43]). Therefore, since an effect of the exercise program was only observed for current source density, this outcome may be more sensitive than tasks performance measures to exercise-induced changes. However, as a result of insufficient studies with a high-quality design (i.e., RCT) and the methodological differences between them, more well-designed RCTs are needed to draw conclusions about the effect of long- term exercise program on working memory and inhibitory control performance.

To the best of our knowledge, there is no previous evidence analyzing the effects of a long-term exercise program on brain activity underlying working memory in children, which hampers comparisons with previous investigations. The novel findings observed in the present study indicated that children with overweight/obesity from the exercise group had a significantly higher increase of brain activation from pre- to post-intervention during a high working memory load task than their peers from the control group. This activation occurred preferably in brain areas of the temporal and frontal lobes, and, to a lesser extent, in areas of the occipital and parietal lobes, and uniquely during the working memory encoding phase of the task (i.e., the processing of the memory set). More concretely, participants from the exercise group had a higher change of current source density during the time where higher demands to the working memory were requested (i.e., retention of 2nd, 3rd, and 4th stimuli) and this change occurred in a broad range of brain areas, rather than in a single one. Taking into account that previous neuroimaging literature has identified a broad network of brain areas (i.e., frontoparietal, motor and cingulate networks) underlying working memory function,[44] an explanation to our findings may be that exercise benefits the functional interaction of a range of brain areas rather than a single system. Despite this, the particular pre- to post-intervention brain activation increments observed in areas such as the cuneus (i.e., BA18), responsible for processing visual information,[45] or the posterior cingulate (i.e., BA30), which plays a crucial role in the storage of visual objects during a delayed period,[46] may be explained by that exercise enhances the capacity to visually process and store information during the memory set - target interval in children with overweight/obesity. Further, exercise may also benefit the access to storage information during working memory processes, as we observed that in the TF when the maintenance phase started, an increment of current source density occurred in prefrontal areas including the middle and inferior frontal gyri (i.g., BA11, BA47).[47] Collectively, all these findings have been shown to be biologically plausible as exercise seems to be beneficial for brain function and cognition reflected in the exercise-induced brain plasticity, angiogenesis and synaptogenesis shown in mammals.[48–50] Future RCTs addressing the long-term effects of exercise on the current source density underlying working memory are needed to confirm or contrast our findings.

A different brain activation pattern was observed during the performance of the incongruent condition of the inhibitory control task. In this case, the exercise group showed a lower increase of current source density from pre- to post-intervention in the frontal lobe, specifically the middle frontal gyrus (i.e., BA10), in comparison with the control group. Two previous RCTs, one in children with overweight/obesity[9] and another other in children with normal-weight,[8] used fMRI to assess the activation pattern of brain areas during a flanker task after an exercise program. While the RCT in children with overweight/obesity showed that the exercise group had a significantly higher increment of activation in the insula and superior temporal gyrus than the control group,[9] the RCT in children with normal-weight showed that the exercise group decreased brain activation in the prefrontal cortex reflecting at the same time better task performance (i.e., greater efficiency to use resources).[8] This decrease activation pattern partially concurs with our findings showing that children from the exercise group, in comparison with those from the control group, had a significantly lower increase of current source density from pre- to post-intervention in the frontal lobe during the information processing phase previous to the answer in the MFT. These findings may indicate a more efficient executive function from the perspective that less brain activation reflects more efficient brain functioning.[51,52] However, we did not observe between-groups differences in task performance and, therefore, it is difficult to make conclusions.

In our study, two different activation patterns were observed depending on the task. During the working memory task, the exercise group showed a higher increase in current source density from pre- to post-intervention in comparison with the control group, whereas during the inhibitory control task, the exercise group showed a lower increase. These differences in activation could be related to the different cognitive demands in each task. Thus, it could be that the DNMS task (i.e., working memory) was a much more complex task for the children than the MFT (i.e., inhibitory control). In this sense, previous research has suggested that some tasks are more sensitive to sustained activation, which is maintained throughout performance of the task, whereas some other tasks are more sensitive to transient activation, which includes processing specifically involved in each trial of the task.[53] In our case, it seems that the DNMS derived in a more transient activation, whereas the MFT derived in a more sustained activation. Taking this into account, it could be that sustained and transient current source density activation were differentially affected by exercise and therefore changes in current source density mainly reflected differences in task strategy between groups, rather than differences in task performance abilities.

The main limitations of the present study are that (i) the results are limited to a sample of children with overweight/obesity and comparison with peers with normal-weight are not possible; (ii) it is unknown whether current source density alterations would persist after a period of detraining; (iii) the sample size included for analyses of the present study might seem relatively small (N = 67), although it is the largest compared to previous studies analyzing the long-term effects of exercise on activity of specific brain areas. However, the previous studies used fMRI which is considered a more spatially precise measurement than brain activation derived from EEG recordings. In summary, the sample size and hence power is not an issue for all significant effects reported in this study; (iv) although several protocols were adopted to reduce the risk of bias in the evaluations (e.g. randomization after baseline assessment, physical trainers not involved in any evaluations, etc.), some of the project staff involved in the post-intervention evaluations were not fully blinded to the group allocation which could add some bias to the measurements. However, since all of the outcomes of the present study were assessed by the same evaluators, we believe is unlikely that the conclusions of this study would largely change due to this limitation. The present study includes also several strengths as follows: (i) To be the first study showing a new approach of EEG analysis based on brain source analyses in relation to exercise effects; (ii) the inclusion of a working memory task together with an EEG measurement and the study of the effects of exercise on current source density changes during this task; and (iii) the general use of standardized and validated instruments.

CONCLUSION

A 4.5-month after-school exercise program consisting in more than one hour of aerobic plus strength training 3 to 5 times per week induces brain activation changes as measured by current source density during working memory and inhibitory control in children with overweight/obesity. Children from the exercise group, in a higher extend than those from the control group, significantly increased the current source density of a broad network of brain areas primarily of the temporal, frontal and limbic lobes during a working memory task. More specifically, these effects were observed during the encoding phase of the high load condition, suggesting that a long-term practice of exercise might enhance the capacity to visually process and store information during working memory processes. On the other hand, children belonging to the exercise group showed a significantly lower increase in current source density of the frontal lobe during the information processing stream previous to the response in an inhibitory control task. However, between-groups differences were not observed in any task performance. Despite this, physical activity seems to alter brain activity what might have a positive influence in executive function. Whereas these findings are confirmed by future research, we believe health institutions should promote policies that increase PA opportunities during the school day and after-school.

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Study 7

Effects of a 4.5-month exercise program on intelligence, cognitive and academic performance, and brain structure in children with overweight/obesity

BACKGROUND

Childhood obesity has reached epidemic proportions globally,[1] which is a major public health problem due to the close link between obesity and disease. It is well known that pediatric obesity predicts future morbidity and mortality, as well as specific cardiovascular diseases, cancer, and psychiatric disorders.[2–4] Moreover, emerging evidence supports that obesity at early stages in life might impair cognitive and brain development.[5–7] This can have negative consequences for future life that go beyond the one directly related with cognition (e.g., more success in educational and professional careers) and affecting also individual's future health. As an example, it is known that the intelligent quotient (IQ) early in life inversely predicts all-cause mortality, as well as specific cardiovascular and cancer mortality.[8–11] In this context, the concept of brain health, as a composite of factors related with cognition, brain and mind has become popular in recent years.[12,13] Stimulating brain health from early stages in life will have an impact in future societies, making them smarter, more productive and healthier. In this context, childhood is a sensitive period for brain stimulation, as well as a crucial period for the establishment of healthy habits, such as a physically active lifestyle. Physical activity has the potential to benefit brain health,[12–14] which of course would be important for every child, but it might be particularly needed in children with overweight/obesity as a risk group for impaired brain health.[5–7]

A recent Position Stand of the American College of Sports Medicine (ACSM) concluded that there is evidence supporting that habitual physical activity can benefit cognition, and particularly executive function (cognitive flexibility, inhibition and working memory).[15] More recently, systematic reviews and meta-analyses have further supported a beneficial effect of physical activity interventions on executive functions and many of them also in academic performance, with the strongest evidence supporting a positive impact on mathematics.[13,16–21] Despite the large amount of studies in this field, the body of evidence is still in its infancy, and further well-designed randomized controlled trial are needed to shed light on many questions that remain unanswered.

First, while many studies focused on executive functions, intelligence has been rarely studied.[22–24] Further individual randomized controlled trials are needed to quantify the effect of physical exercise on crystallized and fluid intelligence. Second, when studying academic performance, school grades should be avoided due to the potential bias and heterogeneity of different teachers and school rating them, also due to the often categorical instead of continuous nature of school grades and ceiling effects. The use of academic performance standardized tests is recommended.[15] Third, while most of studies focused on behavioral outcomes (i.e. executive function, academic performance), only a few trials in children (i.e., the FITKids and FITKids2 trials, The Gerogia Trial and SMART trial), have analyzed the effects of exercise on brain changes as measured by structural (white matter integrity) or functional (resting and task-related) magnetic resonance imaging (MRI/fMRI),[25–32] To the best of our knowledge, no previous exercise trial examined the effects of exercise on grey matter volume in pediatric population. Particularly interesting is the study of hippocampal grey matter volume and yet it has been studied in many animal models, whether exercise can increase hippocampal volume or its subregions in childhood remains unknown.[33] Forth, whereas the Position Stand commented above and other recent systematic reviews have clearly shown a positive relationship of cardiorespiratory fitness (CRF) with brain function and structure, evidence is mainly based on observational studies.[15,34–36] In fact, we have previously reported consistent cross-sectional associations between CRF and grey matter volumes in children.[37–39] However, randomized controlled trials testing whether exercise-induced CRF improvements lead to enhancements in brain health, formally testing mediation, are lacking. Fourth, it is known that exercise interventions can result in a compensatory effect in the intervention group, so that the children enrolled in a new exercise program might reduce their activity levels when out from the exercise program sessions, which might lead to no real increase in overall activity levels of the week.[40,41] Likewise, the control group can be contaminated when participating in an exercise trial and it might increase their activity levels. In order to advance in this field, future randomized controlled trial should objectively monitor physical activity by accelerometry before and during the intervention in both exercise and control groups and be reported in the publications.[42] Fifth, the intensity of the exercise interventions should be carefully monitored so that all children wear individually programmed heart rate monitors in all the sessions during the program for a better interpretation of the actual stimuli applied and effects observed.

The present randomized controlled trial, namely ActiveBrains[43] will contribute to the existing knowledge in all these research directions by reporting the effects of the intervention on the study primary outcomes. Therefore, the primary aim of the present study was to investigate the effects of a 4.5-month exercise program on brain health, and specifically on intelligence, executive functions (cognitive flexibility, inhibition and working memory), academic performance and grey matter volumes in the whole brain, with hippocampus as main candidate region, in children with overweight/obesity. As a secondary aim, we investigated whether changes in CRF mediated the main effects observed in this intervention. In addition, we thoroughly analyzed the potential compensatory and contamination effect of the exercise and control groups respectively on overall activity levels, and the intensity of this exercise intervention. Concerning the primary aim of the study, we hypothesized that our exercise program would improve mainly executive function and to a lower extent academic performance, particularly mathematics. Improvements in intelligence were thought to be less probable, as well as it was unlikely that this relatively short-term exposure to exercise would lead to large changes in grey matter volumes.

METHODS

Design and Participants

The ActiveBrains project was a randomized controlled trial designed to examine the effects of a 4.5-month physical exercise program on brain, and cognitive and academic performance, as well as on selected physical and mental health outcomes in children with overweight/obesity (http://profith.ugr.es/activebrains, web available in Spanish and English). Details of the ActiveBrains project have been described elsewhere.[43] Briefly, the study was conducted in three waves temporarily differentiated due to recruitment and practical reasons. Eligible participants were children meeting the following inclusion/exclusion criteria: (1) To be 8 to 11.9 years-old; (2) to be classified as overweight or obese at baseline based on sex and age specific World Obesity Federation cut-off points;[44,45] (3) to not suffer from physical disabilities or neurological disorders that impeded them to exercise; (4) in the case of girls, not to have started the menstruation at the moment of baseline assessments; (5) to report no use of medications that influenced central nervous system function; (6) to be right-handed (i.e., measured by the Edinburgh inventory)[46] since right-handed individuals substantially differ in brain hemisphere structure (i.e., dominant and non-dominant hemisphere) from left-handed ones; and (7) to not report an attention-deficit hyperactivity disorder (ADHD) over the 85th percentile measured by the ADHD rating scale.[47]

Recruitment process started by contacting families with children with overweight/obesity from databases at the Unit of Pediatrics of the University Hospitals San Cecilio and Virgen de las Nieves (Granada, Spain). Additional strategies included contacting the head teacher of both, public and private schools, of Granada to spread informative pamphlets. Furthermore, advertising related to the project was broadcasted in the local media throughout newspaper, radio, and television. Finally, a total of 109 children with overweight/obesity met the general inclusion/exclusion criteria[43] to participate in this study and 89 children were included in the per-protocol analyses (see Statistical section) after meeting the following protocol criteria: (1) To complete the pre- and post-intervention assessments, (2) to attend to at least a 70% of the recommended 3 sessions/week (i.e., exercise group); and (iii) to keep their usual lifestyle (i.e., control group). The flowchart of the study is presented in **Figure 1**.

The participants were randomly allocated to an exercise group, which participated in the physical exercise program, or to a wait-list control group. The wait-list control group strategy has been previously used,[29,48] and implied that the individuals belonging to this group also received the exercise program after all the assessments of the project had been completed. Random allocation of the participants into exercise or control group was done using a computer random number generator in SPSS software for Windows (version 20.0; Armonk, NY, USA). This method allows for the equal probability of being allocated to one group or another. In order to reduce the risk of bias, several protocols were followed: (1) The computer random generation was conducted by a person not involved in the outcome evaluations; (2) randomization was done immediately after the baseline evaluation; and (3) the physical trainers running the exercise program were not involved in the outcome evaluations. However, due to practical reasons (limited number of project staff due to budget restrictions), some of the staff involved in the post-exercise evaluations were not fully blinded to the participants' group allocation (see discussion about this in the Limitation's section). All data were collected at baseline and post-exercise program from November 2014 through June 2016. The ActiveBrains project was approved by the Human Research Ethics Committee of the University of Granada, and it was registered in ClinicalTrials.gov (identifier: NCT02295072).

Physical exercise program

Characteristics of the exercise program

The physical exercise program had a duration of 4.5 months, and its design was based on meeting the international physical activity guidelines (http://www.health.gov/paguidelines/). Participants had the possibility to attend to the program daily from Monday to Friday (i.e., we offered them 5 sessions/week, 90 min/session). They were recommended to attend at least 3 times/week, yet we

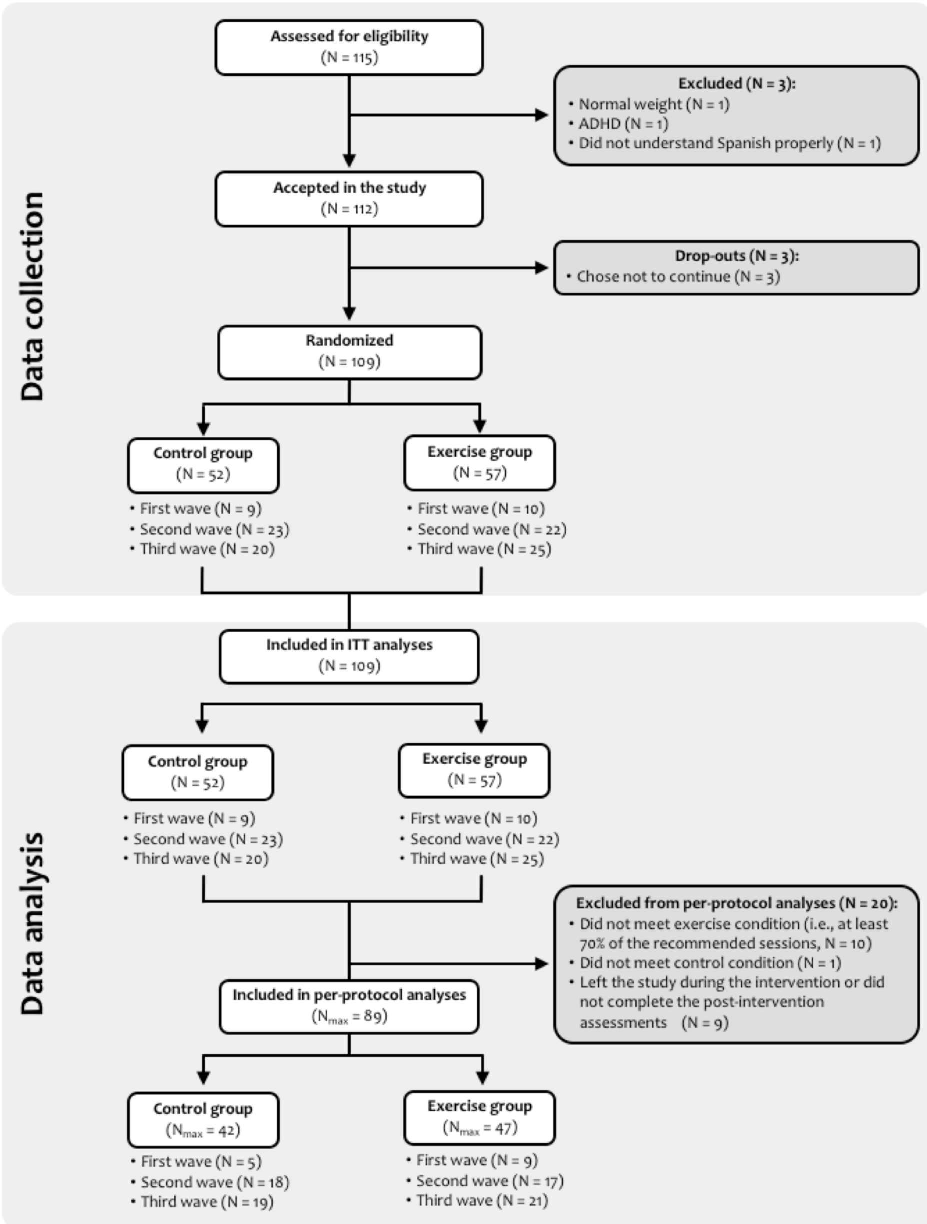

Figure 1. Flowchart of the study. ADHD = Attention-deficit hyperactivity disorder; ITT = Intention-to-treat. For final ITT analyses, those participants that left the study during the intervention or did not complete the post-intervention assessments were imputed (see Statistical section). N_{max} = Maximum N for analyses, it changes depending on the variable, see **Tables 1** and **2**, and **Figure 2**, for specific sample sizes for the main study outcomes.

advised the families that "the more, the better" up to the 5 sessions/week. Accordingly, the attendance criterion was set as a minimum of 3 times/week.

The physical exercise program was based on physical multi-games, with a noticeable emphasis on the playful component in order to increase the adherence to the program. Each session lasted 90 minutes and was structured in three parts: (1^{st}) A 5-10 min warm-up consisting of 1-2 physical games of 5 min each; (2^{nd}) a 60-min aerobic part consisting of around four to five physical multi-games demanding moderate-to-vigorous intensities, with special emphasis on high intensity activities; (3^{rd}) a 20-min resistance training consisting of muscle- and bone-strengthening game-based activities. The strength part included large-muscle-groups strength exercises in sets of 10-12 repetitions using therabands, fitballs and/or own bodyweight; and (4^{th}) a 5-10 min cool-down part consisting of stretching and relaxation exercises. Our intervention was mainly based on active games and can therefore be considered to have a moderate level of cognitive demands, more than just running on a treadmill or cycling on a stationary ergometer, but less than exercises specifically designed to be cognitively demanding.

Monitoring the intensity of the exercise program

The intensity of the exercise program was controlled in all the children and in all the sessions. Every child wore always the same heart rate monitor (POLAR RS300X, Polar Electro Oy Inc., Kempele, Finland) that was individually programmed based on the maximum heart rate that each child had previously achieved in the maximal incremental test (see CRF description below). The intensity progress was weekly checked by trained personnel to: (1) Adapt the intensity of the program progressively according to the improvements of the participants; (2) and also to identify whether any child was training at lower intensities than intended, requiring therefore higher motivation during the exercise sessions. The heart rate data were available for both parts of the session, i.e., aerobic exercise and resistance training, and the sum of both of them. Heart rate is a good indicator of the intensity of the aerobic training. Whereas heart rate might not directly reflect the intensity of resistance training at a muscle level, it does reflect the stimuli of exercise at heart level.

Control group condition

Children allocated into the control group were advised to continue with their usual life, yet we provided them with a pamphlet including nutrition and physical activity recommendations. All the children in the control group received the exercise intervention after the trial was completed (see wait-list control group description above). We detected that one of the children in the control group got enrolled in a swimming club with a heavy training load and competitions (training load 2h/day for 4-5 days/week). We decided to exclude this participant from the main analyses since he did not meet the control group's condition anticipated for a sample of children with overweight/obesity.

Outcome measurements

Every measurement was carried out always by same trained evaluators. Information on demographics was provided by self-report questionnaires.

Intelligence

The IQ was assessed by the Spanish version of Kaufman Brief Intelligence Test (K-BIT).[49] This test consists of vocabulary and matrices subtests which provided an indicator of crystallized intelligence and fluid intelligence, respectively. The typical punctuation of both, crystallized and fluid indicators of intelligence, were computed and a total intelligence score was obtained from the sum of them.

Executive function

Executive functions usually include the three following core-dimensions that were evaluated in this study: cognitive flexibility, inhibition, and working memory.[33]

Cognitive flexibility and inhibition were assessed through three different sub-scales from the Delis–Kaplan Executive Function System (D–KEFS) whose reliability has been proved elsewhere.[50–52] A full description of cognitive flexibility, inhibition, and working memory tests can be found elsewhere.[53,54] Cognitive flexibility was assessed using the Design Fluency Test and the Trail Making Test. The Design Fluency Test comprised three conditions: filled dots, empty dots, and switching. The participants were instructed to connect dots using only four straight lines to design as many novel shapes as possible during 60 seconds for each condition. The total number of correct drawn designs from all three conditions was registered. The Trail Making Test comprised five different conditions. We used condition 2 and condition 4, known as Part A and Part B, respectively. In Part A (i.e., Number Sequencing) participants had to draw lines to connect numbers 1–25 in ascending order as fast as possible. In Part B (i.e., Number-Letter Switching) participants had to draw a line to connect the numbers numerically and the letters alphabetically as fast as possible, switching each time from a number to a letter (e.g., 1–A–2–B, and so on). Part A had a maximum completion time of 2.5 min and Part B of 4 min. In case the children exceeded the maximum time, the test was stopped. The total completion time of Part A was subtracted from the total completion time of Part B as an indicator of cognitive flexibility.[55] A smaller B – A difference (sec) indicated better cognitive flexibility.

Inhibition was measured by a modified version of the Stroop test including four different conditions. We used condition 1 and condition 3. Condition 1 consisted of naming colors of filled rectangles. In condition 3, color-words were printed in a color that differs from their meaning (e.g. the word "red" printed in green) and the task consisted in naming the color of the word (i.e., green in the example) and avoid reading the word. The inhibition score was obtained by subtracting condition 3 completion time – condition 1 completion time (sec) as previously reported.[56] The lower the difference between tests' times (sec), the better the performance was considered.

Working memory was measured by a modified version of the Delayed Non-Match-to-Sample (DNMS) computerized task.[57] A total of 16 practice trials plus 140 experimental trials were presented focally on a computer screen using E-Prime software (Psychology Software Tools, Pittsburgh, PA). Each trial consisted of two phases (i.e., sample and choice) and two memory loads (i.e., high and low). For the present study, the high working memory load was used (i.e., 100 trials). The pre-target phase included a memory set of four different sequential stimuli (i.e., Pokemon cartoons) and participants were asked to memorize them. After the last stimuli, a target consisting of two different Pokemons was shown during the choice phase and participants were asked to select the cartoon that had not been previously shown. The response accuracy (%) in the high load was used as an indicator of working memory. Higher response accuracy refers to better performance.

The paper-pencil based tests were given altogether in a session of 40-50 min. All of them were scored and automatically entered by two separated investigators and in case of disagreement they checked it together to reach a conclusion. The DNMS working memory computerized task was given in a separate assessment that lasted 45-50 min.

Academic performance

Academic performance was assessed by the Spanish version of the Woodcock-Johnson III Tests of Achievement, which has been shown as a valid measure.[58] A total of 12 tests were individually administered by a trained evaluator in a session of 100-120 min. Of these 12 tests, there were 3 tests of reading, 3 tests of mathematics, 2 tests of oral language, 3 tests of written language and 1 test of social sciences and humanities. All tests were double-corrected, processed in the Compuscore and profile software version 3.1 (Riverside Publishing Company, Itasca, IL, USA) and standard scores of reading, mathematics, writing, academic skills (i.e., sum of tests based on basic skills such as reading decoding, mathematics calculation and spelling), academic fluency (i.e., sum of tests based on reading, calculation and writing fluency), problem solving (i.e., sum of tests based on solving academic problems in reading, mathematics and writing) and total achievement (i.e., overall measure of academic performance based on reading, mathematics and writing) were obtained.

Brain structure

Magnetic resonance imagining (MRI) data acquisition

All images were collected on a 3.0 Tesla Siemens Magnetom Tim Trio scanner (Siemens Medical Solutions, Erlangen, Germany) with a 32-channel head coil. High-resolution, T1-weighted images were acquired using a 3D MPRAGE (magnetization-prepared rapid gradient-echo) protocol. The acquisition parameters were the following: Repetition time (TR) = 2,300 ms; echo time (TE) = 3.1 ms; inversion time (TI) = 900 ms; flip angle = 9°; 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 = 6 min and 34 s.

Structural image processing

Hippocampal volumetric analyses. FMRIB's Integrated Registration and Segmentation Tool (FIRST), a semi-automated model-based segmentation tool in FMRIB's Software Library (FSL) version 5.0.7, was used for the hippocampal volumetric analyses. FIRST uses Bayesian framework from shape and appearance models obtained from manually segmented images from the Center for Morphometric Analysis, Massachusetts General Hospital, Boston, MA, USA.[59] Briefly, FIRST runs a two-stage affine registration to a standard space template (i.e., MNI space) using 12 degrees of freedom and uses a subcortical mask to exclude voxels outside subcortical regions. Second, subcortical regions, including hippocampus, are segmented for both hemispheres separately. Manual volumetric region labels are parameterized as surface meshes and modeled as a point distribution model. In addition, the hippocampus segmentation from FIRST was then split based on the center of gravity of the region into anterior and posterior sub-regions for each hemisphere separately. This resulted in separated anterior and posterior hippocampal segmentations for each participant, for each hemisphere.[60,61] The final segmentations were visually inspected for quality. The volume of each region was obtained from FIRST in mm^3.

Whole-brain volumetric analyses. Statistical Parametric Mapping software (SPM 12; Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab (MathWorks, Inc., Natick, MA) was used for the whole-brain volumetric analyses. Imaging pre-processing steps included quality control and alignment, segmentation into gray matter tissue, white matter tissue and cerebrospinal fluid. 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). Detailed information about pre-processing steps is described elsewhere.[39]

Body composition and biological maturational

Body weight was measured with an electronic scale (SECA 861, Hamburg, Germany) and height (cm) with a stadiometer (SECA 225, Hamburg, Germany). Both measurements were performed twice with participants barefoot and wearing light underclothes, and the averages were recorded. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Children were categorized as overweight, obesity grade I and obesity grade II/III according to age- and sex-specific BMI cutoff points.[44,45] Peak height velocity (PHV) is a common indicator of maturity in children and adolescents.[62] PHV was calculated from age and anthropometric variables following Moore's equations.[63] Years from PHV were calculated by subtracting the age of PHV from the chronological age. The difference in years was defined as a value of maturity offset.

Cardiorespiratory fitness

CRF was evaluated under laboratory conditions using a gas analyzer (General Electric Corporation) while performing a maximal incremental treadmill (hp-cosmos ergometer) test modified for unfit children.[64] Briefly, participants walk on a treadmill at a constant speed (4.8 km/h) with a starting 6% slope with grade increments of 1% every minute until volitional exhaustion. Maximal oxygen consumption (VO_2max) relative-to-body weight (mL/kg/min) and final completion time of the test (min), namely time-to-exhaustion, were registered and used in analyses.

Physical activity assessment before and during the intervention

We wanted to test whether the children in the exercise group increased the overall physical activity levels from baseline to during (i.e. right in the middle of the intervention, at week 10th) the exercise program, since it is known from previous literature that a compensatory effect might occur in the overall activity level.[40,41] Likewise, we wanted to test whether the control group kept its activity level or on the contrary, they increased their activity level as a contamination effect of being enrolled in a study or due to the pamphlet given to participants at the beginning of the trial, which included physical activity guidelines.

For these purposes, physical activity patterns at baseline and during the program were assessed by accelerometer (GT3X+, ActiGraph, Pensacola, Florida). The accelerometer data were processed as described elsewhere,[65] in accordance to the systematic review on data processing recently conducted by our group.[66] Participants were asked to wear the accelerometer on non-dominant wrist and right hip for 7 consecutive days (24 hours). A minimum of 4 valid days (i.e., ≥16 hours/day), including at least 1 weekend day, was required to be included in the analyses. For the present study, raw acceleration data from the right hip were used to compute Euclidean Norm Minus One G (ENMO) using the GGIR R package.[67] This was used as an indicator of overall activity,[67,68] yet the wrist-data were also used in sensitivity analyses.

Statistical analysis

Characteristics of the study sample are presented as mean and standard deviation (SD), or frequency and percentage as appropriate. The analyses of the effects of the ActiveBrains program on children with overweight/obesity were performed on the following primary outcomes: intelligence, executive functions, academic performance and grey matter volumes. Effects of the ActiveBrains program on CRF, as well as the mediation role of changes in CRF were also analyzed. First, an exploratory analysis was performed to test whether sex, biological maturation (PHV), or wave of participation should be treated as confounders in all analyses. For that purpose, we tested the role of these variables in main analyses (i.e., see analysis described below) and since the inclusion of them did not alter the results, these main analyses were performed without including these variables as confounders. All the statistical procedures were performed using the SPSS software for Mac (version 22.0, IBM Corporation). A significance difference level of $P < 0.05$ was set.

Primary Aim: Main effects of the ActiveBrains exercise program

For the main analysis, the effects on the outcomes for each dimension were tested according to the per-protocol principle with analysis of covariance (ANCOVA) using post-exercise program data as dependent variables, group (i.e., exercise vs. control) as fixed factor, and baseline data as covariates. Raw scores from each outcome were first winsorized (when needed) to limit the influence of extreme values; this method consists of replacing extreme high/low values for the closest (highest/lowest) valid value.[69] The z-scores for each outcome at post-exercise program were also formed by dividing the difference of the raw score of each participant from the baseline mean by the baseline SD (i.e., (post-exercise individual value – baseline mean) / baseline SD), as in previous randomized controlled trials.[69] Since this variable means how many SDs the outcome has changed from baseline, we interpreted it as an effect size indicator, in which a value around 0.2 is considered a small effect size, 0.5 is considered a medium effect size and 0.8 is considered a large effect size.[70]

Composite z-scores for cognitive flexibility (i.e., Design Fluency Test and Trail Making Test) and executive function (i.e., cognitive flexibility, inhibition, and working memory) were computed by averaging the z-scores for their individual components and renormalizing the average of z-scores to have a mean of 0 and a SD of ± 1 at baseline. For the computation of these composite scores, the averages were obtained only if there were available data for all the components included in the average. In addition, for the whole-brain volumetric analyses, we used the General Lineal Model (GLM) approach implemented in SPM12. Further, the previous ANCOVA analyses were also performed separately by sex (i.e., boys vs. girls), and years from PHV (i.e., < 2.16 vs. ≥ 2.16 years if below or above median values) to graphically show the effects of the ActiveBrains program by these sub-groups. To note is that this study was not powered to run analyses separately by sub-groups, so this analysis does not aim to test significance in the separate groups, but just to graphically see the consistency of the effect sizes.

Secondary Aim: Mediation role of cardiorespiratory fitness

We tested whether the effects of the intervention on the main study outcomes were mediated by changes in CRF following the bootstrapping method.[71] Mediation analyses were performed using the PROCESS macro for SPSS (SPSS Inc., Chicago, Illinois) with a resample procedure of 5,000 bootstrap samples. These mediation analyses were performed for the outcomes for which significant differences were observed between exercise and control groups in main effect analyses. The unstandardized (B) and standardized (β) regression coefficients are presented for four equations: Equation 1 regressed the mediator (i.e., change in CRF) on the independent variable (group). Equation 2 regressed the dependent variables (i.e., cognitive or academic performance outcomes) on the independent variable. Equations 3 regressed the dependent variables on both the mediator (equation 3) and the independent variable (equation 3'). We also included the cognitive or academic performance outcome of interest at baseline as confounder. The indirect effects along with its confidence intervals (CIs) were also presented and the significance was considered if the indirect effect significantly differed from zero (i.e., zero is not contained within the CIs). Finally, the percentage of total effect was computed to know how much of the total effect was explained by the mediation, as follows: (indirect effect / total effect) × 100. This mediation analysis was performed using the variable time-to-exhaustion during the treadmill test (min) as mediator variable since larger effects of the exercise program were observed on this CRF outcome. The analyses were also replicated using VO_2max (mL/kg/min) as mediator variable (data not shown).

Exploratory analysis 1: Intention-to-treat and dropout analyses

Supplementary analyses were conducted using the intention-to-treat principle (N = 109). For this purpose, multiple imputation was performed using the predictive mean matching approach. We performed 10 iterations to create 5 databases which were then averaged to obtain the imputed values for the intention-to-treat analyses.[72] In addition, we tested through analysis of variance (ANOVA) whether the participants that completed the baseline evaluations and randomization, but left the study during the intervention period or did not completed the post-exercise evaluations, namely the dropouts, differed in the main study variables from the participants who completed the study and post-exercise evaluations, namely the non-dropouts.

Exploratory analysis 2: Testing the potential compensatory effect of the intervention on overall activity levels

We performed a 1-dimension curve analysis using SPM1D package available for MATLAB (http://www.spm1d.org),[73] to study whether acceleration values (i.e., expressed as ENMO [*mg*]) identified a significant physical activity increment during-exercise program in comparison with the physical activity pattern at baseline for the control and exercise groups. SPM1D is a statistical parameter mapping tool using the random field theory and briefly allows to perform conventional statistics on 1-dimensional data, as is the case of the waveform acceleration data. Weekly average acceleration curves were presented separately for exercise and control groups from midnight (i.e., 00:00 AM) to the following midnight, i.e., 24h curves centered at noon (12PM). Paired T-tests over the curves were used to identify significant differences between baseline and during-exercise's physical activity patterns for each group throughout the day. SPM involved 4 steps to compute the t-test analysis:[74] (1) Computing the value of a test statistic at each point in the normalized time series; (2) estimating temporal smoothness on the basis of the average temporal gradient; (3) computing the value of test statistic above which only α = 5% of the data would be expected to reach had the test statistic trajectory resulted from an equally smooth random process; (4) computing the probability that specific suprathreshold regions could have resulted from an equivalently smooth random process.

RESULTS

All the baseline characteristics of the study sample are presented in **Table 1.** The study sample had an average age of 10 years (SD = 1.1), a biological maturation age of 2.3 years (SD = 1.0) before the age at which the maximum PHV occurs, and an average BMI of 26.7kg/m^2 (SD = 3.6) at baseline.

Primary aim: Effects of the exercise program on brain health outcomes

The main effects of the ActiveBrains exercise program are presented in **Table 2, Table 3, and Figure 2**, which show the post-exercise program differences between the exercise and the control group after adjustment for baseline values of the study outcomes according to the per-protocol analyses.

The largest effect of the whole exercise intervention was observed on total intelligence and particularly in crystallized intelligence, i.e., 0.62 (P = 0.00008) and 0.71 SDs (P < 0.0000001) showing respectively higher improvements in the exercise group compared to the control group, which can be considered a medium to large effect size (**Table 2**). In addition, the exercise program improved an averaged composite of cognitive flexibility derived from two different cognitive flexibility tests (0.45 SDs, P = 0.003). Particularly, the largest improvement was observed on the performance in the Design Fluency Test (Cognitive Flexibility 1), with the exercise group improving 0.5 SDs more than the control group (P = 0.001), which can be considered a medium effect size. A smaller non-significant effect was observed in the Stroop Color-Word Test (Cognitive Flexibility 2 0.28 SDs, P = 0.15). On the other hand, the exercise intervention had a null effect on the other two dimensions of the executive function, i.e., inhibition and working memory (P = 0.80).

Moreover, the exercise program improved total academic achievement, and particularly mathematics; a composite score of problem solving which includes not only mathematical problems but also problems related with reading and writing; and a composite score of academic skills which includes basic skills on reading decoding, mathematical calculations and spelling (**Table 3**). Overall the effect sizes observed on academic performance were of small magnitude, ranging from 0.22 in total academic achievement to 0.38 SDs in problem solving (P values ranged from 0.020 to 0.004). The exercise program had a small non-significant effect on reading and writing scales (0.16 and 0.20, respectively, P = 0.10) and had absolutely no effect on academic fluency, a composite score indicating how fast the reading, calculation and writing tasks were performed (P = 0.9).

The last primary outcome was grey matter volumes with hippocampus as a candidate region (**Figure 2**). Although the exercise group significantly (i.e. CI not including zero) increased the grey matter

Table 1. Descriptive baseline characteristics of the *ActiveBrains* participants meeting per-protocol criteria.

	All		Exercise group		Control group	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Sex						
Girls (n %)	35	39%	16	34%	19	45%
Boys (n %)	54	61%	31	66%	23	55%
Age (years)	89	10.02 ± 1.10	47	9.99 ± 1.12	42	10.06 ± 1.10
Weight (kg)	89	55.97 ± 11.11	47	57.39 ± 12.65	42	54.39 ± 9.0
Height (cm)	89	144.24 ± 8.12	47	143.92 ± 8.87	42	144.59 ± 7.29
Body mass index (kg/m^2)	89	26.69 ± 3.63	47	27.40 ± 4.07	42	25.90 ± 2.92
Peak height velocity (years)	89	−2.30 ± 0.96	47	−2.39 ± 0.92	42	−2.19 ± 1.0
Wave of participation (%)						
First (n %)	14	16%	9	19%	5	12%
Second (n %)	35	39%	17	36%	18	43%
Third (n %)	40	45%	21	45%	19	45%
Cardiorespiratory fitness						
Time in treadmill test (min)	89	8.55 ± 2.70	47	7.89 ± 2.65	42	9.30 ± 2.59
Relative VO_2max (mL/kg/min)	89	37.41 ± 4.77	47	36.34 ± 4.73	42	38.60 ± 4.57
Academic performance (standard score)						
Academic skills*	88	120.68 ± 14.89	47	122.66 ± 15.48	41	118.41 ± 14.0
Academic fluency†	88	104.06 ± 11.76	47	105.02 ± 10.25	41	102.95 ± 13.33
Problem solving‡	88	99.65 ±8.73	47	101.53 ± 8.83	41	97.49 ± 8.19
Reading	88	108.73 ± 12.44	47	110.66 ± 13.05	41	106.51 ± 11.47
Mathematics	88	102.19 ± 10.40	47	104.53 ± 10.61	41	99.51 ± 9.59
Writing	88	103.55 ± 8.91	47	103.06 ± 8.34	41	104.10 ± 9.59
Total achievement**	88	110.35 ± 11.33	47	112.28 ± 11.44	41	108.15 ± 10.92
Intelligence						
Crystallized intelligence (typical punctuation)††	89	47.65 ± 5.88	47	47.47 ± 5.56	42	47.86 ± 6.28
Fluid intelligence (typical punctuation)††	89	97.28 ± 13.07	47	95.77 ± 14.25	42	98.98 ± 11.55
Total intelligence (typical punctuation)††	89	98.27 ± 11.96	47	97.51 ± 12.73	42	99.12 ± 11.13
Executive functions						
Cognitive flexibility 1 (total correct designs)‡‡	89	20.32 ± 6.47	47	20.00 ± 5.89	42	20.67 ± 7.12
Cognitive flexibility 2 (sec)***	83	87.71 ± 43.61	47	88.13 ± 41.91	36	87.17 ± 46.33
Cognitive flexibility composite z-score†††	83	0.48 ± 1.01	47	−0.01 ± 0.92	36	0.13 ± 1.13
Inhibition (sec)‡‡‡	89	41.68 ± 19.67	47	41.64 ± 20.16	42	41.73 ± 19.34
Working memory (% response accuracy)****	85	65.08 ± 16.99	45	67.62 ± 14.60	40	62.21 ± 19.12
Executive function composite z-score††††	81	0.12 ± 1.0	45	0.03 ± 0.81	36	−0.02 ± 1.2
Hippocampal volume (mm^3)						
Whole Hippocampus	84	7142.12 ± 659.90	45	7082.52 ± 684.47	39	7210.89 ± 632.16
Right hippocampus	84	3643.23 ± 378.79	45	3599.50 ± 395.58	39	3693.69 ± 356.83
Right anterior hippocampus	84	2096.88 ± 238.28	45	2065.03 ± 241.50	39	2133.63 ± 232.14
Right posterior hippocampus	84	1545.38 ± 150.77	45	1534.48 ± 164.41	39	1557.96 ± 134.34
Left hippocampus	84	3498.89 ± 365.40	45	3483.02 ± 342.15	39	3517.20 ± 394.26
Left anterior hippocampus	84	2013.99 ± 212.74	45	2003.16 ± 200.36	39	2026.48 ± 228.20
Left posterior hippocampus	84	1484.90 ± 162.71	45	1479.88 ± 156.07	39	1490.72 ± 171.93

Values are expressed as means ± standard deviations (SD), unless otherwise indicated. *Academic skills are the sum of components based on basic skills such as reading decoding, mathematics calculation, and spelling. †Academic fluency is the sum of the components based on reading, calculation, and writing fluency. ‡Problem solving is the sum of the components based on solving academic problems in reading, mathematics, and writing. **Total achievement is the overall measure of the academic performance based on reading, mathematics, and writing. ††Crystallized, Fluid, and Total Intelligence were measured by the Kaufman Brief Intelligence Test. ‡‡Cognitive flexibility 1 was measured by the Design Fluency Test and expressed as number of total correct designs of the three conditions.

***Cognitive flexibility 2 was measured by the Trail Making Test and expressed as the total completion time (sec) of Part A subtracted from the total completion time (sec) of Part B. A smaller B – A difference score (sec) indicated better cognitive flexibility. †††Cognitive flexibility composite z-score was calculated as the re-normalized mean of the z-scores for Cognitive flexibility 1 and Cognitive flexibility 2. ǂǂǂInhibition was measured by the Stroop Color-Word Test. The inhibition score was obtained by subtracting condition 3 completion time – condition 1 completion time (sec). The lower the difference between tests' times, the better the performance was considered. ****Working memory was measured by the Delayed Non-Match-to sample task. ††††Executive function composite z-score was calculated as the re-normalized mean of the z-scores for Cognitive flexibility, Inhibition, and Working memory.

volume of the whole hippocampus (mean increase and 95% CI expressed in SDs, 0.20, 0.05–0.35), and no significant change was observed in the control group (0.08, −0.08–0.24), the differential change between both groups was non-significant (*P* = 0.28). No significant differences between groups were observed when the hippocampus was analyzed separately by the right and left hemispheres, nor when separated by anterior or posterior hippocampus (*P* > 0.05; **Table 4**). Likewise, a voxel-wise whole brain volumetric approach showed no significant effect of the exercise intervention in grey matter volume of any region of the brain nor in the total brain volumes (data not shown).

In **Figure 3**, we graphically show the effects of the ActiveBrains exercise program on the primary outcomes that were significantly improved and whether these effect sizes differed by sub-groups of sex and biological maturation (i.e., PHV). It can be observed that the effect size of the intervention was rather consistent across sex and maturation for cognitive flexibility, problems solving, mathematics and total academic achievement. On the contrary, a marked difference is observed in crystallized intelligence, in which the exercise program was less effective in girls and in less mature participants compared to boys and more mature participants respectively. Nevertheless, the effects observed in girls and less mature participants on crystallized intelligence were roughly of 0.5 SDs, which even if smaller than for boys it can still be considered a medium effect size. **Figure 3** shows also a lower effect in girls compared to boys in academic skills.

Secondary aim: Mediation role of cardiorespiratory fitness

In order to investigate the potential mediation role of CRF in the exercise intervention effects, we first studied the effect of the intervention on CRF **(Table 5)** and then conducted a formal mediation analysis (**Figure 4**). The exercise program improved the CRF performance as indicated by the time-to-exhaustion (i.e. the participant performed longer in the maximal incremental test), with 0.44 SDs larger improvement in the exercise vs. control group (*P* = 0.04; **Table 5)**. A smaller and borderline non-significant improvement was observed in the capacity of the cardiorespiratory system to provide oxygen to the muscles for a given body weight, i.e., maximal oxygen consumption, VO_2max expressed in mL/kg/min (0.32 SDs, *P* = 0.097; **Table 5**).

In **Figure 4**, we present the mediation modelling to test whether the effects of the exercise program observed on intelligence, cognitive flexibility and academic achievement was mediated by improvements in CRF performance, i.e. time-to-exhaustion. We used this indicator of CRF since it was the most influenced by the exercise program. A significant mediation effect ranging from roughly 10 to 20% was observed for crystallized intelligence, total academic achievement and problem solving. The

Table 2. Per-protocol effects of the *ActiveBrains* exercise program on raw and z-score post-exercise (i.e. z-score of change from baseline) intelligence and executive function dimensions

			Mean (95% CI)				
	N_{all}	N	Exercise group	N	Control group	Difference between groups	P
Crystallized intelligence*	89	47		42			
Raw score (typical punctuation)			111.04 (108.79 to 113.30)		101.97 (99.58 to 104.35)	9.07 (5.79 to 12.36)	**<0.0000001**
z-score			0.62 (0.44 to 0.80)		−0.098 (−0.29 to 0.09)	0.71 (0.45 to 0.97)	
Fluid intelligence*	89	47		42			
Raw score (typical punctuation)			103.13 (99.82 to 106.44)		100.23 (96.73 to 103.73)	2.90 (−1.92 to 7.73)	0.235
z-score			0.44 (0.18 to 0.69)		0.21 (−0.04 to 0.48)	0.22 (−0.14 to 0.58)	
Total intelligence*	89	47		42			
Raw score (typical punctuation)			106.29 (103.81 to 108.78)		98.75 (96.12 to 101.38)	7.55 (3.92 to 11.17)	**0.00008**
z-score			0.69 (0.48 to 0.89)		0.07 (−0.15 to 0.28)	0.62 (0.32 to 0.92)	
Cognitive flexibility 1†	89	47		42			
Raw score (total correct designs)			24.27 (22.95 to 25.60)		21.02 (19.61 to 22.42)	3.25 (1.32 to 5.18)	**0.001**
z-score			0.65 (0.44 to 0.85)		0.14 (−0.07 to 0.36)	0.50 (0.20 to 0.80)	
Cognitive flexibility 2‡	83	47		36			
Raw score (sec)			74.61 (63.37 to 85.84)		87.00 (74.16 to 99.83)	−12.39 (−29.45 to 4.67)	0.152
z-score			−0.35 (−0.60 to −0.09)		−0.06 (−0.36 to 0.23)	−0.28 (−0.67 to 0.10)	
Cognitive flexibility composite z-score**	83	47	0.25 (0.06 to 0.45)	36	−0.19 (−0.41 to 0.02)	0.45 (0.16 to 0.74)	**0.003**
Inhibition††	89	47		42			
Raw score (sec)			31.96 (28.28 to 35.64)		32.32 (28.47 to 36.17)	−0.60 (−5.92 to 4.70)	0.821
z-score			−0.52 (−0.73 to −0.30)		−0.48 (−0.71 to −0.25)	−0.04 (−0.35 to 0.28)	
Working memory‡‡	85	45		40			
Raw score (% accuracy)			65.23 (61.72 to 68.74)		66.03 (62.31 to 69.76)	−0.80 (−5.95 to 4.35)	0.757
z-score			0.01 (−0.20 to 0.22)		0.06 (−0.17 to 0.28)	−0.05 (−0.36 to 0.26)	
Executive function composite z-score***	81	45	0.13 (−0.06 to 0.33)	36	−0.09 (−0.31 to 0.12)	0.22 (−0.06 to 0.51)	0.125

z-score values indicate how many standard deviations have the post-exercise program values changed with respect to the baseline mean and standard deviation. E.g., a 0.50 z-score means that the mean value at post-exercise program is 0.50 standard deviations higher than the mean value at baseline, indicating a positive change, with negative values indicating the opposite. All data presented were adjusted for baseline values. *Crystallized, Fluid, and Total Intelligence were measured by the Kaufman Brief Intelligence Test. †Cognitive flexibility 1 was measured by the Design Fluency Test and expressed as number of total correct designs of the three conditions. ‡Cognitive flexibility 2 was measured by the Trail Making Test and expressed as the total completion time (sec) of Part A subtracted from the total completion time (sec) of Part B. A smaller B – A difference score (sec) indicated better cognitive flexibility. **Cognitive flexibility composite z-score was calculated as the re-normalized mean of the z-scores for Cognitive flexibility 1 and Cognitive flexibility 2. ††Inhibition was measured by the Stroop Color-Word Test. The inhibition score was obtained by subtracting condition 3 completion time – condition 1 completion time (sec). The lower the difference between tests' times, the better the performance was considered. ‡‡Working memory was measured by the Delayed Non-Match-to sample task. The response accuracy (%) in the high load was used as an indicator of working memory. Higher response accuracy refers to better performance. ***Executive function composite z-score was calculated as the re-normalized mean of the z-scores for Cognitive flexibility, Inhibition, and Working memory.

Table 3. Per-protocol effects of the *ActiveBrains* exercise program on raw (standard score) and z-score post-exercise (z-score of change from baseline) academic performance dimension (Woodcock-Muñoz standardized test)

			Mean (95% CI)				
	N_{all}	N	Exercise group	N	Control group	Difference between groups	*P*
Reading	88	47		41			
Raw score			111.57 (109.64 to 113.49)		109.44 (107.37 to 111.51)	2.12 (−0.72 to 4.97)	0.142
z-score			0.22 (0.07 to 0.38)		0.06 (−0.11 to 0.22)	0.16 (−0.05 to 0.39)	
Mathematics	88	47		41			
Raw score			105.70 (103.59 to 107.81)		101.99 (99.73 to 104.26)	3.70 (0.56 to 6.84)	**0.021**
z-score			0.35 (0.16 to 0.55)		0.01 (−0.20 to 0.22)	0.34 (0.05 to 0.63)	
Writing	88	47		41			
Raw score			118.83 (116.64 to 121.02)		116.30 (113.96 to 118.65)	2.53 (−0.68 to 5.74)	0.121
z-score			0.31 (0.13 to 0.48)		0.11 (−0.07 to 0.29)	0.20 (−0.05 to 0.45)	
Academic skills*	88	47		41			
Raw score			124.20 (121.72 to 126.68)		119.93 (117.27 to 122.58)	4.27 (0.62 to 7.92)	**0.022**
z-score			0.27 (0.11 to 0.43)		−0.00 (−0.17 to 0.17)	0.27 (0.04 to 0.50)	
Academic fluency†	88	47		41			
Raw score			106.10 (104.15 to 108.05)		106.23 (104.17 to 108.30)	−0.077 (−2.874 to 2.72)	0.957
z-score			0.16 (0.00 to 0.32)		0.16 (−0.00 to 0.34)	−0.01 (−0.24 to 0.23)	
Problem solving‡	88	47		41			
Raw score			103.11 (101.51 to 104.70)		99.62 (97.92 to 101.33)	3.48 (1.11 to 5.85)	**0.004**
z-score			0.41 (0.24 to 0.59)		0.03 (−0.15 to 0.22)	0.38 (0.12 to 0.64)	
Total achievement**	88	47		41			
Raw score			113.57 (112.01 to 115.12)		110.90 (109.23 to 112.57)	2.66 (0.37 to 4.96)	**0.023**
z-score			0.31 (0.18 to 0.44)		0.06 (−0.05 to 0.22)	0.22 (0.03 to 0.41)	

z-score values indicate how many standard deviations have the post-exercise program values changed with respect to the baseline mean and standard deviation. E.g., a 0.50 z-score means that the mean value at post-exercise program is 0.50 standard deviations higher than the mean value at baseline, indicating a positive change, with negative values indicating the opposite. All data presented were adjusted for baseline values. *Academic skills are the sum of components based on basic skills such as reading decoding, mathematics calculation, and spelling. †Academic fluency is the sum of the components based on reading, calculation, and writing fluency. ‡Problem solving is the sum of the components based on solving academic problems in reading, mathematics, and writing. **Total achievement is the overall measure of the academic performance based on reading, mathematics, and writing

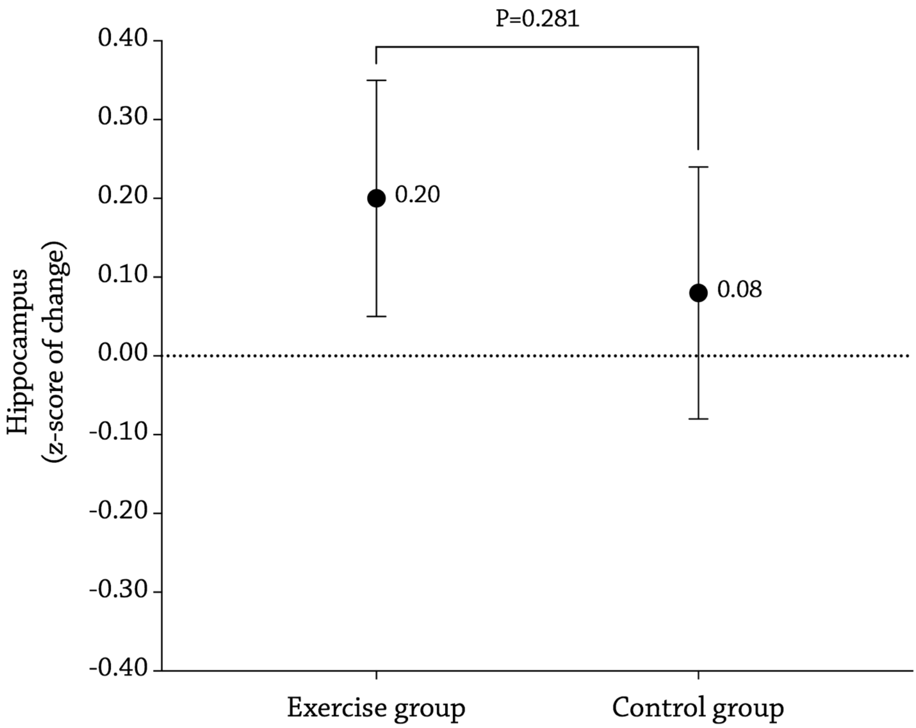

Figure 2. Per-protocol effects of the *ActiveBrains* exercise program on z-score post-exercise hippocampal grey matter volume separated by groups. Analysis was adjusted by baseline outcomes. Dots represent z-score values of change with respect to the baseline mean and standard deviation. Bars represent 95% confidence intervals.

mediation role of CRF on these primary outcomes remained significant when using VO_2max as indicator of CRF instead of the time-to-exhaustion (data not shown).

Exploratory analyses

Intention-to-treat and dropout analyses

All main analyses (i.e., **Tables 2**, **Table 3** and **Table 4**) were replicated following the intention-to-treat principle, i.e. including participants not meeting the requirement of a minimum 70% attendance to the exercise program as well as the dropouts during the intervention by multiple imputation of the missing values at post-exercise, and the results are presented in **Table 6 to 8**. Overall, the effects shown in the intention-to-treat analyses were attenuated compared to the per-protocol effects. Nevertheless, the strongest effects observed in total intelligence and crystallized intelligence, as well as in cognitive flexibility remained significant (*P* values ranging from 0.01 to <0.00001; **Table 6**). The effects on academic achievement were also attenuated becoming borderline non-significant in most of cases (*P* values of ~ 0.08) and remaining significant in the case of academic skills (*P* = 0.02; **Table 7**). Effects on hippocampus that were not significant in per-protocol analyses remained as such in intention-to-treat analyses (**Table 8**). The dropout analyses showed that none of the study outcomes at baseline differed between dropouts and non-dropouts, except for the

Table 4. Per-protocol effects of the *ActiveBrains* exercise program on raw (mm³) and z-scores of post-exercise hippocampal grey matter volume.

			Mean (95% CI)				
	N_{all}	N	Exercise group	N	Control group	Difference between groups	*P*
Hippocampus	84	45		39			
Raw score			7209.92 (7106.91 to 7312.94)		7127.23 (7016.53 to 7237.92)	82.70 (−68.88 to 234.27)	0.281
z-score			0.20 (0.05 to 0.35)		0.08 (−0.08 to 0.24)	0.12 (−0.10 to 0.34)	
Right hippocampus	84	45		39			
Raw score			3692.57 (3633.11 to 3752.03)		3653.59 (3589.68 to 3717.50)	38.97 (−48.65 to 126.61)	0.379
z-score			0.23 (0.08 to 0.38)		0.13 (−0.04 to 0.29)	0.10 (−0.13 to 0.33)	
Right anterior hippocampus	84	45		39			
Raw score			2124.45 (2085.99 to 2162.91)		2101.53 (2060.19 to 2142.88)	22.91 (−33.84 to 79.67)	0.424
z-score			0.21 (0.06 to 0.37)		0.12 (−0.05 to 0.30)	0.09 (−0.14 to 0.32)	
Right posterior hippocampus	84	45		39			
Raw score			1565.22 (1539.08 to 1591.36)		1547.77 (1519.68 to 1575.85)	17.44 (−20.97 to 55.87)	0.369
z-score			0.22 (0.05 to 0.40)		0.11 (−0.08 to 0.29)	0.11 (−0.14 to 0.37)	
Left hippocampus	84	45		39			
Raw score			3515.76 (3447.13 to 3584.39)		3475.46 (3401.73 to 3549.18)	40.30 (−60.47 to 141.08)	0.429
z-score			0.13 (−0.05 to 0.31)		0.03 (−0.17 to 0.22)	0.11 (−0.16 to 0.37)	
Left anterior hippocampus	84	45		39			
Raw score			2021.21 (1981.61 to 2060.80)		1993.49 (1950.96 to 2036.03)	27.71 (−30.44 to 85.87)	0.346
z-score			0.12 (−0.05 to 0.30)		−0.00 (−0.19 to 0.19)	0.12 (−0.14 to 0.38)	
Left posterior hippocampus	84	45		39			
Raw score			1494.46 (1459.24 to 1529.68)		1477.86 (1440.02 to 1515.69)	16.60 (−35.10 to 68.30)	0.525
z-score			0.13 (−0.08 to 0.34)		0.03 (−0.19 to 0.26)	0.10 (−0.21 to 0.40)	

z-score values indicate how many standard deviations have the post-exercise program values changed with respect to the baseline mean and standard deviation. E.g., a 0.50 z-score means that the mean value at post-exercise program is 0.50 standard deviations higher than the mean value at baseline, indicating a positive change, with negative values indicating the opposite. All data presented were adjusted for baseline values.

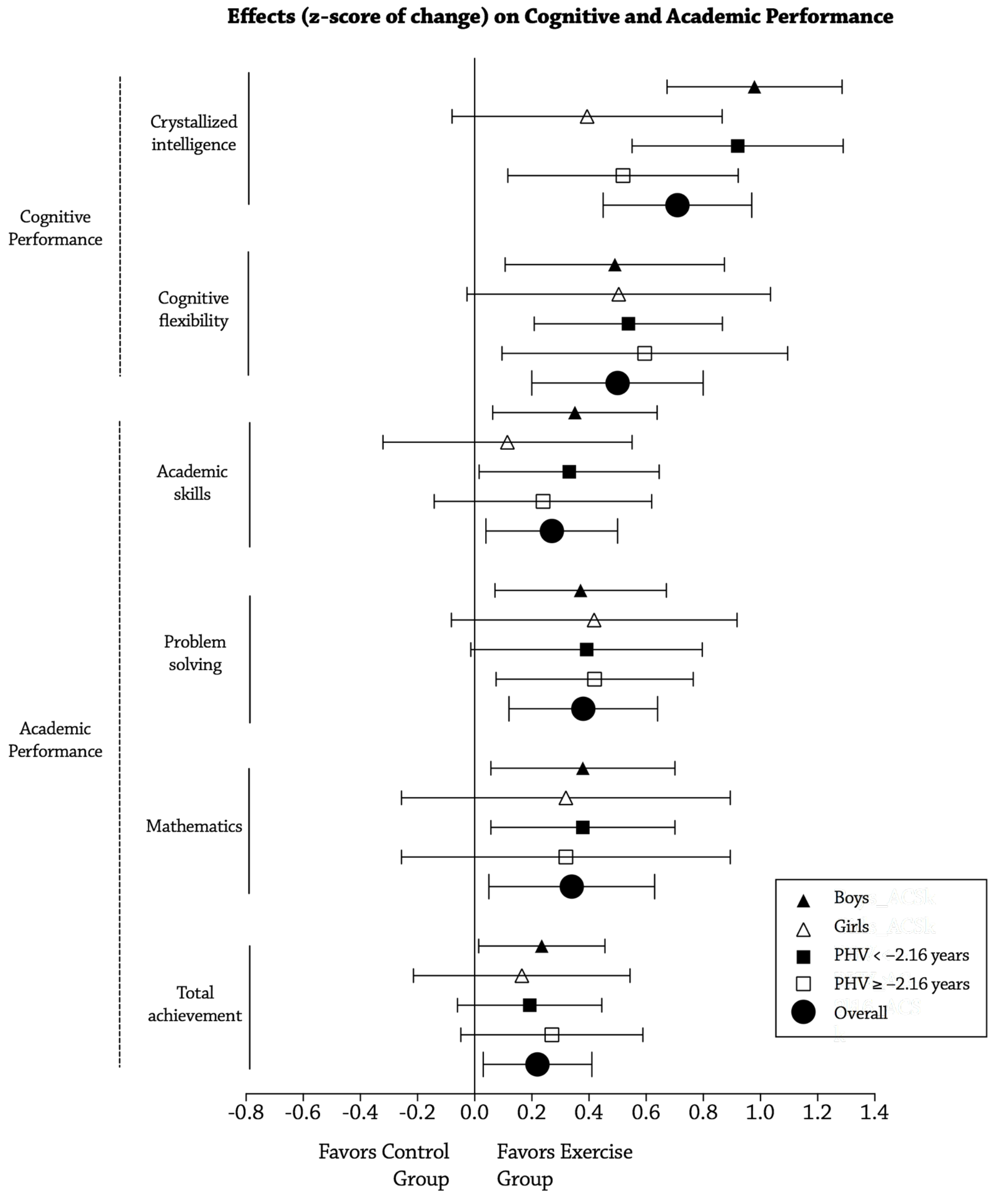

Figure 3. Per-protocol overall and by sex and biological maturation sub-groups effects of the *ActiveBrains* exercise program on the significantly influence post-exercise program outcomes. Each analysis was adjusted by baseline outcomes. Dots represent z-score values of change with respect to the baseline mean and standard deviation. Bars represent 95% confidence intervals. Years from peak height velocity (PHV) were calculated by subtracting the age of PHV from the chronological age. The difference in years was utilized as a measure of maturity. Academic skills are the sum of components based on basic skills such as reading decoding, mathematics calculation, and spelling. Problem solving is the sum of the components based on solving academic problems in reading, mathematics, and writing. Total achievement is the overall measure of the academic performance based on reading, mathematics, and writing. Crystallized intelligence was measured by the Kaufman Brief Intelligence Test. Cognitive flexibility was measured by the Design Fluency Test.

Table 5. Per-protocol effects of the *ActiveBrains* exercise program on raw and z-scores of post-exercise cardiorespiratory fitness.

			Mean (95% CI)				
	N_{all}	N	Exercise group	N	Control group	Difference between groups	*P*
Time-to-exhaustion in the maximal incremental test	89	47		42			
Raw score (min)			9.91 (9.15 to 10.67)		8.73 (7.92 to 9.53)	1.18 (0.05 to 2.30)	**0.040**
z-score			0.56 (0.28 to 0.84)		0.12 (-0.18 to 0.42)	0.44 (0.02 to 0.86)	
VO_2max	89	47		42			
Raw score (mL/kg/min)			38.98 (37.77 to 40.20)		37.47 (36.18 to 38.75)	1.51 (−0.28 to 3.31)	0.097
z-score			0.39 (0.13 to 0.64)		0.07 (−0.21 to 0.34)	0.32 (−0.06 to 0.70)	

z-score values indicate how many standard deviations have the post-exercise program values changed with respect to the baseline mean and standard deviation. E.g., a 0.50 z-score means that the mean value at post-exercise program is 0.50 standard deviations higher than the mean value at baseline, indicating a positive change, with negative values indicating the opposite. All data presented were adjusted for baseline values. VO_2max = Maximal Oxygen Consumption.

hippocampus grey matter volumes that were in some cases significantly lower in the dropouts (*P* < 0.05).

Testing the potential compensatory effect of the intervention on overall activity levels

We measured overall physical activity before (baseline) and during the intervention (week 10^{th}) with accelerometer data processed to derive ENMO (mg). The SPM1D analysis of the whole 24h activity curve (mg) shows how the children enrolled in the exercise group significantly increased their activity levels (suprathreshold cluster: P < 0.001), when the exercise program took place, and did not change their activity levels in the hours out from the exercise program. The children enrolled in the control group kept the same levels of activity all through the day before and during the intervention (**Figure 5**). Noteworthy is that results were similar when using the data derived from the wrist-attached accelerometer (data not shown).

Characterization of the intensity achieved in the exercise program

The analyses of the heart rate monitored through all the sessions of the exercise program showed that, during the aerobic part of the training sessions, the children had an average heart rate of 148bpm (standard deviation, SD = 9bpm). Considering their individual maximum heart rate reached in the incremental maximal test, we observed that the children trained during the aerobic part 47min (SD = 3min) at an average intensity of 75% (SD=3%) of their maximal heart rate. The resistance training part of the sessions lasted in average 20min, during which the children had an average heart rate of 127bpm (SD = 8bpm), which will be equivalent to 64% (SD = 4%) of their maximum heart rate. This result in a total of 66min (SD = 3min) of aerobic plus resistance training exercise, at an average heart rate of 138bpm (SD = 8bpm), which means that the children trained for more than 1h at 70% of their maximum heart rate.

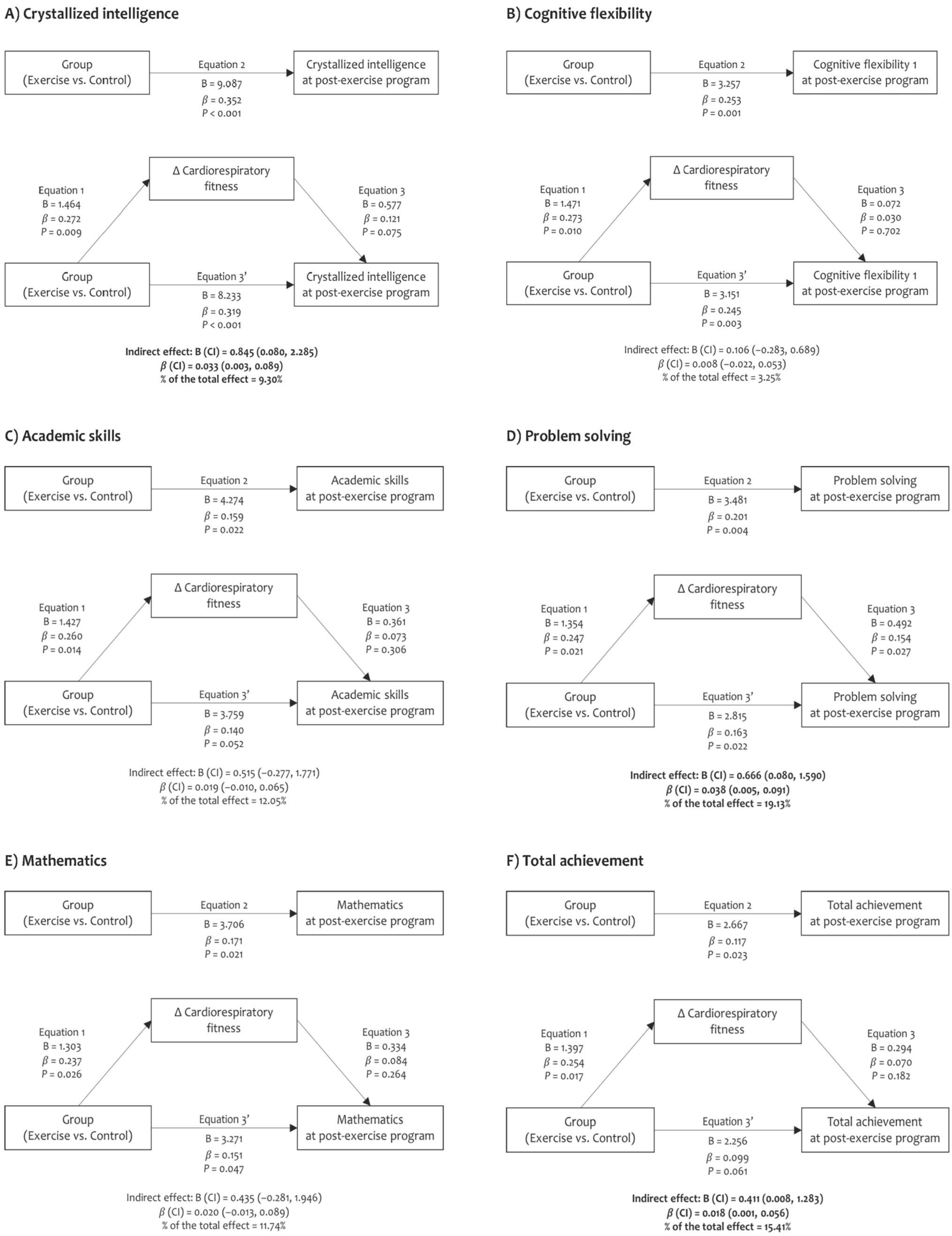

Figure 4. Cardiorespiratory fitness' change mediation models of the relationship of group (i.e., exercise vs. control) with cognitive and academic performance outcomes in children with overweight/obesity. Each analysis was adjusted by the respective cognitive or academic performance outcome at baseline. Bold font indicates significant indirect effect at P<0.05. Delta cardiorespiratory fitness expresses the change in total completion time (min) of the treadmill test at post-exercise program with respect to the total completion time (min) at baseline, since it was the main cardiorespiratory fitness outcome influenced by the exercise program. Academic skills are the sum of components based on basic skills such as reading decoding, mathematics calculation, and spelling. Problem solving is the sum of the components based on solving academic problems in reading, mathematics, and writing. Total achievement is the overall measure of the academic performance based on reading, mathematics, and writing. Crystallized intelligence was measured by the Kaufman Brief Intelligence Test. Cognitive flexibility 1 was measured by the Design Fluency Test.

Table 6. Intention-to-treat effects of the *ActiveBrains* exercise program on raw and z-scores of post-exercise intelligence and executive functions.

			Mean (95% CI)				
	N_{all}	N	Exercise group	N	Control group	Difference between groups	*P*
Crystallized intelligence*	109	57		52			
Raw score (typical punctuation)			109.78 (107.73 to 111.83)		102.13 (99.96 to 104.3)	7.65 (4.66 to 10.63)	**<0.0000001**
z-score			0.52 (0.36 to 0.68)		−0.07 (−0.24 to 0.09)	0.59 (0.36 to 0.82)	
Fluid intelligence*	109	57		52			
Raw score (typical punctuation)			103.53 (100.62 to 106.44)		99.49 (96.41 to 102.56)	4.04 (−0.20 to 8.28)	0.062
z-score			0.46 (0.24 to 0.68)		0.15 (−0.09 to 0.38)	0.31 (−0.02 to 0.63)	
Total intelligence*	109	57		52			
Raw score (typical punctuation)			105.69 (103.40 to 107.99)		98.79 (96.36 to 101.21)	6.91 (3.56 to 10.25)	**0.00001**
z-score			0.57 (0.38 to 0.75)		0.02 (−0.18 to 0.21)	0.55 (0.28 to 0.82)	
Cognitive flexibility 1†	109	57		52			
Raw score (total correct designs)			24.07 (22.84 to 25.29)		21.52 (20.23 to 22.81)	2.55 (0.77 to 4.33)	**0.005**
z-score			0.63 (0.44 to 0.82)		0.24 (0.04 to 0.44)	0.39 (0.12 to 0.67)	
Cognitive flexibility 2‡	109	57		52			
Raw score (sec)			77.38 (66.97 to 87.79)		87.61 (74.98 to 100.24)	−10.23 (−26.60 to 6.14)	0.237
z-score			−0.29 (−0.52 to −0.06)		−0.09 (−0.33 to 0.15)	−0.20 (−0.53 to 0.13)	
Cognitive flexibility composite z-score**	109	57	0.16 (−0.02 to 0.34)	52	−0.18 (−0.37 to 0.01)	0.34 (0.08 to 0.60)	**0.012**
Inhibition††	109	57		52			
Raw score (sec)			31.20 (27.96 to 34.44)		33.80 (30.37 to 37.22)	−2.59 (−7.31 to 2.12)	0.278
z-score			−0.57 (−0.76 to −0.37)		−0.41 (−0.61 to −0.21)	−0.15 (−0.43 to 0.12)	
Working memory‡‡	109	57		52			
Raw score (% accuracy)			66.75 (63.49 to 70.01)		64.84 (61.39 to 68.29)	1.91 (−2.86 to 6.67)	0.429
z-score			0.09 (−0.11 to 0.28)		−0.03 (−0.24 to 0.18)	0.12 (−0.17 to 0.40)	
Executive function composite z-score***	109	57	0.15 (−0.03 to 0.32)	52	−0.16 (−0.34 to 0.02)	0.31 (0.06 to 0.56)	**0.016**

z-score values indicate how many standard deviations have the post-exercise program values changed with respect to the baseline mean and standard deviation. E.g., a 0.50 z-score means that the mean value at post-exercise program is 0.50 standard deviations higher than the mean value at baseline, indicating a positive change, with negative values indicating the opposite. All data presented were adjusted for baseline values. *Crystallized, Fluid, and Total Intelligence were measured by the Kaufman Brief Intelligence Test. †Cognitive flexibility 1 was measured by the Design Fluency Test and expressed as number of total correct designs of the three conditions. ‡Cognitive flexibility 2 was measured by the Trail Making Test and expressed as the total completion time (sec) of Part A subtracted from the total completion time (sec) of Part B. A smaller B – A difference score (sec) indicated better cognitive flexibility. **Cognitive flexibility composite z-score was calculated as the re-normalized mean of the z-scores for Cognitive flexibility 1 and Cognitive flexibility 2. ††Inhibition was measured by the Stroop Color-Word Test. The inhibition score was obtained by subtracting condition 3 completion time – condition 1 completion time (sec). The lower the difference between tests' times, the better the performance was considered. ‡‡Working memory was measured by the Delayed Non-Match-to sample task. The response accuracy (%) in the high load was used as an indicator of working memory. Higher response accuracy refers to better performance. ***Executive function composite z-score was calculated as the re-normalized mean of the z-scores for Cognitive flexibility, Inhibition, and Working memory.

Table 7. Intention-to-treat effects of the *ActiveBrains* exercise program on raw (standard score) and z-scores of post-exercise academic performance dimension (Woodcock-Muñoz standardized test).

			Mean (95% CI)				
	N_{all}	N	Exercise group	N	Control group	Difference between groups	*P*
Academic skills*	109	57		52			
Raw score			124.06 (121.57 to 126.54)		119.78 (117.15 to 122.41)	4.28 (0.65 to 7.90)	**0.021**
z-score			0.34 (0.18 to 0.49)		0.06 (−0.10 to 0.23)	0.27 (0.04 to 0.50)	
Academic fluency†	109	57		52			
Raw score			104.99 (103.17 to 106.80)		106.00 (104.08 to 107.92)	−1.01 (−3.65 to 1.64)	0.451
z-score			0.08 (−0.07 to 0.23)		0.16 (0.00 to 0.32)	−0.08 (−0.30 to 0.14)	
Problem solving‡	109	57		52			
Raw score			102.38 (100.69 to 104.07)		100.16 (98.37 to 101.94)	2.22 (−0.27 to 4.72)	0.08
z-score			0.29 (0.10 to 0.47)		0.05 (−0.15 to 0.24)	0.24 (−0.03 to 0.51)	
Reading	109	57		52			
Raw score			110.81 (108.98 to 112.65)		109.42 (107.48 to 111.36)	1.39 (−1.30 to 4.08)	0.307
z-score			0.19 (0.05 to 0.33)		0.08 (−0.07 to 0.23)	0.11 (−0.10 to 0.32)	
Mathematics	109	57		52			
Raw score			105.17 (103.02 to 107.31)		102.33 (100.05 to 104.60)	2.84 (−0.32 to 6.01)	0.078
z-score			0.29 (0.09 to 0.49)		0.03 (−0.18 to 0.24)	0.26 (−0.03 to 0.55)	
Writing	109	57		52			
Raw score			118.41 (116.12 to 120.71)		116.18 (113.75 to 118.60)	2.24 (−1.10 to 5.57)	0.187
z-score			0.34 (0.16 to 0.52)		0.16 (−0.02 to 0.35)	0.17 (−0.09 to 0.43)	
Total achievement**	109	57		52			
Raw score			113.00 (111.25 to 114.74)		110.76 (108.92 to 112.61)	2.24 (−0.32 to 4.79)	0.085
z-score			0.30 (0.15 to 0.44)		0.11 (−0.05 to 0.26)	0.19 (−0.03 to 0.40)	

z-score values indicate how many standard deviations have the post-exercise program values changed with respect to the baseline mean and standard deviation. E.g., a 0.50 z-score means that the mean value at post-exercise program is 0.50 standard deviations higher than the mean value at baseline, indicating a positive change, with negative values indicating the opposite. All data presented were adjusted for baseline values. *Academic skills are the sum of components based on basic skills such as reading decoding, mathematics calculation, and spelling. †Academic fluency is the sum of the components based on reading, calculation, and writing fluency. ‡Problem solving is the sum of the components based on solving academic problems in reading, mathematics, and writing. **Total achievement is the overall measure of the academic performance based on reading, mathematics, and writing.

Table 8. Intention-to-treat effects of the *ActiveBrains* exercise program on raw (mm^3) and z-scores of post-exercise hippocampal grey matter volume.

			Mean (95% CI)				
	N_{all}	N	Exercise group	N	Control group	Difference between groups	*P*
Hippocampus	109	57		52			
Raw score			7132.53 (7025.48 to 7239.59)		7162.19 (7049.01 to 7275.37)	−29.66 (−185.46 to 126.15)	0.707
z-score			0.13 (−0.03 to 0.29)		0.18 (0.01 to 0.35)	−0.04 (−0.28 to 0.19)	
Right hippocampus	109	57		52			
Raw score			3651.61 (3594.83 to 3708.38)		3670.46 (3610.43 to 3730.48)	−18.85 (−101.53 to 63.83)	0.652
z-score			0.17 (0.02 to 0.32)		0.22 (0.06 to 0.38)	−0.05 (−0.27 to 0.17)	
Right anterior hippocampus	109	57		52			
Raw score			2097.44 (2061.06 to 2133.82)		2112.97 (2074.5 to 2151.43)	−15.53 (−68.52 to 37.47)	0.563
z-score			0.15 (−0.00 to 0.30)		0.22 (0.05 to 0.38)	−0.07 (−0.29 to 0.16)	
Right posterior hippocampus	109	57		52			
Raw score			1551.87 (1528.30 to 1575.45)		1552.71 (1527.78 to 1577.63)	−0.83 (−35.15 to 33.48)	0.962
z-score			0.18 (0.02 to 0.34)		0.19 (0.02 to 0.36)	−0.01 (−0.24 to 0.23)	
Left hippocampus	109	57		52			
Raw score			3480.70 (3412.97 to 3548.42)		3491.99 (3420.39 to 3563.59)	−11.29 (−109.85 to 87.26)	0.821
z-score			0.07 (−0.12 to 0.25)		0.10 (−0.10 to 0.29)	−0.03 (−0.30 to 0.24)	
Left anterior hippocampus	109	57		52			
Raw score			1999.35 (1959.03 to 2039.68)		2002.39 (1959.76 to 2045.02)	−3.04 (−61.72 to 55.64)	0.918
z-score			0.06 (−0.13 to 0.24)		0.07 (−0.13 to 0.27)	−0.01 (−0.29 to 0.26)	
Left posterior hippocampus	109	57		52			
Raw score			1481.90 (1450.11 to 1513.69)		1485.83 (1452.22 to 1519.44)	−3.93 (−50.19 to 42.34)	0.867
z-score			0.08 (−0.11 to 0.28)		0.11 (−0.10 to 0.31)	−0.02 (−0.31 to 0.26)	

z-score values indicate how many standard deviations have the post-exercise program values changed with respect to the baseline mean and standard deviation. E.g., a 0.50 z-score means that the mean value at post-exercise program is 0.50 standard deviations higher than the mean value at baseline, indicating a positive change, with negative values indicating the opposite. All data presented were adjusted for baseline values.

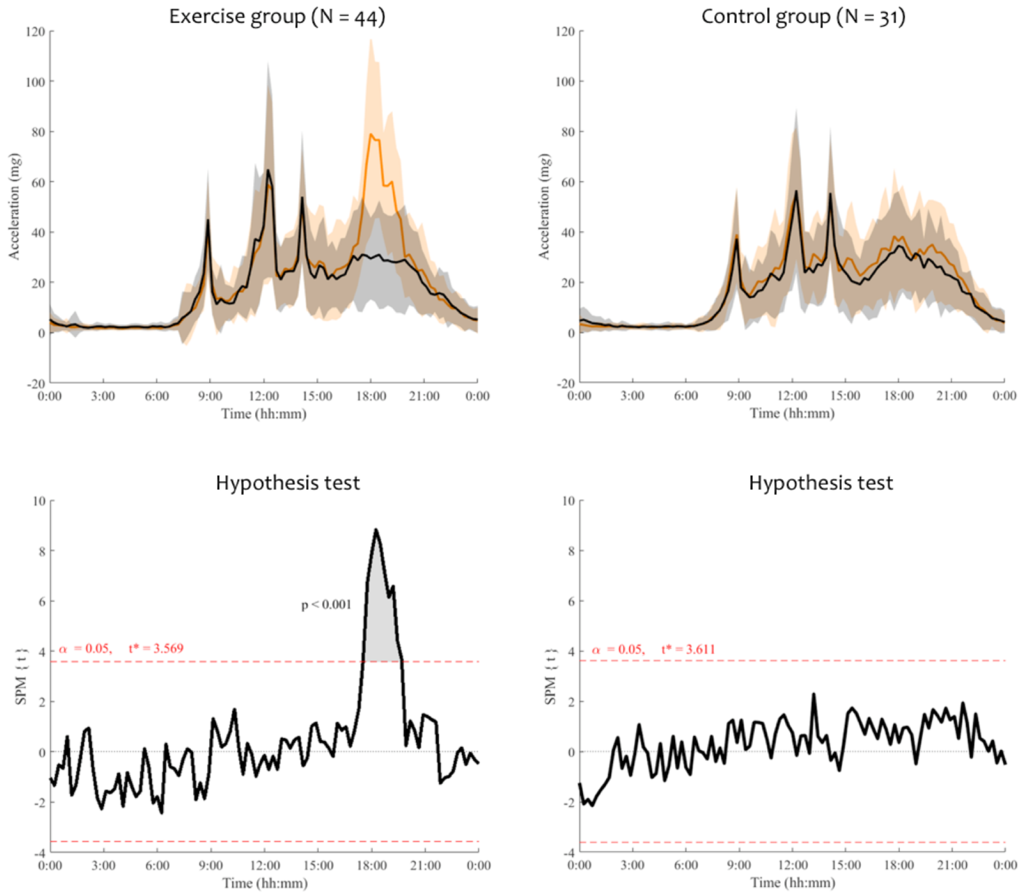

Figure 5. Comparison of the 24h physical activity patterns (i.e., Euclidean Norm Minus One accelerations from the hip-worn accelerometer) at baseline (i.e., black line) and during-exercise program (i.e., orange line) in exercise and control groups. The hypothesis test shows the threshold (t^* = 3.569) at which there are significant physical activity patterns' differences between baseline and during-exercise periods.

DISCUSSION

Main findings

The present study contributes to the existing literature with a number of novel and important findings: (1) A 4.5-month exercise program based on relatively high intensity (i.e. 70% of maximum heart rate for more than 60min) aerobic plus resistance training improved total and crystallized intelligence, cognitive flexibility and academic performance in children with overweight/obesity; (2) other executive function dimensions, such as cognitive inhibition and working memory were absolutely unaffected by this exercise program; (3) while a modest and significant increase in the hippocampus grey matter volume was observed in the exercise group, the between-groups effect was non-significant in this region nor in any other region of the brain; (4) the effects of the exercise intervention observed in crystallized intelligence, total academic achievement and problem solving were mediated by the exercise-induced improvements in CRF. Noteworthy is that although some of the effects observed were partially (10-20%) mediated by improvements in CRF, the largest part of the effects of physical exercise on intelligence, cognitive flexibility and academic performance was not mediated by CRF, which suggest that the benefits of physical exercise occur beyond the improvements in CRF.

In addition to these main findings, two important exploratory analyses were conducted and suggests that: (1) As expected when including less committed participants (i.e., participants attending to less than 70% of exercise sessions or those who left the study-dropouts), the effects shown in the intention-to-treat analyses were attenuated compared to the per-protocol effects, yet remained significant in most of cases, suggesting efficacy and effectiveness of this randomized controlled trial; (2) the thorough analyses of the potential compensatory and contamination effects before and during the intervention using objectively-measured physical activity, demonstrated that the exercise group effectively increased its activity level with this intervention, while remained unchanged in the control group. This kind of objective validation of the activity levels in the study groups is rarely conducted/reported in exercise randomized controlled trials and is important for a more accurate interpretation of the trials' effects.

Effects of exercise on brain health

Effects on intelligence

To the best of our knowledge, 3 previous intervention studies tested the chronic effects of exercise on intelligence outcomes in children. One of the studies compared a yoga program with a daily physically active group,[22] so that it cannot be known what is the effect of physical exercise compared to a non-active control condition. Another study compared a school-based intervention (cluster randomized) including daily physical activity for a whole academic year with a control group also doing daily physical activity but only one of the two semesters and another part of the control group doing 1-day physical activity per week.[23] This study showed some positive effects on fluid intelligence in the experimental group compared with the control group, yet with the study design used is difficult to quantify the exact effect on intelligence of doing physical exercise *versus* no exercise. Finally, our group previously conducted a pilot cluster-randomized controlled trial including 3 study groups who did (1) the habitual 2 physical education sessions/week, (2) 4 physical education sessions/week, and (3) 4 physical education sessions/week of high intensity in adolescents.[24] We observed that the group which doubled the number and intensity of physical education sessions per week improved significantly more than the control group in the overall score of intelligence as assessed by the Spanish Overall and Factorial Intelligence Test,[75] as well as in all the sub-scales studied, i.e., non-verbal and verbal abilities, abstract reasoning, spatial ability, verbal reasoning and numerical ability. Yet these results were promising and support a chronic effect of exercise on intelligence, the small sample size (N ≤ 24 per study group) and the fact that only 3 school classes were randomized made us to consider these findings as preliminary. Therefore, and to the best of our knowledge, the ActiveBrains randomized controlled trial provides the strongest evidence so far on a causal effect of physical exercise on total intelligence, and particularly on crystallized intelligence. The effect size observed in crystallized intelligence was the largest observed out of all study outcomes, i.e., exercise group improved 0.7 SDs more than the control group, which can be considered an unusually large effect when compared with the effects of exercise on brain health outcomes reported in recent meta-analyses.[17,19,20,76,77] Noteworthy is that whereas fluid intelligence was not significantly improved, i.e., no significant difference between groups were observed, the within-group change in the exercise group was of 0.44 SDs (0.18 to 0.69) compared to a change in the control group of 0.21 (-0.04 to 0.48), suggesting that some adaptations in fluid intelligence as a result of exercise actually occurred. Interestingly, although previous evidence on chronic effects of exercise on intelligence is limited, more evidence is available for acute effects.[12] The 2018 Physical Activity Guidelines Advisory Committee Scientific Report concluded that there was strong evidence for improvement in crystallized intelligence after a single bout of moderate-to-vigorous physical activity.[12]This support our findings, since if a single bout of physical activity has this acute response, it is reasonable to think that chronic stimulation with exercise can lead to improvements in crystallized intelligence. Future randomized controlled trial will confirm or contrast these findings.

Effects on executive function

Our exercise program had a significant effect on some dimensions of executive functions but not others. Particularly, exercise had medium effect size (i.e. 0.5 SDs) on cognitive flexibility, while had a null effect on inhibitory control and working memory (i.e. 0.04 and 0.05 SDs respectively). The FITKids trial[78] observed a significant improvement on cognitive flexibility, in agreement with our findings, yet also on inhibition in contrast to us. Overall, systematic reviews and meta-analyses have reported a significant effect of exercise on overall executive function,[17,19,21,76] with differences in the conclusions among reviews when referring to the specific dimensions of executive functions. For example, the meta-analyses from Alvarez-Bueno et al.,[76] observed a significant effect of exercise on inhibition and working memory, but not on cognitive flexibility, which would be in contrast with our findings. Similarly, the meta-analysis conducted by Xue et al.,[17] observed significant effects on inhibition, but not in the other dimensions of executive function. Noteworthy is that cognitive flexibility seems to have a critical period of fast development between 7 to 9 years of age, becoming relatively mature by the age of 12 years.[79] This might have influenced that the children of our ActiveBrains trial aged 8 to 11 could have been especially sensitive to improvements in cognitive flexibility as response to exercise.

Another important issue when assessing effects on executive function dimensions, is the different tests available to assess them, which could explain some inconsistencies in the findings. In the ActiveBrains trial, we found an effect of the exercise program on an averaged composite score of cognitive flexibility. This composite score was derived from two different tests that have been previously considered as indicators of cognitive flexibility (i.e., Design Fluency Test and Trail Making Test).[51,52] When studying the exercise effects on cognitive flexibility from these two tests separately, a markedly larger and significant effect of the exercise program was observed on the performance in the Design Fluency Test (i.e. 0.5 SD more improvement in the exercise group), while a modest (yet in the expected direction) and non-significant effect was observed on the Trail Making Test (i.e. 0.28 SDs more improvement in the exercise group). We believe that the differential effect observed in these two tests could be due to the different nature of the tests. Although typically classified as a cognitive flexibility test, the Design Fluency Test has a multifactorial nature which includes not only cognitive flexibility but also on other multiple cognitive processes including problem solving, creativity, response inhibition, or working memory.[52,80,81] The problem-solving dimension was also positively affected by this intervention as assessed within the academic performance indicators, which strengthens this conclusion. In addition, creativity, also assessed by the Design Fluency Test, could have been improved by this exercise program, which is not the case probably for inhibition and working memory, since we used separate tests for these executive functions in our trial and observed no effects. On the other hand, the Trail Making Test may be considered a test with a more concrete nature mainly based on the cognitive flexibility assessment, and without the problem solving or creativity abilities involved in the Design Fluency Test.[80] These differences could potentially explain the different effects observed in the two tests used.

Effects on academic performance

Our exercise program had a positive effect on total academic performance, as well as in some specific domains such as mathematics, problem solving (a composite including problems in reading, mathematics, and writing), and academic skills (a composite including basic skills such as reading decoding, mathematics calculation, and spelling), as measured by the Spanish version of the Woodcock-Johnson III Tests of Achievement.[58] One of the most recent meta-analysis on this topic concluded that there is strong evidence supporting a beneficial effect of exercise interventions on mathematics,[18] which is in line with our findings. Other meta-analyses also observed a significant positive effect on mathematics,[20] yet one meta-analysis focused only on children with overweight/obesity found a borderline non-significant effect (standardized mean difference=0.49, CIs=-0.04 to 1.01)[19] and another focused only on preadolescent children did not find a significant effect on mathematics. These two meta-analyses focused on only part of the population logically had less studies included and therefore less power for detecting significant effects, which might partially explain the discrepancy in the conclusions between different meta-analyses. Another important issue to bear in mind is that school grades might be biased and prone to ceiling effects, which might also contribute to explain the inconsistences in the findings. Our ActiveBrains trial, using a standardized test for overall and specific academic domains, supports a beneficial small-medium effect size of exercise on mathematics (0.34 SDs more improvements in the exercise group). In addition, we observed a significant effect on problem solving (the largest effect among the academic domains, 0.38 SDs more improvements in the exercise group) and on basic academic skills. These two composites include tasks not only related to mathematics, but also to reading and writing, suggesting that the beneficial effect of exercise might reach also other dimensions of academics, yet clearly, mathematics is the most sensitive domain responding to exercise.

Effects on grey matter volumes

We observed small but significant increase in the grey matter volume of the hippocampus in the exercise group, while no change was observed in the control group, yet the differences between the change observed in both groups did not reach significance. These novel findings suggest that some adaptations occurred as response to exercise, yet either the intervention did not last long enough or the sample size was not large enough for these inter-group differences to be significant. Although these results are promising, future larger trials and with a longer intervention period will confirm or contrast, whether exercise at this critical period of life can positively stimulate hippocampus growth. No significant effect was observed when the hippocampus was analyzed separately by left and right hemisphere, or anterior and posterior hippocampus, nor we found any significant effect when using a whole-brain voxel-based approach, nor in total brain volumes. We found one multidisciplinary study that tested the combined effect of 5-months exercise, diet and cognitive training on grey matter brain volumes in children with obesity.[82] The authors observed a positive effect on total grey matter volume and cerebellum grey matter volume. However, we cannot compare these findings and ours, since it cannot be elucidated which was the individual contribution of diet, exercise or cognitive stimulation. Other exercise interventions (4 different trials) in pediatric population focused on white matter integrity and/or fMRI (resting or task-related), and observed some positive effects of exercise on these outcomes.[25–32]

Sex- and maturational-differences on the effects

Although our study was not powered to conduct separate analyses for sex and maturation sub-groups, we run an exploratory analysis stratifying for these two factors in order to better understand their role in modulating the exercise intervention effects. Our findings suggest that the main significant effects hereby reported were rather consistent across sex and maturation groups, except for crystallized intelligence and academic skills the effects observed were larger in boys than in girls. This notion is partially supported by one cluster-randomized controlled trial which observed that girls responded worse than boys in academic performance.[83] However, there is a chance that the role of sex as moderator of the exercise effects can be dependent of the age group studied. In this context, it has been suggested that in older adults, exercise could have larger effects in women than in men, yet existing evidence is very limited.[13,84] Regarding maturation, only for crystallized intelligence there was a differential effect, with the less mature children having a larger benefit than the more mature children. We did not find other studies testing this moderation effect on PHV as indicator of biological maturation, so we cannot compare our results with others.

Mechanisms

Reviews have identified 3 broad categories of neurobiological mechanisms responsible for the effects of exercise on brain health: (i) Cells, molecules, and circuits that, with current scientific methods are only detectable in animal studies (e.g., neurogenesis, angiogenesis, synaptogenesis), (ii) biomarkers (e.g., grey matter volume, cerebral blood volume, flow); and (iii) peripheral biomarkers (e.g., circulating growth factors, inflammatory markers) that can be observed in human studies.[85,86] Among all the potential mechanisms, the brain-derived neurotrophic factor (BDNF) is probably the one that has received the most attention. Accumulating evidence suggest that exercise leads to an increase in the BDNF in the central nervous system which lead improvement in cognition.[87] Animal studies have identified peripheral mechanisms potentially stimulated by exercise that could increase BDNF levels in brain which in turn may be related with higher neurogenesis and synaptogenesis mainly in the hippocampus. Peripheral factors induced by exercise and candidates to increase BDNF levels in the hippocampus are: FNDC5-irisin, fibronectin type III domain–containing protein 5 that is released into the circulation as Irisin; BHB, B-hydroxybutyrate; and CTSB, Cathepsin B.[88–90] However, available evidence comes mostly from animal models and future studies will examine the effects on these peripheral biomarkers.

Mediation role of cardiorespiratory fitness

We observed a borderline significant effect on CRF as measured by performance in the test, i.e., time-to-exhaustion (i.e. 0.44 SDs more improvements in exercise vs. control group), and a borderline non-significant effect on maximal oxygen consumption, VO_2max expressed in mL/kg/min (i.e. 0.32 SDs more improvements in exercise vs. control group, which is equivalent to an improvement of 1.5 mL/kg/min). This matches exactly the effect size observed on VO_2max in the FitKids trial, i.e. an effect size of 0.34 and 1.3 mL/kg/min for group difference in pre-post changes observed.[78] However, it is unknown whether these improvements in CRF explained the intervention effects observed.

Cross-sectional studies have consistently reported that children with a higher CRF have a healthier brain, as indicated by brain structure outcomes[37–39] or behavioral outcomes.[15,34–36] It is therefore time to step further and test whether exercise-induced improvements in CRF lead to improvements in brain health, formally testing the potential mediation role of CRF. To the best of our knowledge, none of the previous exercise trails on brain health have formally tested the potential mediator role of CRF on the intervention effects. Our mediation analyses suggest that effects of the exercise intervention observed in crystallized intelligence, total academic achievement and problem solving were mediated by the exercise-induced improvements in CRF. However, this mediation was only partial (10-20%) and non-significant in outcomes such as cognitive flexibility or mathematics, which suggest that the benefits of physical exercise may occur beyond the improvements in CRF. These findings have important implication from both a scientific and a public health point of view providing an empirical support to the classic theoretical model proposed by Bouchard and Shephard in 1994.[91] Our findings suggest that physical activity can improve health, in this case brain health, both directly and indirectly through improvements in CRF.

Limitations and Strengths

The sample size of the ActiveBrains trial might seem relatively small (N~100), however it is the second largest trial (after the FITKid2 trial with N= 143)[25] examining effects of exercise on structural or functional brain as measured by MRI. In addition, sample size and hence power is not an issue for all significant effects reported in this trial (intelligence, cognitive flexibility and academic performance). Moreover, we believe that it is unlikely that the non-significant effects observed in inhibition and working memory could be due to lack of power in our analyses, since the intervention effect size observed was nearly zero (0.04-0.05 SDs) and consequently not even a very large trial would have reached significance in this case. Finally, we believe that the differential changes observed in the hippocampus between exercise and control groups would have been significant with a larger sample size (and consequent reduction in CIs) and perhaps also if the intervention would have lasted longer.

Although several protocols were adopted to reduce the risk of bias in the evaluations (e.g. randomization after baseline assessment, physical trainers not involved in any evaluations), some of the project staff involved in the post-exercise evaluations were not fully blinded to the group allocation which could add some bias to the measurements. Nevertheless, there are outcomes such inhibition or working memory were completely unaffected by the intervention and other outcomes such as cognitive flexibility or intelligence that were largely affected by the intervention. Since all these outcomes were assessed for the same evaluators, we believe is unlikely that the conclusions of this study would largely change due to this limitation.

Strengths of this study include: (1) To be one of the few studies examining the chronic effects of exercise on behavioral and brain outcomes in a single article, and the first in pediatric population studying the effect of exercise on grey matter volume; (2) the formal mediation modelling in order to better understand the role of CRF in the effects of exercise intervention on brain health; (3) the focus on intelligence that has been rarely studied before in relation to an exercise program; (4) the thorough monitoring of exercise intensity in all session and children using individualized and a-priori programmed heart rate monitors; (5) numerous exploratory analyses such as the intention-to-treat and dropout analyses; (6) the sophisticated analyses of accelerometer-measured physical activity all throughout the day (24h curves) to accurately examine whether changes in overall activity levels took place in any of the study group and at what time of the day; and (7) the use of standardized and valid instruments and protocols such as the test used for academic performance instead of grades, incremental test with gas analyzer for assessing CRF, and advance processing on accelerometer raw data.

CONCLUSIONS

A 4.5-month after-school program consisting in more than one hour of aerobic plus resistance training at an average intensity of 70% of the maximum heart rate 3 times per week improved brain health in children with overweight or obesity. More specifically, our findings support that physical exercise benefits total and crystallized intelligence, cognitive flexibility (measured with a test that involves also creativity and other high cognitive processes), and total academic performance, and particularly mathematics, problem solving and academic skills. Some of these effects seem to be mediated by CRF. The mediation effect ranged between 10 and 20%, which is a significant but partial mediation effect, suggesting that physical exercise can benefit brain health in childhood indirectly through improvements in CRF but also directly regardless of improvements in CRF. On the other hand, this exercise program had a null effect on other executive functions such as inhibition and working memory. Exercise seems to lead to small adaptations in hippocampal volume in children with overweight or obesity, and longer and larger randomized-controlled trials will confirm whether hippocampus grey matter volume can be significantly enhanced by exercise in childhood. Our thorough analysis on 24h-curves of activity levels demonstrated that this intervention actually increased activity levels in the exercise group, without a significant contamination in the control group, validating the intervention and its effects.

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GENERAL DISCUSSION

MAIN FINDINGS OF THE PRESENT INTERNATIONAL DOCTORAL THESIS

The present thesis has contributed to the existing literature on physical fitness, physical activity, sedentary time, exercise and brain health in children with overweight/obesity with a number of novel findings that are summarized in **Table 1**.

SECTION 1. CROSS-SECTIONAL STUDIES: ROLE OF PHYSICAL FITNESS, PHYSICAL ACTIVITY AND SEDENTARY TIME IN EXECUTIVE FUNCTION, BRAIN ACTIVITY DURING TASK PERFORMANCE AND NEUROTROPHIC FACTORS

In the present International Doctoral Thesis, we generally report a positive association of physical fitness components with executive function and underlying neuroelectric activity in children with overweight/obesity. Particularly, in our **Study 1** we found both speed-agility and cardiorespiratory fitness positively related to cognitive flexibility in children with overweight/obesity. Speed-agility was further the only fitness component positively associated with inhibition, and muscular strength the only one related to planning ability. In general, cardiorespiratory fitness has been the physical fitness component more related to executive function and underlying neuroelectric activity across studies of the present Thesis. Apart from being positively associated with cognitive flexibility in **Study 1**, higher levels of cardiorespiratory fitness were consistently associated with better performances (i.e., shorter reaction times, RTs) and higher amplitudes of P3 component in tasks of working memory (**Study 2**) and inhibitory control (**Study 3**), therefore indicating increased attentional resource allocation during stimulus engagement. These positive findings were finally confirmed in our **Study 4** in which we went a step forward from a neuroelectric activity perspective, and we used for the first time a brain source analysis to identify the brain areas whose current source density was related with fitness. Therefore, in **Study 4**, cardiorespiratory fitness was the only fitness component positively associated with brain current source density during all processes of working memory (i.e., encoding, maintenance, and retrieval) in a broad range of Brodmann areas (BAs) of the frontal, limbic, temporal and occipital regions. This has been supported by a previous study carried out in the same sample as ours in which cardiorespiratory fitness was the fitness component associated with more regions in the brain.[1] The stimulation of brain by exercise,[2] as a major determinant of cardiorespiratory fitness, reflected in increases of cell proliferation and survival,[3] increases of levels of Brain-derived neurotrophic factor (BDNF),[3] increases of synaptic efficiency,[4] and increases of angiogenesis and blood flow,[5] make the relationship found for cardiorespiratory fitness biologically plausible.

Together with cardiorespiratory fitness, speed-agility should be also highlighted as a component of fitness showing a strong relationship with executive function and underlying neuroelectric activity. This affirmation is based on that this component was also related with cognitive flexibility in **Study 1** and better performances and higher amplitudes of P3 during working memory in **Study 2** and inhibitory control in **Study 3**. However, speed-agility did not show any association with current source density in any area of the brain in **Study 4.** In comparison to cardiorespiratory fitness, speed-agility showed an association with inhibition in **Study 1**. The

Table 1. Main findings of the present International Doctoral Thesis.

Studies	Main findings
Study 1	• Speed-agility, CRF, and an overall physical fitness score were positively related to cognitive flexibility. • Speed-agility was the only fitness component positively associated with inhibition, and muscular strength was the only component related to planning ability. • No significant associations were found between PA, sedentary time and any of the executive function indicators.
Study 2	• CRF and speed-agility were associated with shorter RT, and larger P3 amplitude and shorter latency during working memory. • Inconsistent findings were observed for muscular strength, with upper-limbs absolute strength associated with lower response accuracy, and lower-limbs relative strength associated with larger P3 amplitude. • The relationship of PA with working memory and neuroelectric activity was intensity-dependent and consistent across accelerometer locations and cut-points (i.e., hip and wrist). • No association was observed for sedentary time in relation to working memory and neuroelectric activity.
Study 3	• CRF and speed-agility were positively associated with shorter RT and larger P3 amplitude of inhibitory control, whereas no associations were observed for muscular strength. • Collectively, the associations for CRF and speed-agility were generally observed in the incongruent condition of the task engendering greater amounts of inhibitory control. • Sedentary time and PA were not associated with inhibitory control performance, regardless of the location of the accelerometer. However, only when using hip–worn accelerometer data we observed that higher vigorous physical activity was associated with larger P3 amplitude, whereas higher moderate physical activity was associated with longer P3 latency.
Study 4	• CRF was the only physical fitness component positively associated with brain current source density mainly during pre-target phases of the working memory task (i.e., encoding and maintenance) and in BA of the frontal, limbic, and occipital regions. • CRF was in a lesser extent associated with current source density during the retrieval phase only in a BA of the temporal lobe. • No positive associations were observed for speed-agility and muscular fitness in relation to brain current source density. • The CRF-related brain current source density observed in limbic regions was further associated with higher response accuracy in the high load condition of the working memory task.
Study 5	• Light, moderate, and MVPA were related to the BDNF, being the associations of moderate and MVPA consistent from either hip or wrist accelerometer data. • A peak of 60-min steps cadency as well as the time spent in purposeful movements (i.e., 40–59 steps per minute) and slow walking (i.e., 60–79 steps per minute) were associated with BDNF. • No significant association was observed between sedentary time and BDNF, nor between PA, sedentary time and steps, and VEGF and IGF-1.
Study 6	• The exercise and control groups improved working memory and inhibitory control performance, although no between-groups differences were observed. • The exercise group had a significantly higher increase of brain activation from pre- to post-intervention mainly in areas of the frontal and temporal lobes, and, specifically, during the encoding phase of the high load condition of the working memory task. • The exercise group presented a lower current source density change from pre- to post-intervention in a single brain area of the frontal lobe during the phase of information processing previous to the answer of the incongruent condition of the inhibitory control task.
Study 7	• The exercise group significantly improved intelligence, cognitive flexibility and academic performance over the control group. • The exercise group showed a modest and significant increase in the hippocampus grey matter volume, although there was not a between-groups effect. • The effects of exercise program on crystallized intelligence, total academic achievement and problem solving were mediated by the exercise-induced improvements in CRF.

BA = Brodmann area; BDNF = Brain-derived neurotrophic factor; CRF = Cardiorespiratory fitness; IGF-1 = Insulin growth factor-1; MVPA = Moderate-to-vigorous physical activity; PA = physical activity; RT = Reaction time; VEGF = Vascular endothelial growth factor relationship between complex motor skills (i.e., speed, agility, coordination) and higher-order cognitive skills such as inhibition has been previously reported,[6] suggesting that those skills that involve precise execution movements may activate a neuronal network that connects brain regions associated with both motor and cognitive functions.[7] Finally, muscular strength was possibly the fitness component less related to executive function and underlying neuroelectric activity. This component was related somehow only with planning ability in **Study 1**, and with better performance and higher P3 amplitude during working memory in **Study 2**. However, these associations varied depending on the muscular strength outcome analyzed (i.e., upper- versus lower-limb strength). Altogether, our results suggest that the association between physical fitness and the different executive function domains and neuroelectric activity parameters might be fitness-component specific. Overall, cardiorespiratory fitness and, to a slightly lesser extent, speed-agility, might be the two health-related physical fitness components with the potential to improve brain development from a neuroelectric activity perspective resulting in better executive function.

Whereas physical fitness components were generally related with executive function and underlying neuroelectric activity in **Study 1**, **2** and **3**, the association for physical activity was more inconsistent across these studies. Hence, we reported in our **Study 5** that objectively-measured moderate and moderate-to-vigorous physical activity regardless of the accelerometer location (i.e., non-dominant wrist or hip), as well as the time spent in walking at slow cadences were positively related with BDNF. These findings suggest that different intensities and types of physical activity, mainly moderate ones, as well as walking behaviors may be an enough stimulus to increase plasma BDNF levels in children with overweight/obesity. Despite these positive findings have been supported by a previous intervention study in which exercise had an effect on BDNF in children with obesity,[8] findings from most observational studies suggest a null or even an inverse relationship between physical activity and peripheral BDNF levels.[9–11] This inconsistency can be also found across some of the studies that make up our Thesis. Hence, whereas we observed a null association of physical activity with either pencil-based executive function (**Study 1**) or computerized-based working memory and inhibitory control performance (**Study 2** and **3**, respectively), a positive association of physical activity with neuroelectric activity underlying working memory and inhibitory control was found in **Study 2** and **3**, respectively. However, these associations seemed to be intensity and accelerometer-location dependent. Therefore, a consistent finding across studies was that only vigorous physical activity seemed to have an implication for the amplitude of P3 during either working memory or inhibitory control tasks. On the other hand, whereas the association between physical activity and P3 amplitude was consistent across accelerometer locations in **Study 2**, the positive association found between vigorous physical activity and P3 amplitude underlying inhibitory control in **Study 3** was observed only when physical activity data were used from the hip accelerometer-location. Given these inconsistencies, future studies should care about different methodological considerations (i.e., accelerometer-location, cut-points, etc.) that may influence analyses.[12] Taking together findings from **Study 2** and **3**, vigorous physical activity intensity data extracted from hip accelerometer-location seems more strongly related to neuroelectric indices of working memory and inhibitory control than other physical activity intensities and accelerometer locations.

With respect to sedentary time, none of the studies including this measurement (i.e., **Study 1**, **2**, **3** and **5**) observed an association with the outcomes. This consistency might not be surprising taking into consideration previous studies that reported that only certain sedentary behaviors (e.g., watching TV, playing videogames), but not total sedentary time, may have an association with brain health.[13] Interestingly, there is limited evidence to conclude whether associations between sedentary behaviors, and executive function and neuroelectric activity outcomes are explained by the type of sedentary behaviors or the time spent in any sedentary activity. Therefore, it seems reasonable to speculate that the sedentary time did not shown any association in the present Thesis since it may also include the time spent in non-screen-based or educational-based sedentary behaviors (i.e., reading, painting, studying) which may be beneficial for executive function operations. However, little information is available in this regard. Thus, it seems important for further studies to determine how different sedentary behaviors could influence executive function and underlying neuroelectric activity in children.

SECTION 2. INTERVENTION STUDIES: EFFECTS OF EXERCISE ON BRAIN ACTIVITY DURING TASK PERFORMANCE, COGNITIVE AND ACADEMIC PERFORMANCE, AND BRAIN STRUCTURE

The present International Doctoral Thesis includes two studies (i.e., **Study 6** and **7**) addressing the effects of a 4.5-month exercise program based on high intensity aerobic plus resistance training on different brain health outcomes in children with overweight/obesity. In **Study 6**, we used for the first time an EEG-based brain source analysis approach that takes advantage of the high temporal resolution of the EEG and the spatial representation of fMRI (although in much lower resolution) to analyze the effects of exercise on brain current source density underlying working memory and inhibitory control. More concretely, in **Study 6** we show that children from the exercise group had a significantly higher increase of brain current source density from pre- to post-intervention in brain areas preferably of the temporal and frontal lobes, and, specifically, during the encoding phase of the high working memory task than their peers from the control group. With respect to the brain current source density underlying an inhibitory control task, we show that participants from the exercise group presented a lower current source density change from pre- to post-intervention in a single brain area of the frontal lobe during the phase of information processing previous to the answer in comparison with the exercise group. These differences of activation patterns between the exercise and control groups have been previously seen in other studies using fMRI during different tasks in children.[14–16] This may be due to the huge differences in the design of each task as well as in the different cognitive demands of them. But what seems clear is that exercise alters brain activation and that this alteration happens in a relevant phase of working memory such as the encoding one. Further, although to a lesser extent, exercise seems to alter also brain activity during the information processing phase of inhibitory control. Apart from this, another important finding that must be highlighted is that experimental findings of the **Study 6** showing exercise-induced activations of brain areas such as the prefrontal cortex (i.e., BA8, 9, 11), the posterior cingulate (i.e., BA30) and the cuneus (i.e., BA 18) during the encoding phase of working memory support observational evidence from **Study 4** in which cardiorespiratory fitness was positively associated with these areas during the same phase. Since, the associations found in **Study 4** during the maintenance or the retrieval phases are not accompanied by an effect of the exercise in **Study 6**, we can conclude that the phase of working memory that seems to be more stimulated by exercise and strongly associated with cardiorespiratory fitness is the encoding one.

Study 7 presents a number of novel and important findings. First, the exercise program improved total and crystallized intelligence, cognitive flexibility and academic performance, whereas no effect was seen for working memory or inhibition in children with overweight/obesity. Interestingly, in **Study 1** we had already observed that cognitive flexibility seemed to be the executive function indicator more strongly associated with physical fitness. Therefore, the observational findings of **Study 1** are supported by **Study 7** in which we observe a specific effect of exercise on cognitive flexibility but not in inhibition or working memory. This could be explained by two different aspects. First, by the developmental trajectory of the executive functions whereby cognitive flexibility is the last executive function indicator to reach maturity, whereas inhibition, for example, emerges and is fully developed during early ages.[17] Thus, cognitive flexibility might not be fully developed during childhood and, may therefore, be more easily influenced by physical fitness. Second, cognitive flexibility is regulated by areas such as the prefrontal cortex or the anterior cingulate cortex which showed an alteration in terms of brain current source density due to exercise in **Study 6**.[2] On the other hand, the non-significant effect of exercise on working memory, indicated by the response accuracy in the DNMS, and inhibition, measured by the Stroop test, observed in **Study 7** is partially consistent with observational evidence of **Study 1** showing no association of most of fitness components with inhibition measured by this test, and of **Study 2** showing only associations with RT during the DNMS task, but not response accuracy. We believe that the differential associations and effects observed on executive function dimensions could be due to the different nature of each test as well as to the different construct that they assessed, being some of them more sensitive to exercise than others.

Study 7 also shows a modest and significant increase in the hippocampus gray matter volume in the exercise group, although there were no between-groups significant effect of exercise. This is not surprising since previous studies in animals and older adults have shown the hippocampus as the most affected region from exercise.[18] Although solid conclusions cannot be drawn as consequence of the non-significant differences between groups, there seems to be a trend effect of exercise on this region supported from a functional perspective by cortical activity alterations observed in **Study 6** in areas surrounding hippocampus such as the parahippocampal gyrus. However, future research analyzing effects of exercise on cortical and subcortical regions of the children's brain are needed in order to get further insights.

OVERALL LIMITATIONS AND STRENGTHS OF THIS THESIS

An integrative view of the general limitations and strengths of the present International Doctoral Thesis can be found in **Table 2**.

SECTIONS	Limitations	Strengths
SECTION 1	• All studies in this SECTION 1 applied a cross-sectional design, and therefore, drawing causal associations is not possible. • Different types of physical activity such as bicycling and swimming cannot be well captured by the accelerometers used in the present Thesis. • Identification of sedentary time was not sensitive to postures, so we cannot differ between different sedentary behaviors and, therefore, some standing activity could be erroneously classified as sedentary time. • Although we explored the role of potential confounders in the analyses of this SECTION, it is not possible to guarantee that other confounders not measured in the present Thesis could explain the observed associations. • BDNF was measured from the plasma, and therefore a measurement of BDNF in the brain is not possible in humans. • Although we generally used a specific way to analyze physical activity (i.e., linear regressions), nowadays it is complex to choose a single way to analyze it, what is reflected in the wide variety of statistical approaches (i.e., partial least squares regression, isotemporal analsysis, compositional analysis, etc.) to analyze physical activity in the literature.	• The study of, not only cardiorespiratory fitness, but also other health-related physical fitness components such as speed-agility or muscular strength. • The inclusion of a complete, objective and standardized assessment of the physical fitness components, executive function aspects and neuroelectric activity. • The use of an objective, valid and reliable measurement such as accelerometry to assess physical activity and sedentary time. Further, the use of two different body locations (i.e., non-dominant wrist and hip) to attach the accelerometers and several cut-points. • The analysis of different intensities of physical activity, as well as step-related behaviors. • The inclusion, for the first time, of a brain source analysis in relation to physical fitness components in children. • The use of the most advanced technology to analyze neurotrophic factors (i.e, Luminex IS 100/200 system). • The evaluations of all outcomes of this SECTION were performed always by the same evaluators.
SECTION 2	• The effects observed in this SECTION 2 are limited to a sample of children with overweight/obesity, and it is therefore unknown the extent to which the findings would apply to children with normal-weight. • It is unknown whether the effects observed would have been larger if the intervention would have lasted longer. • The use of sLORETA software to measure brain activity is limited to the determination of activity in cortical regions and not in subcortical ones, such as the hippocampus. • Although several protocols were adopted to reduce the risk of bias in the evaluations, some of the project staff involved in the post-intervention evaluations were not fully blinded to the group allocation which could add some bias to the measurements.	• The inclusion of a new approach of electroencephalography analysis based on brain source analyses in relation to exercise effects. • The general use of standardized and validated instruments to assess all the outcomes. • The evaluations of all outcomes of this SECTION were performed always by the same evaluators. • The inclusion of a formal mediation analysis on the influenced outcomes by exercise, which has been rarely conducted in previous randomized controlled trials in this field. • The thorough monitoring of exercise intensity in all session and children using individualized and a-priori programmed heart rate monitors.

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functional MRI activation associated with cognitive control in children: a randomized controlled intervention. *Front Hum Neurosci*. 2013;7:72.

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CONCLUSIONS AND FUTURE PERSPECTIVES

CONCLUSIONS

Overall conclusion

The results of the present International Doctoral Thesis suggest that health-related physical fitness components, mainly cardiorespiratory fitness and speed-agility, are positively associated with executive function and neuroelectric activity during working memory and inhibitory control tasks. Moreover, physical activity is positively associated with BDNF and neuroelectric activity, yet not with executive function. Results from this Thesis do not support an association between sedentary time and any of the outcomes studied. Finally, and most importantly, this Thesis supports the notion that a 4.5-month exercise program alters brain activity during a working memory task, as well as improves intelligence, cognitive flexibility and academic achievement in the exercise group compared to the control group, in children with overweight/obesity.

Specific conclusions

SECTION 1. Cross-sectional studies: Role of physical fitness, physical activity and sedentary time in executive function, brain activity during task performance, and neurotrophic factors

- **Study 1**: Speed-agility, cardiorespiratory fitness and, to a lesser extent, muscular strength are positively associated with executive function in children with overweight/obesity. Furthermore, cognitive flexibility seems to be the executive function indicator with a higher association with physical fitness, whereas planning ability and inhibition might be fitness component specific. No associations were found for physical activity and sedentary time with executive function.
- **Study 2**: Speed agility and cardiorespiratory fitness are the fitness components more consistently related to both working memory performance and neuroelectric activity. The association of physical activity with working memory and neuroelectric activity is intensity-dependent, since only vigorous physical activity demonstrates a consistent relationship. No associations are found for sedentary time.
- **Study 3**: Speed agility and cardiorespiratory fitness are associated with inhibitory control and P3 amplitude in children with overweight/obesity. There are no significant associations between muscular strength, physical activity, or sedentary time and inhibitory control. The associations observed for vigorous physical activity with P3 amplitude, and for moderate physical activity with P3 latency are accelerometer location-dependent, (i.e., only associated when using physical activity data from the hip).
- **Study 4**: Cardiorespiratory fitness, but not speed-agility nor muscular strength, is positively associated with the brain current source density of regions of the frontal, limbic and occipital lobes during the encoding and maintenance phases of working memory. Further, cardiorespiratory fitness is associated with the current source density in regions of the temporal lobe during the retrieval phase of working memory. The fitness-related association with brain current density of the posterior cingulate and parahippocampal gyri was further related to better working memory performance.
- **Study 5**: Light to moderate physical activity intensity and step-related behaviors, but not sedentary time, are positively associated to

BDNF in children with overweight/obesity. Particularly, the time spent in walking at slow cadences may be stimulus enough to influence the levels of BDNF in children with overweight/obesity. No associations are found between physical activity, sedentary time, and VEGF and IGF.

SECTION 2. Intervention studies: Effects of exercise on brain activity during task performance, cognitive and academic performance, and brain structure

- **Study 6**: Children from the exercise group, in a higher extend than those from the control group, significantly increased the current source density of a broad network of brain areas primarily of the temporal, frontal and limbic lobes during the encoding phase of the high load condition of a working memory task after a 4.5-month exercise program. On the other hand, children belonging to the exercise group showed a significantly lower increase in current source density of the frontal lobe during the moment of information processing previous to response in an inhibitory control task. However, no between-groups differences where observed in any task performance.
- **Study 7**: Children in the exercise group improved significantly more than the control group their crystallized intelligence, cognitive flexibility, and total academic achievement. Further, they significantly increased their hippocampal brain volumes yet it was not significantly different than the control group. Many of these effects were mediated by cardiorespiratory fitness with a mediation effect ranging from 10 to 20%. Our thorough analysis on 24h-curves of activity levels demonstrated that this intervention actually increased overall activity levels in the exercise group, without a significant contamination in the control group, validating the intervention and its effects.

FUTURE PERSPECTIVES

- Brain source analysis should be incorporated in future studies using neuroelectric measurements such as electroencephalography in relation to physical fitness and exercise.
- Apart from this analysis, the study of a global connectivity index as a novel and valid indicator of the brain activity measured by electroencephalography have not been addressed yet in relation to fitness and exercise and future studies should include this outcome.
- Future studies are needed in order to shed light on which physical activity-based accelerometer location better predicts brain health.
- Evidence on the effects of physical activity interventions on brain health in children is growing but this body of evidence is still in its infancy, and further well-designed randomized controlled trials are needed to confirm or contrast our findings.
- Whereas the mechanism that are given to explain the effect of exercise on brain health have been widely studied in animals, there is still need to gain insights into the mechanism that explain this effects in humans. For instance, studies addressing the role of physical activity in neurotrophic factors are needed.

ANEXES

PAPERS DERIVED FROM THE THESIS

Published/accepted papers

1. **Mora-Gonzalez J**, Migueles JH, Esteban-Cornejo I, Cadenas-Sanchez C, Pastor-Villaescusa B, Molina-Garcia P, Rodriguez-Ayllon M, Rico MC, Gil A, Aguilera CM, Escolano-Margarit MV, Gejl AK, Andersen LB, Catena A, Ortega FB. Sedentarism, physical activity, steps, and neurotrophic factors in obese children. ***Med Sci Sports Exerc***, in press.

2. **Mora-Gonzalez J**, Esteban-Cornejo I, Cadenas-Sanchez C, Migueles JH, Rodriguez-Ayllon M, Molina-Garcia P, Hillman CH, Catena A, Pontifex MB, Ortega FB. Fitness, physical activity, working memory and neuroelectric activity in children with overweight/obesity. ***Scand J Med Sci Sports*** 2019, doi: 10.1111/sms.13456.

3. **Mora-Gonzalez J**, Esteban-Cornejo I, Cadenas-Sanchez C, Migueles JH, Molina-Garcia P, Rodriguez-Ayllon M, Henriksson P, Pontifex MB, Catena A, Ortega FB. Physical fitness, physical activity, and the executive function in children with overweight and obesity. ***J Pediatr*** 2019; 208:50-56.e1.

Papers in preparation/submitted

1. **Mora-Gonzalez J**, Esteban-Cornejo I, Solis-Urra P, Migueles JH, Cadenas-Sanchez C, Molina-Garcia P, Rodriguez-Ayllon M, Hillman CH, Catena A, Pontifex MB, Ortega FB. Fitness, physical activity, sedentary time, inhibitory control and neuroelectric activity in children with overweight/obesity: The ActiveBrains project. ***Psychophysiology***. *Submitted*.

2. **Mora-Gonzalez J**, Esteban-Cornejo I, Migueles JH, Rodriguez-Ayllon M, Molina-Garcia P, Cadenas-Sanchez C, Solis-Urra P, Plaza-Florido A, Kramer AF, Erickson KI, Hillman CH, Catena A, Ortega FB. Physical fitness and brain source analysis during a working memory task in children with overweight/obesity: The ActiveBrains project. ***Cereb Cortex***. *Submitted*.

3. **Mora-Gonzalez J**, et al. A 4.5-month after-school exercise program induces changes in current source density, but not performance, during executive function tasks in children with overweight/obesity: The ActiveBrains randomized controlled trial. *In preparation*.

4. Ortega FB*, **Mora-Gonzalez J***, et al. Effects of a 4.5-months exercise program on brain health in children with overweight/obesity: The ActiveBrains randomized controlled trial. ***In preparation.***

*These authors share first authorship.

INTERNATIONAL RESEARCH INTERNSHIPS

2018 Northeastern University, Boston, USA. Center for Cognitive and Brain Health. Prof. Charles H. Hillman. Duration: 3 months. Funded by: FPU Mobility Program.

2017 Michigan State University, East Lansing, USA. Health Behaviors and Cognition Laboratory. Dr. Matthew B. Pontifex. Duration: 3.5 months. Funded by: PhD International Mobility Program.

RESEARCH PROJECTS

2008 – 2020 The SmarterMove project. Funded by: Spanish Ministry of Economy and Competitiveness (100.000,00€). Principal Investigators: Francisco B. Ortega, Jonatan R. Ruiz.

2014 – 2017 The ActiveBrains project. Funded by: Spanish Ministry of Economy and Competitiveness (120.000,00€). PI: Francisco B. Ortega.

2014 – 2017 ACTIBATE. Funded by: Spanish Ministry of Economy and Competitiveness (600.000,00€). PI: Jonatan R. Ruiz.

2013 – 2016 The PREFIT project. Funded by: Spanish Ministry of Economy and Competitiveness (15.000,00€) PI: Francisco B. Ortega.

TEACHING INNOVATION PROJECTS

2017 – 2018 The University as promoter of health in youth: design of a gamification teaching material. Funded by: University of Granada (500,00€). PI: Isaac Pérez López

ACADEMIC/EDUCATIONAL ACTIVITY

2016 – 2019 Lecturer in the degree of Sport Sciences, Faculty of Sport Sciences. University of Granada, Granada, Spain

2017 Erasmus Lecturer in the degree of Sport Sciences, University School of Physical Education, Krakow, Poland.

PATENTS OR RECORDS OF THE INTELECTUAL PROPERTY

2013 – 2019 Co-developer of "Cofito and Cofita evaluate your physical fitness" (IPR-813). https://www.safecreative.org/work/1906181192161-cofito-y-cofita-evaluan-tu-condicion-fisica

PUBLICATIONS

1. **Mora-Gonzalez J**, Migueles JH, Esteban-Cornejo I, Cadenas-Sanchez C, Rodriguez-Ayllon M, Molina-García P, Pastor-Villaescusa B, Rico MC, Gil A, Aguilera CM, Catena C, Ortega FB. Sedentarism, Physical Activity, Steps, and Neurotrophic Factors in Obese Children. ***Med Sci Sports Exerc***, in press.
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3. **Mora-Gonzalez J**, Esteban-Cornejo I, Cadenas-Sanchez C, Migueles JH, Rodriguez-Ayllon M, Molina-Garcia P, Hillman CH, Catena A, Pontifex MB, Ortega FB. Fitness, physical activity, working memory and neuroelectric activity in children with overweight/obesity. ***Scand J Med Sci Sports*** 2019, doi: 10.1111/sms.13456.
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preschoolers, children and adolescents: A systematic review and meta-analysis. ***Sports Med*** 2019, doi: 10.1007/s40279-019-01099-5.

5. Molina-Garcia P, Migueles JH, Cadenas-Sanchez C, Esteban-Cornejo I, **Mora-Gonzalez J**, Rodriguez-Ayllon M, Plaza-Florido A, Vanrenterghem J, Ortega FB. A systematic review on biomechanical characteristics of walking in children and adolescents with overweight/obesity: Possible implications for the development of musculoskeletal disorders. ***Obes Rev*** 2019; 20(7):1033-1044.
6. **Mora-Gonzalez J**, Esteban-Cornejo I, Cadenas-Sanchez C, Migueles JH, Molina-Garcia P, Rodriguez-Ayllon M, Henriksson P, Pontifex MB, Catena A, Ortega FB. Physical fitness, physical activity, and the executive function in children with overweight and obesity. ***J Pediatr*** 2019; 208:50-56.e1.
7. Esteban-Cornejo I, Rodriguez-Ayllon M, Verdejo-Roman J, Cadenas-Sanchez C, **Mora-Gonzalez J**, Chaddock-Heyman L, Raine LB, Stillman CM, Kramer AF, Erickson KI, Catena A, Ortega FB, Hillman CH. Physical fitness, white matter volume and academic performance in children: Findings from the ActiveBrains and FITKids2 projects. ***Front Psychol*** 2019; 10:208.
8. Gomez-Bruton A, Arenaza L, Medrano M, **Mora-Gonzalez J**, Cadenas-Sanchez C, Migueles JH, Muñoz-Hernández V, Merchan-Ramirez E, Martinez-Avila WD, Maldonado J, Oses M, Tobalina I, Gracia-Marco L, Vicente-Rodriguez G, Ortega FB, Labayen I. Associations of dietary energy density with body composition and cardiometabolic risk in children with overweight and obesity: role of energy density calculations, under-reporting energy intake and physical activity. ***Br J Nutr*** 2019; 121(9):1057-1068.
9. Merino-de Haro I*, **Mora-Gonzalez J***, Cadenas-Sanchez C, Borras PA, Benito PJ, Chiva-Bartoll O, Torrijos-Niño C, Samaniego-Sánchez C, Quesada-Granados JJ, Sánchez-Delgado A, Dorado García C, García-Martínez JM, Vicente-Rodriguez G, Labayen I, Ortega FB. Higher socioeconomic status is related to healthier levels of fatness and fitness already at 3 to 5 years of age: The PREFIT Project. ***J Sport Sci*** 2019; 37(12):1327-1337. *Same contribution.
10. Migueles JH, Cadenas-Sanchez C, Tudor-Locke C, Löf M, Esteban-Cornejo I, Molina-Garcia P, **Mora-Gonzalez J**, Rodriguez-Ayllon M, Garcia-Marmol E, Ekelund U, Ortega FB. Comparability of published cut-points for the assessment of physical activity: Implications for data harmonization. ***Scand J Med Sci Sports*** 2019; 29(4):566-574.
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13. Rodriguez-Ayllon M, Cadenas-Sanchez C, Esteban-Cornejo I, Migueles JH, **Mora-Gonzalez J**, Henriksson P, Martín-Matillas M, Mena-Molina A, Molina-García P, Estévez-López F, Enriquez GM, Perales JC, Ruiz JR, Catena A, Ortega FB. Physical fitness and psychological health in overweight/obese children: A cross-sectional study from the ActiveBrains project. ***J Sci Med Sport*** 2018;21(2):179-184.
14. **Mora-Gonzalez J**, Cadenas-Sánchez C, Martinez-Tellez B, Sanchez-Delgado G, Ruiz JR, Léger L, Ortega FB. Estimating VO2max in children aged 5-6 years through the adapted 20m shuttle-run test (PREFIT). ***Eur J Appl Physiol*** 2017; 117(11):2295-2307.
15. Esteban-Cornejo I, Cadenas-Sanchez C, Contreras-Rodriguez O, Verdejo-Roman J, **Mora-Gonzalez J**, Migueles JH, Henriksson P, Davis CL, Verdejo-Garcia A, Catena A, Ortega FB. A whole brain volumetric approach in overweight/obese children: Examining the association with different physical fitness components and academic performance. The ActiveBrains project. ***Neuroimage*** 2017; 159:346-354.
16. Migueles JH, Cadenas-Sanchez C, Ekelund U, Delisle Nyström C, **Mora-Gonzalez J**, Löf M, Labayen I, Ruiz JR, Ortega FB. Accelerometer Data Collection and Processing Criteria to Assess Physical Activity and Other Outcomes: A Systematic Review and Practical Considerations. ***Sports Med*** 2017; 47(9):1821-1845.

17. **Mora-Gonzalez J**, Rodríguez-López C, Cadenas-Sanchez C, Herrador-Colmenero M, Esteban-Cornejo I, Huertas-Delgado FJ, Ardoy DN, Ortega FB, Chillón P. Active commuting to school was inversely associated with academic achievement in primary but not secondary school students. ***Acta Paediatr*** 2017; 106(2):334-340.
18. Cadenas-Sanchez C, Sanchez-Delgado G, Martinez-Tellez B, **Mora-Gonzalez J**, Löf M, España-Romero V, Ruiz JR, Ortega FB. Reliability and Validity of Different Models of TKK Hand Dynamometers. ***Am J Occup Ther*** 2016; 70(4):7004300010.
19. Cadenas-Sanchez C, Martinez-Tellez B, Sanchez-Delgado G, **Mora-Gonzalez J**, Castro-Piñero J, Löf M, Ruiz JR, Ortega FB. Assessing physical fitness in preschool children: Feasibility, reliability and practical recommendations for the PREFIT battery. ***J Sci Med Sport*** 2016; 19(11):910-915.
20. Cadenas-Sánchez C, **Mora-González J**, Migueles JH, Martín-Matillas M, Gómez-Vida J, Escolano-Margarit MV, Maldonado J, Enriquez GM, Pastor-Villaescusa B, de Teresa C, Navarrete S, Lozano RM, de Dios Beas-Jiménez J, Estévez-López F, Mena-Molina A, Heras MJ, Chillón P, Campoy C, Muñoz-Hernández V, Martínez-Ávila WD, Merchan ME, Perales JC, Gil Á, Verdejo-García A, Aguilera CM, Ruiz JR, Labayen I, Catena A, Ortega FB. An exercise-based randomized controlled trial on brain, cognition, physical health and mental health in overweight/obese children (ActiveBrains project): Rationale, design and methods. ***Contemp Clin Trials*** 2016; 47:315-24.
21. Martinez-Tellez B, Sanchez-Delgado G, Cadenas-Sanchez C, **Mora-Gonzalez J**, Martín-Matillas M, Löf M, Ortega FB, Ruiz JR. Health-related physical fitness is associated with total and central body fat in preschool children aged 3 to 5 years. ***Pediatr Obes*** 2016; 11(6):468-474.
22. Cadenas-Sanchez C, Nyström C, Sanchez-Delgado G, Martinez-Tellez B, **Mora-Gonzalez J**, Risinger AS, Ruiz JR, Ortega FB, Löf M. Prevalence of overweight/obesity and fitness level in preschool children from the north compared with the south of Europe: an exploration with two countries. ***Pediatr Obes*** 2016;11(5):403-10.
23. Sanchez-Delgado G, Cadenas-Sanchez C, **Mora-Gonzalez J**, Martinez-Tellez B, Chillón P, Löf M, Ortega FB, Ruiz JR. Assessment of handgrip strength in preschool children aged 3 to 5 years. ***J Hand Surg Eur Vol*** 2015; 40(9):966-72.
24. Cadenas-Sánchez C, Alcántara-Moral F, Sánchez-Delgado G, **Mora-González J**, Martínez-Téllez B, Herrador-Colmenero M, Jiménez-Pavón D, Femia P, Ruiz JR, Ortega FB. Assessment of cardiorespiratory fitness in preschool children: adaptation of the 20 metres shuttle run test. ***Nutr Hosp*** 2014; 30(6):1333-43.
25. Ortega FB, Cadenas-Sánchez C, Sánchez-Delgado G, **Mora-González J**, Martínez-Téllez B, Artero EG, Castro-Piñero J, Labayen I, Chillón P, Löf M, Ruiz JR. Systematic review and proposal of a field-based physical fitness-test battery in preschool children: the PREFIT battery. ***Sports Med*** 2015;45(4):533-55.

ACCEPTED CONGRESS COMUNICATIONS AS FIRST AUTHOR

1. **Mora-Gonzalez J**, Esteban-Cornejo I, Cadenas-Sanchez C, Migueles JH, Catena A, Pontifex MB, Ortega FB. [The associations between physical fitness components and neuroelectric activity underlying a working memory task in children with overweight/obesity: ActiveBrains project.] *I Congreso Nacional de Investigadores del PTS.* Granada, Spain. 13–15 February, 2019.
2. **Mora-Gonzalez J**, Esteban-Cornejo I, Cadenas-Sanchez C, Migueles JH, Rodriguez-Ayllon M, Molina-Garcia P, Catena A, Pontifex MB, Ortega FB. Physical fitness and neuroelectric activity underlying a working memory task in overweight/obese children: the ActiveBrains project. *58th Annual Meeting of the Society for Psychophysiology Research.* Quebec, Canada. 3–7 October, 2018.
3. **Mora-Gonzalez J**, Cadenas-Sanchez C, Esteban-Cornejo I, Hidalgo-Migueles J, Henriksson P, Catena A, Ortega FB. Is physical fitness associated to executive function in overweight/obese children? Results from the ActiveBrains project. *ActiveBrains for all: Exercise, cognition and mental health - International Symposium.* Granada, Spain. 12 June, 2017.
4. **Mora-Gonzalez J**, Cadenas-Sanchez C, Esteban-Cornejo I, Hidalgo-Migueles JH, Henriksson P, Ortega FB. [Upper-limbs muscular strength is associated with the planning skills of overweight/obese children: The

The ActiveBrains project]. *II training researchers' conferences: promoting interdisciplinary.* Granada, Spain. 17–19 May, 2017.

5. **Mora-Gonzalez J**, Cadenas-Sanchez C, Esteban-Cornejo I, Migueles JH, Henriksson P, Ortega FB. Higher physical fitness is associated with a better executive function in overweight/obese children: the ActiveBrains project. *I International CIMCYC Workship: from representations to cognitive theory.* Granada, Spain. 19–20 April, 2017.
6. **Mora-Gonzalez J**, Rodríguez-López C, Cadenas-Sánchez C, Herrador-Colmenero M, Ávila-García M, Huertas-Delgado FJ, Ardoy DN, Ortega FB, Chillón P. [Those who actively commute to school has lower odd of having high grades in comparison with passive ones in Primary Education, but not in Secondary]. *I International Seminar on the Knowledge Updating in Health Sciences. EXERNET.* Granada, Spain. 20 January, 2017.
7. **Mora-Gonzalez J**, Cadenas-Sanchez C, Migueles JH, Esteban-Cornejo I, Henriksson P, Catena A, Ortega FB. [Cardiorespiratory fitness is positively associated with cognitive performance and intellectual quotient in overweight-obese children: Preliminary results of ActiveBrains projects]. *Symposium EXERNET: Research in physical exercise and health: present and future in Spain.* Cádiz, Spain. 14–15 October, 2016.
8. **Mora-Gonzalez J**, Cadenas-Sanchez C, Migueles JH, Esteban-Cornejo I, Henriksson P, Catena A, Ortega FB. Physical activity, physical fitness, fatness and their association with executive function in overweight and obese children: The ActiveBrains project. *HEPA Conference (12th Annual Meeting and 7th Conference of HEPA Europe).* Belfast, Northern Ireland. 28–30 September, 2016.
9. **Mora-Gonzalez J**, Cadenas-Sanchez C, Sanchez-Delgado G, Ortega FB. Assessment of cardiorespiratory fitness in 5-6 year-olds: construct validity between the original and the preschool-adapted (PREFIT) 20 meters shuttle- run tests. *I training researchers' conferences: promoting interdisciplinary.* Granada, Spain. 18 May, 2016.
10. **Mora-González J**, Cadenas-Sánchez C, Migueles JH, Esteban-Cornejo I, Henriksson P, Catena A, Ortega FB. Association between health-related physical fitness, physical activity, sedentary behaviour and cognitive performance in overweight and obese children: the ActiveBrains study. *Early Nutrition Academy Postgraduate Course 2016 (ENA): role of early nutrition on non-communicable diseases development.* Granada, Spain. 4 May, 2016.
11. **Mora-González J**, Rodríguez López C, Cadenas-Sánchez C, Herrador-Colmenero M, Ávila-García M, Huertas-Delgado FJ, Ardoy DN, Ortega FB, Chillón P. Is active commuting to school associated to academic achievement in youth? *Symposium EXERNET. Research in physical exercise and health: present and future in Spain*. Granada, Spain. 7–8 November, 2014.

INVITED CONFERENCES/LECTURES

1. **Mora-Gonzalez J**. Fitness, physical activity and neuroelectric activity in children**.** *The ActiveBrains-SmarterMove International Seminar: Exercise, Cognition and Brain in Childhood and Older Age.* Granada, Spain. 8 March, 2018.
2. **Mora-Gonzalez J**. Past, Present and Future of the ActiveBrains, SmarterMover, and CoCa projects. *ActiveBrains for all: Exercise, cognition and mental health - International Symposium*. Granada, Spain. 12 June, 2017.
3. **Mora-González J.** Active commuting to school and academic achievement in children and adolescents. *Active commuting: investigations and experiences of transference. Workshop.* Granada, Spain. 1 July, 2015.
4. **Mora-Gonzalez J.** Ejemplo de una propuesta de innovación real en el área de Educación Física: diseño y claves de su éxito. *Lecturer in Foundations in Physical Education (Degree in Sport Sciences)*. Granada, Spain. 5 June, 2014.

OTHER MERITS

2019	Abstract entitled "Physical activity and slep-related behaviors, but not sedentary time, are associated with brain-derived neurotrophic factor in children with overweight/obesity" with Mora-Gonzalez J as first author was selected for a Travel Award of 500€ at the 2019 ECNP Congress, Copenhagen, Denmark.
2018	Finalist of the "3 Minute Thesis Competition" with the talk "Fiction or reality" (https://www.youtube.com/watch?v=_xpgV1ssleA&t=105s). International School for Postgraduate Studies, University of Granada, Granada, Spain.
2015	Special mention award to the best Final Master Degree project in the Master's degree in Physical Activity and Sport Research. University of Granada, Granada, Spain.

AGRADECIMIENTOS

LA AVENTURA DEL APRENDIZ

PRÓLOGO

Retazos de un comienzo

Había llegado el día. Pepe Mora, un chico inquieto y de carácter simpático, de tez clara, generosa nariz y cabellos rubios cortados y estilizados con sumo cuidado, se enfrentaba a aquello por lo que tanto había luchado, a aquello para lo que había sido entrenado y formado durante varios años: *El Examen del Título de Maestro Doctor*. Pepe Mora era lo que por entonces se conocía como un *Aprendiz*. Solo aquellos denominados como tal eran llamados a instruirse día y noche en el arte de la ciencia y la investigación para algún día llegar a ser verdaderos *Maestros Doctores*. Todo *Aprendiz*, al decidir querer optar al mencionado título, aceptaba paralelamente sumergirse en una gran aventura que se caracterizaba por el conocimiento y encuentro de diversos personajes de todo tipo, por la experiencia de momentos únicos e irrepetibles y por el aprendizaje de diferentes técnicas y poderes. A pesar de lo llamativo de esta aventura, durante ella eran también muy numerosas las temidas batallas a las que había que hacer frente y que, a su vez, llevaban a los *Aprendices* a sacar lo peor y mejor de sí mismos. El camino hacía el *Titulo de Maestro Doctor* no era un camino de rosas, sino que todo aquel que lo afrontaba asumía el reto de enfrentarse a sí mismo, a sus miedos, a sus defectos y a sus inseguridades. Este camino, era un camino de aprendizaje y de conocimiento de uno mismo y de los demás al que solo los más valientes estaban dispuestos a hacer frente. Quizás un día estuvieses en la cima, pero al día siguiente te arrastrarías por los más oscuros avernos del infierno, deseando agarrar una mano amiga que te sacara de allí. Y es que, solo aquellos dotados del poder de la constancia, la responsabilidad y el tesón llegarían vivos al día del *Examen del Título de Maestro Doctor*.

Pepe Mora vivía junto a sus padres y sus dos hermanos en una casita alejada de los grandes castillos y murallas que dibujaban la ciudad de *Granada*. La casita estaba situada en un barrio de gente humilde, frente a un frondoso jardín de dimensiones interminables. Desde la casita se podía respirar la humedad que desprendían las hojas de los cansados robles que se apostaban a los extremos del jardín y, en épocas primaverales, el arcoíris que dibujaban las diferentes familias de flores que comformaban el corazón del jardín era de una belleza inigualable. En la casita, Pepe Mora habitaba junto a sus dos hermanos: Pablo, de aspecto corpulento y tímido carácter que acompañaba con una habilidad innata para bromear, y Nacho (o como a Pepe le gustaba llamar y él, en cambio, detestaba: Ignacio), capaz de dormir, cantar, jugar, estudiar, comer y luego, por supuesto, volver a dormir en décimas de segundo. Sus padres, Lola y Pepe, eran ambos alegres, generosos y cariñosos. La casita contó también con la agradable presencia de Kasita, una joven princesa de cabellos dorados y sonrisa dulce que había decidido dejarlo todo en Polonia, su país, para acompañar a quién un día se convertiría en su futuro marido, Pepe Mora.

Pepe Mora vivía muy feliz junto a su familia y su amor, y nunca llegaría a imaginar que, sin tener que abandonarla físicamente, acabaría ausentándose del día a día de la casita y de los seres que la llenaban a consecuencia de embarcarse en la aventura por convertirse en *Maestro Doctor*. Esta es, por tanto, la historia de un joven granadino que un día de septiembre de 2013 conoció a su *Mentor* y se embarcó en una aventura que le cambiaría la vida…

1

El encuentro

Recuerdo a la perfección aquel martes 10 de septiembre de 2013. Había decidido, sin ser muy consciente de lo que ello conllevaba, acercarme a conocer más profundamente a uno de los *Mentores* que me habían instruido durante mi época de estudiante. Ya por aquel entonces, había quedado prendado de su humildad y sencillez características. Además, su talento inconmensurable había despertado en mí cierto interés por el arte que este *Mentor* dominaba: la ciencia e investigación. Esa mañana recuerdo que todo sucedió muy deprisa. Tras pasar una noche prácticamente en vela movido por la emoción que suponía para mí encontrarme frente a frente al *Mentor*, apenas di dos bocados a mi regular tostada de todas las mañanas y me apresuré hacia uno de los *Centros de Entrenamiento* para *Aprendices* más conocido como *La Academia (INEF)*, donde había recibido mi formación previa.

Mis nudillos se posaron sobre la puerta de su despacho y golpearon ésta sin apenas darme cuenta de lo que estaba haciendo. Una voz joven y llena de vida desde dentro llamó a que entrara y, dudando un segundo, giré el pomo de la puerta para entrar. Abrí la puerta y allí estaba él. **Fran Ortega** se encontraba escondido tras su ordenador y de forma enérgica se levantó para darme la bienvenida. Se encontraba tal y como lo recordaba de mi época de estudiante, para ser sinceros, seguía manteniendo la poca pinta de un *Mentor* al uso. Desprendía una alegría campechana que te invitaba a relajarte y olvidarte de cualquier cosa que te atormentara. Su capacidad para combinar colores que no tenían nada que ver entre sí era hasta graciosa, y su cercanía amigable conquistaba a cualquiera que se preciara a mantener una conversación con él. Fran me acogió con los brazos abiertos. Él, desde el primer día, me instruyó en el arte de la ciencia y la investigación regalándome todo su talento a disposición y convirtiéndose en mi primer *Mentor Oficial*. Aún recuerdo mi batalla contra el primer *Paper*, más conocido como *Commutator*. Fran se dedicó en cuerpo y alma a que aprendiera todas las técnicas mágicas para sacar lo mejor de mi y acabar encadenando a *Commutator* a una revista para siempre. Además de su talento científico, Fran era una de las personas más bondadosas que yo había conocido. Aún hoy siento un agradecimiento profundo por escucharme en los momentos más difíciles de mi aventura, por darme siempre la mano y buscar constatemente la salida más sana y saludable para el bien de todos los que, poco a poco, fuimos conformamos su escuadrón. Fran no es un *Mentor Oficial* más. Fran ha conseguido que yo aprenda no solo los más valiosos poderes y habilidades científicas, sino a ser mejor persona, a querer a los demás, a ser generoso y empático. Fran es el mejor *Mentor Oficial* que jamás alguien podría desear, capaz de enseñarte, por un lado, los más valiosos poderes de engatusamiento de los más que temibles *Editors* y, por otro, las habilidades más ocultas de compadreo y colegueo acompañadas siempre de una buena birra en las tabernas y posadas de *Granada*. Hoy le digo de viva voz: "Gracias Fran por darme la oportunidad de crecer junto a ti, aprender junto, caerme junto a ti…" Ojalá nunca dejes de *Mentorizarme*.

La cita con Fran fue todo un éxito y rápidamente me impulsó a conocer a mi primer escuadrón de entrenamiento con quien experimentaría mi primera gran aventura. Unos días después de mi cara a cara con Fran, volví a ese despacho de aspecto sencillo, pero cálido, y me encontré rodeado de un inquieto **Borja**, un seguro **Guille** y una comprometida **Cristina**. Junto a ellos, había otro gran *Mentor* llamado **Jonatan** y que, sin duda, compartía con Fran muchas de las habilidades que nosotros aspirábamos a tener algún día. Jonatan, siempre apostó por mí entre otros y, por ello, le estoy muy agradecido. Recuerdo además una anécdota graciosa que aún hoy compartimos para reírnos conjuntamente. Una de mis mejores habilidades como *Aprendiz* era registrar con grabaciones ocultas las diferentes reuniones que teníamos con los *Mentores* y Jonatan, que era de una gran inteligencia, me pilló y, aún hoy, siempre de broma, me lo sigue recordando. Fran y Jonatan nos hicieron llamar *Los 4 magníficos* y nos embarcaron en nuestra primera misión, *El Proyecto PREFIT*, en el cual nos uniríamos a los graciosos *Cofito y Cofita* para formar y evaluar físicamente a cientos de niños y niñas prescolares en la batalla contra *Grasito.* De entre mis compañeros de entrenamiento, recuerdo especialmente los momentos compartidos con **Borja**. Borja era un chico de pecho descomunal, agraciado físicamente y con una habilidad especial para el cachondeo. Con él, además de formar parte del escuadrón *PREFIT*, tuve la oportunidad de vivir la aventura de *Los Juegos del Rojas*, en la que ambos compartimos el ser, por primera vez y previamente a convertirnos en *Aprendices*, *Docentes en prácticas* y fue algo sencillamente espectacular. Ambos cambiamos nuestra identidad para esta misión y nos hicimos llamar *Salut Mellark y Habit Everdeen*. Favorecidos por nuestro gran manejo del arco, conseguimos convencer a numerosos jóvenes en la lucha contra los hábitos insaludables liderada por *Snow*. Particularmente durante esta aventura, fue tal el apoyo que sentí por parte de Borja que me llevó en volandas por toda la experiencia. Los dos aprendimos juntos, nos caímos juntos, y nos volvimos a levantar apoyándonos el uno en el otro. Todo esto lo acompañamos de momentos de risas y júbilo. Los dos fuimos grandes protagonistas de una aventura inigualable, en la que aparcamos nuestros egos para centrarnos el uno en el otro y cuidarnos mutuamente. Hoy le agradezco que me aconsejara a nivel personal y profesional, que me acompañara en mis locuras y las apoyara con ilusión y desenfreno. Además de Borja, en esa habitación conocí también a **Guille**, popular por su cabeza de dimensiones nunca antes vistas (tanto física como intelectualmente) y también por tener siempre una respuesta que hacía tambalear los cimientos de tu cerebro, sacando lo mejor de uno mismo. De hecho, yo diría que es posiblemente el *Aprendiz* más sabio que conocí en mi aventura y bastaba estar un rato junto a él para terminar sintiendo que habías aprendido más que cualquier año de estudio. Por último, también estaba **Cristi**, líder nata del proyecto PREFIT y el toque de responsabilidad y trabajo del que tanto aprendí. Tanto ella como Guille fueron los dos compañeros ideales para dar mis primeros pasos como *Aprendiz*. De ellos aprendí desde el punto de vista técnico y personal y les estaré siempre agradecido por ello.

Junto a Fran y Jonatan, el grupo de *Mentores* que conformaban el llamado escuadrón *PROFITH* al que pasé a formar parte, estaba constituido por Jonatan, **Palma** y **Miguel(ón)**. **Palma** fue, junto con Fran, la *Mentora* que me ayudó en la lucha contra mi primer *Paper*, el conocido como *Commutator*. ¡Qué fácil y necesario es compartir momentos con ella! Para mí, aprender a combatir contra mi primer *Paper* fue sin duda un enorme reto en mi entrenamiento. Sin la ayuda de Palma, jamás hubiera sido posible. Además, cada encuentro con ella a lo largo de esta aventura me regaló grandes consejos que jamás olvidaré. No solo me ayudó en el camino de *Aprendiz de Doctor* sino también, y no menos importante, en el de *Aprendiz de Docente*. Y es un gran honor y orgullo que forme parte del *Tribunal* en mi *Examen del Título de Maestro Doctor.* Es, sin duda, una de esas personas a las que el resto de *Aprendices* desean acudir por tener grandes poderes de cercanía, amabilidad y simpatía. Y de esto sabe también bastante **Miguel(ón)**. ¡Qué capacidad para hacer de cada batalla y momento de tensión y nervios algo agradable, gracioso y distendido! Miguel(ón) es un gran mago del arte de la broma y es que, en este mundo tan lleno de decepciones, tensión, frustraciones, siempre es de agradecer el tener personas con habilidades únicas e irrepetibles para llenar de humor y de color los momentos más difíciles.

Cuando, en mi batalla por encadenar el *Paper* de *Commutator* a una revista estaba casi decidida apareció alguien que se acabaría convirtiendo en una de las personas más importantes de mi aventura: **Irene**. Ire, como ella prefiere ser llamada, apareció de repente y Fran rápidamente le ordenó acercarse a mi para orientarme en mi primera misión frente a *Commutator*. Rápidamente pude detectar que tenía un poder oculto y especial que jamás había visto antes. Ire tenía la capacidad de asustar a todos los miedos e inquietudes de su alrededor y de los alrededores de los que se encontrasen junto a ella. Trabajadora incansable, desde el primer momento me sentí cuidado y arropado por ella. Aún recuerdo el día en que Fran me comentó que podía incorporar a un segundo *Mentor Oficial* que me siguiera guiando en mi aventura como *Aprendiz*. No dudé ni un segundo en convertir a Ire en mi segunda *Mentora Oficial*. Siempre pendiente de mí. Siempre dispuesta a ayudarme y a acompañarme en mi camino pasase lo que pasase. Y es que no todo es color de rosa. Los dos vivímos momentos difíciles, propios de una relación estrecha y única, pero el cariño y respeto entre ambos siempre se imponían. Ella no tuvo problema en acompañarme cuando mis misiones de *Aprendiz* me obligaron a salir fuera de mi zona de confort, a salir fuera de mi país y cruzar el charco. Yo,

persona de grandes inseguridades, me sentía siempre seguro cuando ella estaba junto a mi. Ese poder no lo he conocido en nadie más que en ella. Me cuidó y quiso como una verdadera amiga, incluso como una hermana. No me puedo imaginar separarme de ella pues estoy convencido de que gran parte de mi éxito y de mi formación es gracias a ella. No había día que pasara y que ella dejase de interesarse por cómo estaba. Es, sin duda, mi mano derecha. Una gran amiga y la hermana que nunca tuve.

2

El escuadrón de ActiveBrainers

Tras dar mis primeros pasos como *Aprendiz* y superar las primeras batallas junto al escuadrón *PREFIT*, Fran se embarcó en la aventura conocida como *ActiveBrains*. Pensar en *ActiveBrains* me lleva a pensar en grandes personas, también conocidas como *Aprendices ActiveBrainers* que me han acompañado y ayudado fielmente a ser y convertirme en quien soy hoy. La misión de entrenamiento *ActiveBrains* ha sido, sin duda, la que ha marcado y marcará para siempre mi formación como *Aprendiz*. Pero antes de hacer referencia a mis compañeros *Aprendices*, lo cierto es que al principio simplemente Fran, Cristina y yo conformamos un escuadrón para entrenar e instruir físicamente a los verdaderos protagonistas de esta aventura y a quiénes yo, personalmente, les debo estar aquí hoy. Nuestros **niños y niñas** y sus **maravillosas familias** procedentes de todas partes de Granada acudieron a ser evaluados y entrenados siempre con una sonrisa y dispuestos a ayudar en todo lo posible para poder, a su vez, hacer realidad mi entrenamiento como *Aprendiz*. Los días de evaluación y entrenamiento de los chicos y chicas fueron largos, duros y tediosos pero lo que yo me llevé fue mucho más que eso. Aún recuerdo los abrazos llenos de cariño que recibí de cada uno de ellos al terminar cada evaluación o entrenamiento o el agradecimiento eterno de sus padres y madres. También la emoción por acabar mi particular aventura con ellos o el sentir profundamente la ayuda que estaba brindandoles, permitiéndoles conocer y mejorar su estado de salud. Más allá de todas las técnicas, poderes y habilidades que aprendí en mi instrucción como *Aprendiz a Maestro Doctor*, me quedo, sin lugar a dudas, con la gran cantidad de sentimientos y emociones que estos chicos y sus familias despertaron e instauraron en mi corazón para siempre.

Hacer frente con éxito a todo lo que *ActiveBrains* iba desencadenando en mi camino a *Maestro Doctor* era misión imposible si hubiese de encararlo yo solo. Nada más iniciar la misión, recuerdo con alegría la llegada de **Ana** y **Alejandra**, *Entrenadoras* que fueron de gran ayuda, sobre todo, en el inicio de la misión. Especialmente con Alejandra, tuve la suerte de compartir profundas conversaciones sobre la vida, a la vez que uníamos esfuerzos para entrenar a nuestros pupilos. No mucho más tarde, apareció **Jairo**, un nuevo *Aprendiz* que, en un principio, pareció entrar de puntillas, pero que finalmente acabaría pisando muy fuerte. Jairo, mostró desde un principio ser uno de los *Aprendices*, desde mi punto de vista, con mayor capacidad intelectual. Su inteligencia nos regaló grandes victorias en el campo de batalla y nos permitió conocer profundamente y a la perfección todo nuestro equipamiento, para usarlo de la mejor forma posible. Jairo se mostró siempre dispuesto a ayudar y compartir sus conocimientos con sus compañeros.

Recuerdo especialmente que hubo un tiempo en el que las fuerzas flaqueaban en el seno del escuadrón y el entrenamiento se hacía cada vez más y más duro. Cuando parecía que hincar la rodilla era la solución, aparecieron dos *Aprendices* de gran corazón y que dieron gran alegría al equipo: **Pablo** y **María**, o **María** y **Pablo**. En concreto, Pablo se ganó mi corazón desde un primer momento con su humildad e inocencia características. Personaje de carácter despistado pero gracioso, Pablo conseguía vencer a todos los enemigos de nuestro entorno sin ni siquiera él darse cuenta de ello. Es uno de esos compañeros que todo el mundo querría en su escuadrón, porque todo es sumamente fácil cuando él está cerca. Además, es una persona que me ha regalado momentos de gran alegría y me ha hecho olvidar mis preocupaciones y frustraciones continuas cuando más lo necesitaba. Cuando tenía un mal día o las cosas no salían, siempre era agradable tener a Pablo cerca porque hacía que todo tuviese menos importancia de lo que la tiene. Quizás incluso lo hiciese sin darse cuenta… **María** es posiblemente la compañera más enérgica que nunca haya tenido. Siempre pensaré lo mismo: ¡Qué importante es tener en el escuadrón a gente que genera buen rollo, que desprende energía positiva a raduales! María es ejemplo de alegría, de despreocupación, de tranquilidad. Además, siempre que hay que afrontar cualquier batalla contra un *Paper*, ella demuestra su pasión por lo que hace e incluso llega a contagiar a los que estamos a su alrededor y nos hace sentirnos invencibles ante ellos. Tanto con María como con Pablo he podido, además, compartir conversaciones más personales que nos han hecho sin duda crecer conjuntamente. En definitiva, tanto uno como otro dotaron al escuadrón y, en concreto, a mí, de una alegría, capacidad de trabajo y de compañerismo sin igual.

La labor del escuadrón se ha visto reforzada en numerosas ocasiones por diversos *Entrenadores* y *Aprendices a Maestro Doctor*, algunos de los cuáles aportaron su granito de arena y se marcharon, mientras otros se mantenían y hoy también son *Aprendices ActiveBrainers*. En el ámbito de *Entrenadores* no me puedo olvidar de la labor y esfuerzo que realizaron **Patri**, **Carlitos**, **Antonio** o **Zeus** para que el entrenamiento de los chicos y chicas fuese el mejor posible. En el ámbito de *Aprendices a Maestro Doctor*, compañeros como **José Juan**, **Lucía**, **Juan Pablo** o **Abel** llenaron de vitalidad y ganas de aprender al escuadrón. Hoy somos compañeros *ActiveBrainers* y me hace muy feliz saber que están ahí para lo que necesite. No me olvido ni mucho menos de la incorporación al escuadrón de **Luis** y **Esther** y su labor en concreto para hacer frente a *DXAtor*, una máquina cuya programación nos lo puso bastante difícil. Luis y Esther llegaron dispuestos a ayudar de forma generosa y altruista y eso fue una gran lección para mí. Además de todos ellos, recuerdo con especial cariño la llegada de ayuda y aire freso para el escuadrón procente del extranjero, de la mano de **Pontus** y **Hanna** que rápidamente se ganaron mi corazón con su bondad y alegría. Por último, también llegado desde el extranjero, tuve la suerte de conocer a un compañero con el que compartía diversas habilidades científicas y que también entendía bien el poder del rendimiento cognitivo. **Pato** me ayudó desde un principio a hacer frente a los distintos *Papers* que se me fueron presentando en mi camino y con su conocimiento y pasión pudimos dar respuesta a muchas de las preguntas que se nos plantearon en el campo de batalla. Pato, además de su capacidad para tener éxito en el campo de batalla, ha sido para mí un ejemplo de amor por la familia sin él saberlo. Siempre dispuesto a ayudar, siempre dispuesto a entregarme su tiempo. Un compañero de esos de los que nunca me gustaría separarme. Además de Pato, nunca podré olvidar una de las mayores sorpresas que me pude llevar, que no es otra que la incorporación al escuadrón de mi amigo y "hermano" **Nacho Merino**. Nacho era un chaval atlético, de poco pelo y de energía inagotable. Nos habíamos conocido en otro contexto, fuera del mundo de la ciencia y la investigación. Sin los dos esperarlo, el destino había querido que nos juntásemos y su presencia supuso para mi un gran soplo de aire freso entre tanto entrenamiento y, porqué no decirlo, un poquito de oración. Y es que, entre batalla y batalla, pudimos hacer lo que más nos gusta: Hablar de la vida, de nosotros, de quiénes somos, de adónde vamos, y de cómo seguir creciendo.

ActiveBrains nos planteó numerosas dificultades en cuanto a la necesidad de gozar de una serie de habilidades especiales que muchos de los ya mencionados no poseíamos. En cualquier contexto hubiésemos desfallecido y fracasado en nuestra misión si no hubiese sido por la inestimable ayuda de los llamados *Specialists* que dominaban las artes de la nutrición, la bioquímica o la medicina y gracias a los cuáles pudimos salir airosos de números enfrentamientos. Recuerdo, por ejemplo, a las que bauticé como *Damas de la Nutrición*, **Eli**, **Wendy** y **Victoria**, y su arte para atender y recoger la mayor información posible sobre aspectos nutricionales. Recuerdo también la capacidad de las, también bautizadas por mí, como *Damas de la Centrifugación*, **Belén** y **Mari Cruz**, para recoger y analizar las muestras de nuestros chicos y chicas y dotarnos de esta información fundamental para el proceso de entrenamiento. Y cómo olvidar la inestimable ayuda de captación de nuestros pupilos de parte de *Los Mentores de Bata Blanca* como **Pepe Gómez**, **Victoria**, **María José** o **Gala**, así como la dedicación y trabajo del *Clan del Esfuerzo* formado por **Rosa**, **Carlos de Teresa** y **Socorro**.

Mi labor en la misión *ActiveBrains* fue, al fin y al cabo, relativamente fácil pues siempre conté con la colaboración de un gran grupo de personas que llenaron mi tiempo de compañerismo y buen hacer. No obstante, la batalla más dura aún habría de ser librada y, para afrontarla con garantías, necesitaba de la ayuda de dos *Maestros Sabios* fundamentales en mi aventura…

3

Los Maestros Sabios

Los *Maestros Sabios* son fundamentales y muy importantes para mí en esta aventura y aunque se denominan igual influyen de manera diferente en mi vida de *Aprendiz*. Recuerdo el momento en el que veíamos, tanto el escuadrón como yo, que la misión *ActiveBrains* estaba siendo llevada a cabo con éxito. Los chicos y chicas eran entrenados con éxito y recibíamos de ellos información muy valiosa para hacer frente a los más que temidos *Papers*. En mi caso, todo se torció y el caos aterrizó en mi camino cuando me vi envuelto en la trama del *Electroencefalogrameitor*. Fran e Ire, mis *Mentores Oficiales*, ya me habían informado de la dificultad que conllevaba esta misión. Tendría que hacer frente a un todopoderoso Electroencefalograma, a su ramificación en 64 electrodos y a sus diversas formas de ser analizado. Cuando todo parecía en calma, y con el entrenamiento y evaluación de los chicos y chicas de *ActiveBrainer*s habiendo finalizado con éxito, me entró el pánico y quise huir al verme enfrentado al *Electroencefalogrameitor*. Cada vez que salía al campo de batalla para verme las caras con él, recibía un tremendo revés que me dejaba exhausto durante unos días. Sentía frustración y decepción de mí mismo, de no poder estar a la altura de tal misión, e incluso pensé en abandonar. Pero entonces, cuando todo parecía perdido, apareció él…

Desde lo más alto, en la cima de *La Cartuja* aguardaba un misterioso *Maestro Sabio* llamado **Andrés Catena**. De pelo canoso y sonrisa permanente, los años parecían no pasar por él. Desprendía una gran vitalidad y el tono irónico que caracterizaba a su oratoria convertían cada encuentro con él en un auténtico espectáculo. Durante varios años, estuve acudiendo frecuentemente al encuentro de Andrés, quien me educó en el arte de la Neurociencia y me adiestró para poder enfrentarme a *Electroencefalogrameitor*. Era tal su generosidad, que nunca recibí un “no” por respuesta ante mi insistencia por vernos y aclarar dudas de mi entrenamiento. Además, Andrés me permitió utilizar técnicas de grabado de la información que él me proporcionaba y luego yo podía seguir entrenando por mi cuenta. Nunca miró el reloj y siempre me preguntó y se aseguró de que todo hubiese quedado claro. Su sabiduría no conocía límites y su capacidad para transmitírmela, tampoco. Sin su altruismo para compartir su conocimiento conmigo, jamás hubiese podido enfrentarme con garantías de éxito a *Electroencefalogrameitor* y jamás hubiese podido poner un pie en el *Examen del Título de Maestro Doctor*. A medida que avanzabamos en nuestro entrenamiento, me enfrenté a los primeros *Papers* en los que, sin duda, me encontraría con *Electroencefalogrameitor*. Pude salir airoso en varias ocasiones gracias a la ayuda magistral de Andrés. Cuando afrontar los *Papers* parecía convertirse en éxito rutinario, la sabiduría de Andrés quiso ir más alla y me mostró, de nuevo de forma generosa y altruista, un poder oculto que recibía el nombre de *LORETA*. Nunca llegaría a imaginar la capacidad de ese poder, jamás antes visto, que me regaló grandes victorias y que, sin duda, es uno de los más importantes de mi aventura como *Aprendiz*. Nunca será suficiente el AGRADECIMIENTO, con mayúsculas, a Andrés Catena, nunca. un *Maestro Sabio* que no tuvo reparo en acercarse al más débil y compartir con él todo su conocimiento.

Amigo. Maestro. Guía. Confesor. Ejemplo. Espejo. Referente. Ídolo… Todas estas y muchas más son las palabras que se dibujan y entrelazan en mi mente y que definen claramente la figura del que para mí es el *Maestro Sabio* más importante en mi aventura y en mi vida: **Isaac**. El rol de Isaac en esta aventura se aleja de todo lo relacionado con el entrenamiento específico en el arte de la ciencia y la investigación para conseguir superar el *Examen del Título de Maestro Doctor*, pero no por ello es menos importante. De hecho, su importancia es vital para mí, pues puedo decir que Isaac salvó mi vida en muchos sentidos. Mi relación con él se remonta a mucho antes de que comenzara mi andadura como *Aprendiz*. Fue durante mi formación en *La Academia* donde me encontré con él y mi vida dio un vuelco de 360°. Isaac es un *Maestro Sabio* que domina a la perfección el arte de la *Docencia* y que posee uno de los poderes más efectivos y emocionantes de todos los que se hayan visto sobre la faz de la tierra: *la Gamificcíón*. Me recuerdo a mí mismo, aún bastante inmaduro, autoconvecido de mi escasa creatividad y falta de ingenio. Me recuerdo también voluntarioso, constante en el trabajo, pero cuadriculado, agarrado siempre a unas normas y a una estructura fija que bloqueaban mi progresión. Isaac rompió las cadenas que me apresaban y echó a volar mi imaginación. No fue tarea fácil. Sudé dolor y lágrimas en cada encuentro que tuvimos, pues su forma de instruirme no era sino exigente e inconformista. Cada vez que salía de su mundo lleno de creatividad, de color, de magia, de pasión conocido como *La Guarida*, deseaba que un tren pasase por encima de mí para no tener que levantarme jamás. Con el paso de los años, pude apreciar y valorar todo lo que esas cuatro paredes nos regalaron, me regalaron. Isaac transformo mi corazón. Lo llenó de creatividad, de inconformismo, de sueños, de alegría, de valores, de ganas de superación, de motivación… Y, lo mejor de todo, es que su humildad pura, su cariño brindado de forma tímida, su bondad, su capacidad de empatía, su escucha y su consejo las enlacé junto a mi con el lazo más fuerte y resistente que hay: La amistad. Habiendo pasado los años y habiéndome convertido ya en *Aprendiz*, nuestra conexión permaneció intacta a pesar de que los deberes y labores de cada uno quisieran separarnos. Pude y busqué, en muchas ocasiones, compaginar mi entrenamiento de *Aprendiz* con momentos únicos compartidos con Isaac que me hacían abandonar un poco

el "lado oscuro" de mi camino y abrazar de nuevo mis sueños y pasiones. Isaac fue un soplo de aire fresco que nunca cesó y siempre estuvo ahí. Se hicieron cada vez más frecuentes las escapadas de ambos a lo que me gusta llamar como *El Rinconcito del Desvarío*. Allí Isaac siguió siempre cuidando de mi, atendiendo mis preocupaciones, mis inquietudes y aconsejándome siempre, desde su corazón. A él le debo ser quien soy en muchos sentidos y, sobre todo, el haber crecido como persona. Isaac se entregó de forma altruista y generosa a mi y me enseñó el valor de la coherencia y la responsabilidad. Me siento afortunado de haber podido recibir de él tantas y tantas lecciones de vida y, sobre todo, de haber sentido de su parte un profundo interés por mi bienestar y mi persona. Hoy se sienta como *Tribunal* en el *Examen del Título de Maestro Doctor* y no hay nada que me pueda hacer más feliz que esto. Ambos sabemos que seguramente no vaya a descubrime algo nuevo sobre el arte de la ciencia y la investigación, pero seguro que me vuelve a regalar un momento cuidado de forma exquisita, coherente y lleno de eso que nos unirá para siempre: Amistad.

4

La Academia

Cuando inicié mi etapa como *Aprendiz*, gracias principalmente a la experiencia y trabajo previo de mi *Mentor Oficial* Fran, se me fue otorgada la responsabilidad conocida como *FPU* que me permitía ser adiestrado y formado en el arte de la *Docencia*, mi gran pasión verdadera, durante un entrenamiento paralelo al de *Aprendiz a Maestro Doctor*. Este es, sin duda, el entrenamiento que mas disfruté y donde considero que más poderes y habilidades pude sacar a relucir. En particular, uno de mis grandes poderes ocultos es el dominio de la música, el ritmo y la danza. Durante este adiestramiento se me entregó, a su vez, la responsabilidad de entrenar y formar a diversos grupos de chicos y chicas que comenzaban su andadura en *La Academia* y que desde un principio me exprimieron al máximo para sacar la mejor versión de mis poderes y habilidades. Hoy me enorgullezco de llamarles **mis alumnos/as** y les agradezco que confiaran ciegamente en mis habilidades como *Docente*. También les agradezco su iniciativa, su motivación y sus ganas de aprender y compartir conmigo experiencias de aprendizaje divertidas y únicas.

Mi entrenamiento en el arte de la *Docencia* me regaló a una de las personas más cariñosas y adorables que me he encontrado en mi camino. El dominio de la música y la danza de **Belén de Rueda** me cautivaron desde el principio y no dudé en convertirme en su fiel compañero. Lo más curioso es que mi camino con Belén se cruzó por primera vez cuando yo comencé mi andanza en *La Academia* y para mi supuso un auténtico honor y orgullo poder compartir con ella la misma experiencia, pero desde otra perspectiva, la del *Docente*. Belén era mujer de elegante, risueña y alegre figura. A su alrededor todo parecía descontrolado y caótico, pero siempre sorprendía con un control y manejo de la situación como nadie. Belén me enseñó a entregarme a mis alumnos/as y a priorizar siempre el corazón. Siempre pude contar con ella para todo y siempre encontré en ella una fuente de desahogo. Pero, sin duda, si algo me llevé de Belén, fue su capacidad para hacerme sentir valorado, para hacerme sentir seguro dentro de mi inseguridad y para apreciarme tal y como soy. Curiosamente, nunca jamás tuvimos un problema durante los tres años que estuvimos juntos y es que con Belén todo es sencillo y agradable. Me hizo sentir cómodo y con ella pude dejar reflajado todo mi poder musical y artístico. Los dos formamos un equipo ideal y pudimos hacer frente a todos los retos que se nos plantearon.

Recuerdo, también con agrado, las mañanas en las que, cansado, finalizaba mi labor *Docente* en *La Academia* y acudía a descansar o seguir en otras labores a la *Sala del Becario*. Allí pude conocer a grandes personas a las que hoy agradezco su cariño, escucha y capacidad para hacerme olvidar preocupaciones y frustraciones. Este grupo, de también *Aprendices,* lo forman personas como **Artacho**, **Milkana**, **Inma**, **Carmen**, **Irene Col**, **Javi**, **Manu Herrador**, **Juanma**, **Pedro** y **Fran Acosta** y **Fer**. También, *Aprendices* venidos de tierras lejanas y con los que compartí grandes momentos y de los que guardo un gran recuerdo como **Mireia** y **Adri**. Muchas gracias compañeros por sacarme siempre una sonrisa.

5

El Examen del Título de Maestro Doctor y Los seres que me llenan

Ha llegado el gran día: El día del *Examen del Título de Maestro Doctor*. Levanto ligeramente la cabeza y me encuentro de frente a un *Tribunal* al cual le estoy muy agradecido por estar valorando mi entrenamiento y por darme sus mejores consejos y opiniones. Hago un ligero gesto de afirmación con la cabeza en señal de agradecimiento hacia los ya mencionado Palma e Isaac, y también a **Arthur Kramer**, **Marie Löf** y **Celia**. Me sorprendo a mi mismo al escuchar voces en mi cerebro que hablan en otro idioma: “Thank you very much to you three for coming to Granada and sharing with me your knowledge and your thoughs”. Entonces ocurre. Desde que comencé con mi *Examen* venía experimentando una fuerza capaz de levantarme en cualquier momento y ante cualquier adversidad, un aliento inmaculado que me agitaba suavemente para recordarme que no estaba solo, un escudo protector que me hacía sentir invencible e invulnerable. Me doy cuenta de que todo eso tiene nombre y lo descubro al contemplar, primero con el rabillo del ojo, y segundos después con toda mi capacidad visual que me encuentro respaldado y rodeado por aquellos que siempre estuvieron ahí, que nunca me abandonaron y que siempre me acompañarán: *Los seres que me llenan*. Ese fuego que me levantaba, ese aliento que me recordaba que no estaba solo, ese escudo protector, no es otra cosa sino el amor más puro que alguien puede desear: El amor de mi familia y de mi mujer. Ante la atenta mirada de los asistentes a mi *Examen* y en un clima de silencio, mi corazón impulsa hacia mis labios palabras que no soy consciente que digo y me sorprendo a mi mismo llamándolos a subir a la posición de examinado en la que yo me encuentro. Y entonces, pido que el aplauso por superar el *Examen del Título de Maestro Doctor* no sea para mí sino para ellos. Comienzo entonces a observarlos y mis ojos se detienen sobre los de mi hermano **Pablo** para pasar después a encontrarse con los de mi otro hermano, **Nacho**. Ellos no son ni *Maestros*, ni *Doctores*, ni *Docentes*, ni *Mentores*, pero hace mucho tiempo que superaron con matrícula de honor el

Examen a Mejores Hermanos del Mundo. Al mirarles, confirmo lo que siempre sentí desde que los tres convivíamos en nuestra casita, una tranquilidad absoluta por sentirme completamente protegido por ellos. Y eso que soy el mayor, y por edad debería ser el protector, pero nada más lejos de la realidad. Me acuerdo entonces de una cita de alguien al que los tres conocemos bastante bien. *San Agustín* decía que "*La medida del amor es el amor sin medida*", y es que no hay metro en el mundo que pueda medir el amor fraterno que cada día me regalan. Ojalá algún día pueda llegar a ser la mitad de buen hermano que vosotros habéis sido siempre para mí. Pablo, el hermano poco protagonista, poco carismático, pero héroe "detrás de las cámaras", ese que nos ayuda siempre en todo, que está siempre disponible para lo que se le necesite. Nacho, el hermano un poco despistado, caótico, pero siempre dispuesto a escucharme, siempre dispuesto a ensalzarme y compartir momentos de locura y diversión sea donde sea. Junto con mis hermanos, no me olvido del agradecimiento a mis "hermanas" **Ada** y **Andrea**, que tanto cariño y apoyo me han dado siempre. También a mis **titos** y **primos** por estar siempre ahí. Y, como no, a todos mis **amigos**, que no son amigos sino, como decimos los Granaínos, mis "hermanos".

Cuando empiezo a recobrar la compostura, me doy cuenta de que mi emoción no ha hecho más que empezar. Esto lo descubro cuando miro a mi **padre** y a mi **madre**. No puedo evitar soltar una lágrima al verlos ahí, aparentemente indefensos e inocentes ante la "grandeza" del acto. Pero no me importa, porque lo que ocurre en ese momento es que se me empiezan a aparecer en mi mente todas aquellas veces en las que tanto mi padre como mi madre me ayudaron a llegar donde he llegado. Son, sin duda, los que merecen el mayor de los reconocimientos y protagonismo en este día. Mis padres son el verdadero fruto de mi felicidad. Desde siempre he visto cómo se han dejado la piel por mí y mis hermanos. Entonces miro a mi padre y le digo: "Papi, eres la persona más generosa que he conocido y que conoceré en mi vida. Te quitas el bocado de tu boca para dármelo a mi o a mis hermanos. Siempre queriendo lo peor para ti sabiendo y quedándote con la tranquilidad de que yo, mami y mis hermanos tendríamos lo mejor. No hay mayor muestra de amor que esa. El querer vernos a nosotros plenamente felices, pues solo así sabes que tú puedes ser feliz. Gracias por traer el pan bajo el brazo siempre a casa, por tratar desde un principio de mi nacimiento de buscar trabajar lo menos posible y pasar así el mayor tiempo posible conmigo. Gracias por enseñarme el sentido y la importancia de hacer las cosas bien. Jamás en la vida he escuchado a alguien hablar mal de ti, todo lo contrario. Siento profundo orgullo y felicidad de ver cómo todo el mundo te aprecia y te quiere por tu humildad sin límites. Me has dado la mejor educación que un hijo podría tener, basada en los valores del amor, el respeto, la bondad y la generosidad. Eres, junto a mamá, lo más cercano a la palabra perfección que he podido encontrar en este mundo. Te quiero con toda mi alma y mi corazón". Rápidamente, mis ojos vidriosos se posan sobre los de mi madre que está justo a su lado y le digo: "Mami, ¿qué se le puede decir a una madre que ya no sepa? Pues seguramente mil veces que te quiero. Siento todos los te quieros que no te he dicho durante mi vida. Prometo recuperar cada uno de los que se quedaron dentro de mí. No mereces otra cosa. Me has dado la vida, pero no solo eso, me has enseñado también a vivirla de la mejor forma posible: Desde el amor. Mamá, todo, absolutamente todo lo que haces lo llenas de ese amor madre-hijo que solo tú eres capaz de comprender. Sobran las palabras contigo porque no hay palabras que puedan llegar a definir tanto agradecimiento. Te agradezco ser quién soy, haberme criado y dado tanto amor como lo has hecho. Daría mi vida por ti, por hacerte inmensamente feliz. Desde siempre me has escuchado y tratado de guiar y cuando me he caído, que ha sido mucho, ahí estaba tu mano para levantarme. No he aprendido más en mi vida que contigo. Ahora espero simplemente ser reflejo de lo que tú eres en mi vida. No deseo nada más. Gracias por todas las veces en las que me has abierto el *Hotel Mora González* y me has acogido y dado de comer en mi desenfrenada rutina. Te quiero con toda mi alma y mi corazón". Gracias de todo corazón papá y mamá. Me habéis ayudado siempre que lo he necesitado durante mi camino de *Aprendiz*. Me habéis preparado almuerzos y llevado a donde me ha hecho falta. Me habéis abrazo y dado consuelo cuando, durante mi entrenamiento, me he sentido decepcionado o triste. Siempre, siempre habéis estado ahí. A pesar de todas estas palabras que escribo de agradecimiento a vosotros, nunca podré sentirme verdaderamente en paz con todo lo que me habéis dado. Os quiero. Con locura.

No todos los *Aprendices* tienen la suerte que tuve yo. Me refiero más concretamente a la oportunidad de hacer un parón en mi camino a convertirme en *Maestro Doctor* para vivir el que ha sido hasta ahora el día más feliz de mi vida: mi boda. Mi **Kasitka**, **Gobich** o **Gobiobich**, la mujer de mi vida, se encuentra mirándome emocionada y recordando también aquel maravilloso día que nos unió para siempre. La miro también tratando de devolverle desesperadamente todo el tiempo que mi entrenamiento como *Aprendiz* nos ha robado. Y es que ha sido muy duro ver cómo tenía que rechazar compartir tiempo con ella para dedicarlo a mi adiestramiento. Hoy le pido perdón por todas esas veces que no fuimos a la playa, que no salimos a cenar, que no fuimos al cine, que no la acompañé a comprar, que no cociné con ella, que no limpié con ella, siempre culpando a mis deberes y tareas propias de mi condición de *Aprendiz*. Al igual que le pido perdón, le agradezco su paciencia conmigo, una paciencia inhumana fruto únicamente del amor más profundo que existe. Ella ha vivido de primera mano mis frustraciones, mis enfados, mis cansancios, mis rabietas, mis decepciones y no puedo hacer otra cosa que recordar, por su puesto, una buena cara, un mensaje de ánimo y aliento o unas palabras de consuelo por su parte. Sin duda, el llegar aquí hoy no hubiese sido posible si ella no hubiese estado a mi lado, apoyándome, aconsejándome, escuchándome, amándome. Lo único que deseo en esta vida es que todo el trabajo y esfuerzo de llegar donde he llegado me permita recompensarla como se merece: Con la posibilidad de pasar más tiempo juntos y de mejor calidad. Gracias por cuidarme y darme cariño como lo has hecho. Alegrabas mis días tristes y de bajón. Llenas la casa y mi corazón de color y música y eres mi sostén. Contigo me siento invencible y, sin duda, sacas de mí la mejor versión. Prometo recompensarte por cada una de las cosas que me has regalado durante todo este tiempo y por todo el amor que me das cada día. Eres la mujer de mi vida y mi llegar hasta aquí es por y para ti. Te quiero.

Me doy cuenta entonces que todo el esfuerzo y trabajo dedicado durante varios años a convertirme en *Maestro Doctor* ha merecido la pena por ver a *Los seres que me llenan* con una alegría y orgullo que son difíciles de describir. Obtener el *Título de Maestro Doctor* no es más que una muestra más de todo el apoyo

y cariño recibido por ellos y es, sin duda, el resultado de millones de empujoncitos dados por cada uno de ellos con el único fin de verme feliz. Llenos de alegría y de júbilo por lo logrado, nos marchamos juntos a celebrar lo que, esperemos, sea el comienzo de una nueva aventura llena de aprendizaje y quién sabe si tú, que lees ahora estas líneas, tendrás quizás un papel en ella algún día…

ActiveBrains