Physical fitness and shape of subcortical brain structures in children

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ABSTRACT

A few studies have recently reported that higher cardiorespiratory fitness is associated with higher volumes of subcortical brain structures in children. It is however unknown how different fitness measures relate to shape of subcortical brain nuclei. We aimed to examine the association of the main health-related physical fitness components with shape of subcortical brain structures in a sample of 44 Spanish children aged 9.7±0.2 years from the NUHEAL project. Cardiorespiratory fitness, muscular strength and speed-agility were assessed using valid and reliable tests (ALPHA-fitness test battery). Shape of the subcortical brain structures was assessed by magnetic resonance imaging, and its relationship with fitness was examined after controlling for a set of potential confounders using a partial correlation permutation approach. Our results showed that all physical fitness components studied were significantly related to shape of subcortical brain nuclei. These associations were both positive and negative, indicating that a higher level of fitness in childhood is related to both expansions and contractions in certain regions of Accumbens, Amygdala, Caudate, Hippocampus, Pallidum, Putamen and Thalamus. Cardiorespiratory fitness was mainly associated with expansions, whereas handgrip was mostly associated with contractions in the structures studied. Future randomized controlled trials will confirm or contrast our findings, demonstrating whether changes in fitness modify the shape of brain structures and the extent to which those changes influence cognitive function.

Key words: fitness, muscular strength, speed-agility, brain, shape analysis, children.

INTRODUCTION

Physical fitness in childhood and adolescence is currently considered a strong predictor of health at these ages[1] and later in life[2]. Physical fitness is usually defined as a set of attributes that people have or achieve that relates to the ability to perform physical activity. This set of attributes refer to the components of physical fitness, which are: cardiorespiratory fitness, muscular strength, speed-agility and flexibility[3]. While all of these components have consistently shown to be associated with several health outcomes (such as lower total and central adiposity, lower levels of traditional and emerging cardiovascular disease risk factors, better bone health, less fatigue and better quality of life in cancer survivors, and better mental health, particularly depression, anxiety, mood status and self-esteem)[1], information about their relationship with cognition and brain is scarce. In depth understanding of the relationship between physical fitness and brain in young ages might have important implications, since physical fitness is a factor that can be modified by physical exercise programs which could aim to have a positive effect not only on physical health, but also on cognition and brain.

Several authors have recently reviewed the literature on this novel topic and summarized what is currently known about physical fitness and brain in young people[4–6]. It has been suggested that higher levels of cardiorespiratory fitness could be related to brain both at a functional and structural level. To the best of our knowledge, only two studies (3 articles) conducted by Dr. Chaddock-Heymanand colleagues have explored the associations of cardiorespiratory fitness with brain structures in children[7–9] and none in adolescents. The authors observed that preadolescent children with high levels of cardiorespiratory fitness had a larger volume of Hippocampus[7] and Basal Ganglia, particularly Putamen and Globus Pallidus volumes[8,9]. They did not find associations between fitness and whole brain size (i.e. total grey and/or white matter volumes), suggesting that the effect of cardiorespiratory fitness on brain structures seems to be very specific. In agreement with animal studies, this effect could mainly take place in brain regions in charge of executive control processes (which include scheduling, planning, working or relational memory, multi-tasking and dealing with ambiguity)[10].In spite of the major contributions made by these pioneering studies, many questions remain to be answered in this novel and emerging field.

As mentioned above, previous studies in children have focused on volumetric measurements of subcortical regions of the brain (i.e. hippocampus and basal ganglia)[7–9]. However, although useful, the volume of a subcortical nucleus is a somewhat crude way of summarizing the features of these nuclei and evaluate the potential effects of variables such fitness components. Volume analysis provides information on global changes in the structure, but cannot provide information on regional or local enlargements/contractions. Shape analysis is a sensitive method to detect small changes in brain morphology. Shape is considered as an index of neural development[11], it is related to cognitive performance[12–15]and its study in relation with fitness would provide new insights into this field. Likewise, as previous studies have focused only on cardiorespiratory fitness, there is a need for better understanding how other physical fitness components such us muscular strength or speed-agility relate to brain structures. The present study aimed to examine the association of the main health-related physical fitness components (i.e. cardiorespiratory fitness, muscular strength and speed-agility) with shape of subcortical brain structures in a sample of preadolescents. Since previous studies in children have shown that better cardiorespiratory fitness was associated with larger volumes of subcortical brain regions[7–9], we hypothesized that better cardiorespiratory fitness and perhaps also other fitness components might be associated with the shape of subcortical nuclei. On the other hand, since this is, to the best of our knowledge, the first study examining the potential link between fitness and shape of subcortical brains structures either in children or adults, our study is at the same time explorative and hypothesis generating.

METHODS

Study design and sample

This study is a part of a European project, namely NUHEAL (NUtraceuticals for a HEALthier life; registration no. NCT01180933). The detailed study design, subject recruitment and characteristics, inclusion criteria and outcome measurements have been described previously [16–18]. Briefly, pregnant women attending antenatal care clinics for ultrasound examination between gestation weeks 12 and 20 were approached by study personal and invited to participate. A total of 315 women from Granada (Spain), Munich (Germany) and Pecs (Hungary) agreed to participate in this study. We do not have data about those women who did not agree to participate in the study, so non-response analyses cannot be conducted. The NUHEAL project is a randomized controlled trial in which healthy pregnant women were randomly allocated to one of four treatment groups: (a) fish oil, (b) folic acid, (c) fish oil + folic acid, and (d) placebo. The four groups received this supplementation during the second half of pregnancy with the aim to assess the effect of folate and fish oil intake during pregnancy on infant outcomes. Out of the 315 women that accepted to participate in the study, 243 maternal blood samples could be drawn at delivery, and cord blood samples were obtained in 220 cases. The reasons for dropping from the study have been reported elsewhere and it has also been reported that drop-outs distributed equally among the study groups [16,18].

The present study focused on the cross-sectional outcomes obtained from the Spanish cohort (155 participants with valid data at delivery). These newborns were followed up to 9-10 years, when the children underwent physical fitness and structural magnetic resonance imaging (MRI) examinations. Due to practical and funding reasons, these two examinations took place within a separate median period of 11.5months. However, we controlled for this gap in all of the statistical analyses. The analyses for the present study were done at a cross-sectional level. Since there are fundamental differences in the brain structure depending on which hand is dominant, left-handed and right-handed individuals should not be analyzed together. In our study, 2 children were left-handed and were therefore excluded from the analyses, as is standard in the literature [7–9]. In addition, collected images were visually inspected on the quality and a total of 6 participants were excluded due to movement artifacts in their MRI images. Finally, a total of 44 children aged 9.7±0.2 years had valid data for all physical fitness tests and MRI and were included in the final analyses. These 44 children came from the original 4 study groups with a relatively homogenous distribution among them: fish oil (n=10), folic acid (n=8), fish oil + folic acid (n=10), and placebo (n=16). The supplementation during pregnancy with fish oil and folic acid (separately or in combination) had no effect on children’s brain measures or fitness, therefore there was no need to control for the effect of the study groups in our analyses. In addition, we tested whether the 44 participants in the present study differed from the original sample (n=155) in a number of key variables: age of the mother at gestational week 20, body mass index of the mother at delivery, birth weight of the participant, and weight or BMI at ages 1.5, 4.0, 6.5, 8.5 and 9.5 years (all $p \geq 0.3$). This suggests that the present study sample is representative of the original study sample.

This study was conducted according to the guidelines provided in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the local Ethical Committee of San Cecilio University Clinical Hospital of Granada (Spain). Written [or Verbal] informed consent was obtained from all the children's mothers.

Socioeconomic status and birth weight assessment

The Hollingshead Scale (1975) was used to determine the socioeconomic status of the children by creating a score from a set of variables: self-reported marital status, self-reported maternal and paternal occupation, and self-reported maternal and paternal educational level[19,20]. Weight at birth was also recorded.

Anthropometric assessment

Weight was measured in underwear and without shoes with an electronic scale (Type SECA 861) to the nearest 0.1 kg, and height was measured barefoot in the Frankfort horizontal plane with a telescopic height measuring instrument (Type SECA 225) to the nearest 0.1 cm. Body mass index was calculated as body weight in kilograms divided by the square of height in meters.

Physical fitness assessment

The field-based fitness tests used to assess the different health-related physical fitness components studied have been used in previous European projects (i.e. the HELENA and the ALPHA projects). Detailed information on fitness protocols, operational manual, training workshops and standardized instructions to participants have been published elsewhere[21,22]. In brief, cardiorespiratory fitness was assessed by the 20 m shuttle-run test and maximum oxygen consumption (VO_2max, ml/kg/min) was estimated using the equation reported by Léger et al[23].

Muscular fitness was assessed by means of the handgrip strength[24–26] and standing long jump[25,27]tests. Speed-agility was assessed with the 4x10 m shuttle-run test[28]. All the tests were performed twice and the best score was retained, except the 20-m shuttle run test, which was performed only once. These physical fitness tests have shown to be valid, reliable and related with health in young people [3,21,29,30].

Images acquisition and processing

High-resolution (0.94 mm × 0.94 mm × 1 mm) T1-weighted structural brain images were acquired for all participants using a 3D MPRAGE (Magnetization Prepared Rapid Gradient Echo Imaging) protocol with 160 contiguous axial slices, collected in ascending fashion parallel to the anterior and posterior commissures [echo time (TE) = 3.77 ms, repetition time (TR) = 8.17ms, field of view (FOV) = 240 × 240 mm, acquisition matrix = 256 mm × 256 mm, slice thickness = 1 mm, and flip angle = 8°). All images were collected on a 3T head-only Philips Achieva scanner equipped with a8 channels phased-array head coil.

Total intracranial volume (TIV) was obtained as the sum of grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF). TIV is commonly used to control for local volumes variability associated to participants' size, weight, and sex[31,32]. We used the DARTEL algorithm implemented in SPM8 ((http://www.fil.ion.ucl.ac.uk/spm/) for the segmentation of GM, WM, CSF[33]. Segmentation of subcortical nuclei and shape analysis was carried out using the FSL/FIRST software (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST)[34]. FIRST is a model-based registration tool that use a set of 15 subcortical structures outlined by experts on T1 weighted images obtained from 336 subjects ranging from 4.2 to 72 years, which allow the assessment of changes in typical developing brains. Subcortical nuclei volumes were adjusted using a regression approach: Subcortical Volume = Raw Subcortical Volume - b (TIV- average TIV), being b the slope of the regression of the Subcortical Volume on TIV [35].

Shape analysis

Shape analysis is based on the individual meshes composed by a large number of vertices and triangles. The number of triangles and vertices is the same for each nuclei, allowing the within and between subjects comparison of each vertex. These comparisons are possible because all meshes are aligned to the Montreal Neurological Institute (MNI) space, and pose (rotation and translation) is removed [36]. Our vertex-wise analysis followed methods described previously [15,37]. To assess local changes in each nuclei, we use the radial distance of each vertex to the medial curve of the nuclei (Figure 1). The medial curve can be thought of as the centroid curve of the nuclei boundary in each section [37] or, similarly, as the skeleton of the 3D structure. Note that radial distances related each vertex spatial location to the core line of the structure, are objective measures of regional expansion/contraction of the nuclei, and are indicators of local changes associated to factors affecting the structure shape. It is important to bear in mind that although we use throughout the text shape terms such as expansions/contractions or similar terms commonly used in this field (e.g. enlarged/shortened), our study is cross-sectional and therefore the results cannot be interpreted as changes, but just as the shape of the nuclei at the moment of assessment.

Statistical analysis

Correlations between hemispheres were tested in order to decide whether to analyze them separately or together using average or sum of them. For the analysis of the relationships of the fitness variables (cardiorespiratory fitness, 2 tests of muscular strength and speed-agility) with the shape of each subcortical nuclei (14 nuclei: Left and Right Accumbens, Amygdala, Caudate, Hippocampus, Pallidum, Putamen, and Thalamus) we implemented a partial correlation permutation approach. Thus, the correlation between the radial distance of each vertex and each fitness variable was computed after controlling the variance associated with the following set of potential confounders: age at first evaluation visit (i.e. when MRI scan was done, namely MRI age), the time difference between MRI and fitness assessments, sex, socioeconomic status of the family, birth weight, body mass index and TIV. In order to account for multiple comparisons, we used a t-max approach [38–40]. In short, permutation tests avoid strong assumptions on the distributional features of the variables and compute the empirical distribution of the contrast statistic, by assuming that the outcome-predictor pairs were observed by accident [41]. In each simulation run, the largest partial correlation of the whole set of vertices of the nucleus (more than 640), is used to build the empirical distribution of the statistic. Finally, the p-value for each vertex is computed as the fraction between the number of accidental partial correlation equal or greater than that observed in the study and the total number of permutations (10000 in the present study). We considered significance only when 4 or more vertices were simultaneously significant at the corrected p-level. Results are displayed as color-coded significance maps. We used blue colors to indicate a negative outcome-predictor association (that is, the higher is the value of the predictor the smaller are radial distances), the orange-yellow colors to indicate positive relationships (the higher is the value of the predictor the larger are radial distances) and the grey color to indicate non-significant association. We checked for potential outliers in our analytic sample, as defined by a value beyond 2.5 SD, and we identified no single value over this threshold in the predictors or outcomes. Analyses were run only in subjects with complete valid data on the main predictors (fitness) and outcomes (MRI variables), i.e. no imputation method was used.

RESULTS

The sample was aged 9.7±0.2 years at first assessment visit (MRI evaluation) and was similarly distributed by sex (i.e. 45.5% girls). Characteristics of the study sample are shown in Table 1. Volumes (mm^3) of the subcortical nuclei and the correlations between hemispheres for each nucleus are displayed in Table 2. Correlations between hemispheres were not very high (though significant) for some of the nuclei, particularly between the two hemispheres of Accumbens, Amygdala, Hippocampus, and Pallidum (correlation coefficient ranging from 0.5 to 0.6). Based on this, we decided to perform the rest of analyses separately for left and right hemispheres.

Table 3 (also Figure 2 to 5) shows the results from the vertex-wise permutation tests for each fitness variable as predictor, radial distances (brain nuclei shapes) as the outcome, and the following set of covariates as potential confounders: age at first evaluation visit (i.e. when MRI scan was done, namely MRI age), the time difference between MRI and fitness assessments, sex, socioeconomic status of the family, birth weight and body mass index and TIV. We observed that fitness variables were significantly related to the shape (expansions/contractions) of all subcortical brain structures studied except for right Accumbens and right Caudate. Out of the fitness components studied, cardiorespiratory fitness was the one showing more significant and positive associations with shape of subcortical brain structures; particularly, higher cardiorespiratory fitness was associated with expansions in 6 out of the 14 structures studied, while muscular strength or speed-agility were associated with expansion only in 2 or less structures studied. On the other hand, muscular strength as measured by handgrip strength test, was mainly related with contractions (i.e. reduced radial distances) in 5 of the subcortical brain structures studied, while with expansions only in 2 structures. Muscular strength, as measured by standing long jump, and speed-agility were associated with fewer structures (2 and 4 respectively), and indicating equally expansions and contractions.

Figures 2 to 5 graphically show how fitness variables relate to shape of the subcortical nuclei indicating, by means of a color-coded significance map, the parts of each brain structure that are significantly related to fitness and the direction of that association (positive/negative=expansions/contractions). Figure 2 shows that cardiorespiratory fitness was related to right Putamen, Hippocampus and Amygdala, and left Putamen, Pallidum and Accumbens. All these associations were positive (expansions), except for right Hippocampus and left Accumbens in which we found both expansions and contractions. Figure 3 shows that higher handgrip strength was related to expansions in left Caudate Head and right anterior Thalamus, yet was also related to contractions in right Putamen, Hippocampus (right and left), left Amygdala and left Accumbens. Figure 4 shows that higher muscular strength as measured by standing long jump was related to expansions in left Pallidum and contractions in right Thalamus. Figure 5 shows that speed-agility was negatively related to right Putamen and Pallidum, which due to the fact that this test is expressed inversely (i.e. lower score means higher performance), indicated that higher performance in speed-agility is related to expansions in these subcortical brain nuclei. Better performance in speed-agility was however related to contractions in right Caudate. Finally, in order to graphically represent which are the strongest associations found in our study, we provide as a Supplementary Material (Figure S1 to S4) the same set of figures, but showing only those associations with p-value <0.025 (instead of 0.05 as standard).

DISCUSSION

This study used a novel approach (shape analysis) to examine brain structure in relation with physical fitness in children, contributing to the current knowledge in this field. Overall, our results suggest that all the main health-related physical fitness components (i.e. cardiorespiratory fitness, muscular strength and speed-agility) are significantly related to shape of subcortical brain nuclei in childhood. These associations were mainly positive for most of fitness tests, indicating that a higher level of fitness in childhood is related to expansions in certain parts of Accumbens, Amygdala, Caudate, Hippocampus, Pallidum, Putamen and Thalamus. However, higher fitness was also significantly related to contractions in other parts of these subcortical nuclei, suggesting that fitness might have an effect on brain structures by shaping them in a certain way, and not necessary by increasing the whole volume of such brain structures. These results highlight that shape rather than volume may be a more sensitive measure of the impact of fitness variables on developing brains[15]. The association between fitness and brain structures shape was examined using correlation coefficients (r statistics), which are considered an effect size measure itself [42]. The correlation coefficients obtained inform us that the associations between fitness and subcortical brain structure shapes are of low to medium magnitude. In addition, it is important to highlight that although significant associations were found between fitness and certain regions of the subcortical nuclei studied; other large regions were not associated, suggesting that fitness might be only a modest contributor to brain shaping, with many other genetic and environmental factors influencing brain morphology. On the other hand, it is important to bear in mind that the subcortical regions associated with fitness in the present study, though seem small, include hundreds of thousands neurons, fibers and connections and the effect of these differences in shape on brain functioning is unknown. Whether the associations between fitness and shape of subcortical brain structures is causal or not will be confirmed or contrasted in future randomized controlled trials.

We have not found previous studies analyzing shape of subcortical brain nuclei in young people in relation to physical fitness, which precludes us from doing direct comparisons between our results and those from other studies. Previous literature in youth has focused on volumetric analysis of subcortical brain structures and on cardiorespiratory fitness [7–9]. These authors observed that higher cardiorespiratory fitness level was associated with higher volume of Hippocampus[7] and Basal Ganglia, particularly higher Putamen and Pallidum volumes[8,9].The contribution of our study to current knowledge in the field is therefore two folds: 1) the inclusion of shape analysis of subcortical brain structures in children, and 2) the inclusions of other components of physical fitness such as muscular strength and speed-agility since it is unknown whether they could be related to structural measures of brain in children.

We observed that children with higher cardiorespiratory fitness had enlarged regions in the left Amygdala, left Hippocampus, left and right Putamen and right Pallidum. These results are in line with previous studies that reported higher volumes in 3 (i.e. Hippocampus, Putamen and Pallidum) out of these 4 brain nuclei in children with a high cardiorespiratory fitness level compared with those peers with lower cardiorespiratory fitness level [7–9]. Previous studies in rodents have consistently shown a number of mechanisms that could explain these findings, with special focus on Hippocampus[43]. Aerobic exercise (a major determinant of cardiorespiratory fitness) increases cell proliferation and survival in the dentate gyrus of the hippocampus and increases hippocampal levels of growth factors in the brain such as the brain-derived neuro-trophic factor (BDNF), insulin-like growth factor 1 (IGF-1), and vascular endothelial-derived growth factor, which are involved in neuronal survival, synaptic development and angiogenesis

[44]. Similarly, animal models have demonstrated that aerobic exercise increases the production and secretion of BDNF in the Striatum, which includes Caudate and Putamen associated with cardiorespiratory fitness[45][46]. Recent systematic reviews and meta-analysis in humans have confirmed that doing aerobic exercise regularly has a positive effect on BDNF levels [47,48].

We have not found any study examining the association of muscular strength or speed-agility with brain structure in any age group. There is however emerging evidence suggesting that resistance training (which leads to better muscular strength) could have a beneficial effect on functional plasticity in old women, as measured by functional magnetic resonance imaging, particularly in the anterior portion of the left middle temporal gyrus and the left anterior insula extending into lateral orbital frontal cortex [49]. Recent reviews in humans have concluded that low or high resistance exercise increases levels of IGF-1 (yet not of BDNF), which could at least partially mediate the positive effects of exercise on brain functioning mentioned above [47,50]. Voelcker and Niemann did a comprehensive review on the structural and functional brain changes related to physical activity across the life span and classed both aerobic and resistance training as metabolic exercise arguing that both have important metabolic adaptations[4]. They also grouped other activities as coordinative exercise, which comprises fine and gross motor body coordination such as balance, eye-hand coordination, leg-arm coordination as well as spatial orientation. These authors concluded that with a few exceptions, all published results support that metabolic exercise modulates structural brain plasticity. They also concluded that coordinative exercise might influence brain structure in a different fashion than metabolic exercise, due to its higher cognitive demands and similarly to enriched environments in animal studies. Unfortunately, the information currently available about coordinative exercise and brain is very limited. Our speed-agility test involves a mixture of metabolic and coordinative exercise, while sprinting is an anaerobic physiological effort with a marked impact on metabolism, this tests also includes special orientation (changes in directions) and picking-dropping objects (sponges) which would fit in the definition of coordinative exercise. To the best of our knowledge, this is the first study directly comparing how cardiorespiratory fitness, muscular strength and speed-agility relate to brain structure and our data support that these 3 components are associated with brain shaping in children, though the highest number of significant and positive associations was found for cardiorespiratory fitness, suggesting that it is the strongest predictor of brain structure in young people.

Our findings concerning muscular strength might seem contradictory, since associations with shape of brain structure were mainly positive (i.e. expansions) when muscular strength was assessed by standing long jump test, whereas the associations were mainly negative (i.e. contractions) when muscular strength was assessed by the handgrip strength test. This however, could have an explanation. Muscular fitness or strength can be assessed using body weight dependent or body weight independent tests. The first type includes any activity in which the person has to lift, hold or carry on his/her own body weight, e.g. hanging from a bar or tree branch, standing long jump test, etc. Examples of body weight independent tests include situations in which the person’s body weight *per se* has no influence on the performance, i.e. weight lifting, carry on a suitcase or move a heavy object, handgrip strength test, etc. Consequently, we used standing long jump as an indicator of body weight dependent test, while handgrip strength was used as an indicator of body weight independent test. Overweight-obese children or adolescents have higher levels of fat mass but also higher levels of muscle mass [51,52] which make them to have a higher strength as measure by body weight independent tests, yet lower when using body weight dependent tests (e.g. they jump less due to their high body weight)[53]. We have previously reported that these two ways of assessing muscular strength lead to completely different associations with health outcomes such as cardiometabolic risk factors in young people, and our results might suggest that this is also the case for associations with brains measures[54]. We attempted to eliminate this potential effect by additionally adjusting our models for body mass index, yet we cannot ensure that all the potential confounding effects has been removed.

Limitations and strengths

A major limitation of the present study is the relatively small sample size included (N=44), though it is similar to that from previous studies on this topic conducted in children (i.e. N ranging from 32 to 55)[7,8].Due to this small sample size and therefore small statistical power (and thus high beta error), some potentially significant associations might not have been detected. On the other hand, given this small statistical power, an association becomes significant only when the effect size is relatively large. Our correlation coefficients, an effect size measure regardless of the p-values, ranged between 0.3 and 0.5, which is a decent strength of association if considered that the shape of a brain structure is the result of multiple genetics and environmental factors, with fitness being only one of them modestly contributing to it with a small portion of the total variance explained.

As previously mentioned, due to the cross-sectional nature of this study it is not possible to draw causal relationships. Also, this study was conducted in a sample of preadolescent children and we cannot know the extent to which these results apply to other age groups. In addition, the fact that fitness and MRI were not assessed exactly at the same time, but with a time gap of 11.5 months should be knowledge as a limitation. However, we believe it is unlikely that this could have influenced much our results/conclusions due to several reasons: a) All the statistical analyses conducted in this study adjusted for the time difference between assessments to diminish this potential source of error. b) This time gap was very homogenous among the participants being less than 1 month the difference between the percentile 25th and 75th (i.e. 11.1 and 12.0 months respectively), suggesting that any residual error not corrected by the statistical adjustment applied would be similar across the participants. c) Longitudinal studies on fitness have shown small and non-significant ($P>0.05$) changes in 1-year of follow-up in 10 year-old children (i.e. 1-2 ml/kg/min VO_2max, cardiorespiratory fitness)[55]. d) Fitness has shown to track well across childhood, being this tracking particularly high in short follow-up period (i.e. 1-year follow-up)[55] and in a pre-pubertal phase (less physiological changes compared with puberty), which suggests that fittest participants when measured in our study would also be the fittest participants of the sample if measured 1 year earlier or later.

On the other hand, this study should be acknowledged for the use of highly reliable and valid tests for assessing the main health-related fitness components. The tests used are based on 3 recent systematic reviews and methodological studies conducted under the umbrella of the European ALPHA project (www.thealphaproject.net) [2,3,21,29]. Another major strength of this study is to have used a shape analysis approach which provides new lights into the field of fitness and brain morphology in childhood.

Conclusions

Our results support that cardiorespiratory fitness, muscular strength and speed-agility (the main health-related fitness components) are significantly associated to shape of subcortical brain nuclei. Higher fitness was associated with expansions in certain parts of these brain nuclei, but also to contractions in other parts of these nuclei, suggesting that fitness could potentially influence brain by shaping certain nuclei and not necessarily by increasing their volume. Our results support that cardiorespiratory fitness is the fitness component associated with expansions in more subcortical brain structures, i.e. 6 out of 14 structures studied. Future exercise-based randomized controlled trials will confirm or contrast the present observational evidence. In addition, further research is needed to fully understand the consequences that changes in shape of subcortical brain structures have on brain functioning.

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Conflict of interest

None.

Authorship

All authors helped in the interpretation of results and contributed to manuscript preparation. F.B.O., M.M.M and C.M.Z. designed and participated in the fitness data collection; D.C., F.J.T.E., M.M.M. and C.M.Z. performed the rest of children's examinations. A.C. performed the neuroimaging analysis and interpretation; A.C. and F.O did the statistical analysis and wrote the manuscript; S.A. and C.C.S. critically reviewed the manuscript providing relevant comments and S.A. revised the English grammar. C.C. was the coordinator of the NUHEAL study in Spain and the NUTRIMENTHE EU Project and supervised the manuscript.

REFERENCES

1. Ortega FB, Ruiz JR, Castillo MJ, Sjöström M. Physical fitness in childhood and adolescence: a powerful marker of health. Int J Obes [Internet] 2008 [cited 2014 Jun 12];32(1):1–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18043605
2. Ruiz JR, Castro-Piñero J, Artero EG, Ortega FB, Sjöström M, Suni J, et al. Predictive validity of health-related fitness in youth: a systematic review. Br J Sports Med [Internet] 2009 [cited 2013 May 29];43(12):909–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19158130
3. Ruiz JR, Castro-Pinero J, Espana-Romero V, Artero EG, Ortega FB, Cuenca MM, et al. Field-based fitness assessment in young people: the ALPHA health-related fitness test battery for children and adolescents. Br J Sports Med [Internet] 2011 [cited 2013 Jun 17];45(6):518–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20961915
4. Voelcker-Rehage C, Niemann C. Structural and functional brain changes related to different types of physical activity across the life span. Neurosci Biobehav Rev [Internet] 2013 [cited 2014 Jun 4];37(9 Pt B):2268–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23399048
5. Voss MW, Nagamatsu LS, Liu-Ambrose T, Kramer AF. Exercise, brain, and cognition across the life span. J Appl Physiol [Internet] 2011 [cited 2014 May 27];111(5):1505–13. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3220305&tool=pmcentrez&rendertype=abstract
6. Khan NA, Hillman C. The Relation of Childhood Physical Activity and Aerobic Fitness to Brain Function and Cognition: A Review. Pediatr Exerc Sci [Internet] 2014 [cited 2014 Jun 3];26(2):138–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24722921
7. Chaddock L, Erickson KI, Prakash RS, Kim JS, Voss MW, Vanpatter M, et al. A neuroimaging investigation of the association between aerobic fitness, hippocampal volume, and memory performance in preadolescent children. Brain Res [Internet] 2010 [cited 2013 Jun 5];1358:172–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20735996
8. Chaddock L, Erickson KI, Prakash RS, VanPatter M, Voss MW, Pontifex MB, et al. Basal ganglia volume is associated with aerobic fitness in preadolescent children. Dev Neurosci [Internet] 2010;32(3):249–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20693803
9. Chaddock L, Hillman CH, Pontifex MB, Johnson CR, Raine LB, Kramer AF. Childhood aerobic fitness predicts cognitive performance one year later. J Sports Sci [Internet] 2012;30(5):421–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22260155
10. Hillman CH, Erickson KI, Kramer AF. Be smart, exercise your heart: exercise effects on brain and cognition. Nat Rev Neurosci 2008;9(1):58–65.
11. Sampaio A, Bouix S, Sousa N, Vasconcelos C, Férnandez M, Shenton ME, et al. Morphometry of corpus callosum in Williams syndrome: Shape as an index of neural development. Brain Struct Funct 2013;218:711–20.

12. Lim H-K, Hong SC, Jung WS, Ahn KJ, Won WY, Hahn C, et al. Hippocampal shape and cognitive performance in amnestic mild cognitive impairment. Neuroreport 2012;23:364–8.

13. Xie J, Alcantara D, Amenta N, Fletcher E, Martinez O, Persianinova M, et al. Spatially localized hippocampal shape analysis in late-life cognitive decline. Hippocampus 2009;19:526–32.

14. Cachia A, Borst G, Vidal J, Fischer C, Pineau A, Mangin J-F, et al. The shape of the ACC contributes to cognitive control efficiency in preschoolers. J Cogn Neurosci 2014;26(1):96–106.

15. Sandman C a., Head K, Muftuler LT, Su L, Buss C, Davis EP. Shape of the basal ganglia in preadolescent children is associated with cognitive performance. Neuroimage [Internet] 2014;99:93–102. Available from: http://dx.doi.org/10.1016/j.neuroimage.2014.05.020

16. Decsi T, Campoy C, Koletzko B. Effect of N-3 polyunsaturated fatty acid supplementation in pregnancy: the Nuheal trial. Adv Exp Med Biol [Internet] 2005 [cited 2014 Jun 16];569:109–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16137113

17. Campoy C, Escolano-Margarit M V, Ramos R, Parrilla-Roure M, Csábi G, Beyer J, et al. Effects of prenatal fish-oil and 5-methyltetrahydrofolate supplementation on cognitive development of children at 6.5 y of age. Am J Clin Nutr [Internet] 2011 [cited 2014 Jun 16];94(6 Suppl):1880S – 1888S. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21849596

18. Krauss-Etschmann S, Shadid R, Campoy C, Hoster E, Demmelmair H, Jiménez M, et al. Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. Am J Clin Nutr [Internet] 2007 [cited 2014 Jun 16];85(5):1392–400. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17490978

19. Peckins MK, Susman EJ. Variability in diurnal testosterone, exposure to violence, and antisocial behavior in young adolescents. Dev Psychopathol 2015;in press:1–12.

20. Jacobson SW, Chiodo LM, Sokol RJ, Jacobson JL. Validity of maternal report of prenatal alcohol, cocaine, and smoking in relation to neurobehavioral outcome. Pediatrics 2002;109(5):815–25.

21. Ortega FB, Artero EG, Ruiz JR, Vicente-Rodriguez G, Bergman P, Hagstromer M, et al. Reliability of health-related physical fitness tests in European adolescents. The HELENA Study. Int J Obes 2008;32 Suppl 5:S49–57.

22. Ortega FB, Artero EG, Ruiz JR, España-Romero V, Jiménez-Pavón D, Vicente-Rodriguez G, et al. Physical fitness levels among European adolescents: the HELENA study. Br J Sports Med [Internet] 2011 [cited 2014 Jun 12];45(1):20–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19700434

23. Léger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. J Sports Sci 1988;6(2):93–101.

24. Espana-Romero V, Artero EG, Santaliestra-Pasias AM, Gutierrez A, Castillo MJ, Ruiz JR. Hand span influences optimal grip span in boys and girls aged 6 to 12 years. J Hand

Surgery Am Vol 2008;33(3):378–84.

25. Artero EG, Espana-Romero V, Castro-Pinero J, Ruiz J, Jimenez-Pavon D, Aparicio V, et al. Criterion-related validity of field-based muscular fitness tests in youth. J Sports Med Phys Fitness 2012;52(3):263–72.

26. Espana-Romero V, Ortega FB, Vicente-Rodriguez G, Artero EG, Rey JP, Ruiz JR. Elbow position affects handgrip strength in adolescents: validity and reliability of Jamar, DynEx, and TKK dynamometers. J Strength Cond Res 2010;24(1):272–7.

27. Castro-Pinero J, Ortega FB, Artero EG, Girela-Rejon MJ, Mora J, Sjostrom M, et al. Assessing muscular strength in youth: usefulness of standing long jump as a general index of muscular fitness. J Strength Cond Res 2010;24(7):1810–7.

28. Vicente-Rodriguez G, Rey-Lopez JP, Ruiz JR, Jimenez-Pavon D, Bergman P, Ciarapica D, et al. Interrater reliability and time measurement validity of speed-agility field tests in adolescents. J Strength Cond Res 2011;25(7):2059–63.

29. Artero EG, Espana-Romero V, Castro-Pinero J, Ortega FB, Suni J, Castillo-Garzon MJ, et al. Reliability of field-based fitness tests in youth. Int J Sports Med [Internet] 2011;32(3):159–69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21165805

30. Castro-Pinero J, Artero EG, Espana-Romero V, Ortega FB, Sjostrom M, Suni J, et al. Criterion-related validity of field-based fitness tests in youth: a systematic review. Br J Sports Med [Internet] 2010 [cited 2013 Jun 17];44(13):934–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19364756

31. Raz N, Lindenberger U, Rodrigue KM, Kennedy KM, Head D, Williamson A, et al. Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. Cereb Cortex 2005;15(11):1676–89.

32. Lenroot RK, Gogtay N, Greenstein DK, Wells EM, Wallace GL, Clasen LS, et al. Sexual dimorphism of brain developmental trajectories during childhood and adolescence. Neuroimage 2007;36(4):1065–73.

33. Ashburner J. A fast diffeomorphic image registration algorithm. Neuroimage 2007;38(1):95–113.

34. Patenaude B, Smith SM, Kennedy DN, Jenkinson M. A Bayesian model of shape and appearance for subcortical brain segmentation. Neuroimage 2011;56(3):907–22.

35. Kennedy KM, Erickson KI, Rodrigue KM, Voss MW, Colcombe SJ, Kramer AF, et al. Age-related differences in regional brain volumes: a comparison of optimized voxel-based morphometry to manual volumetry. Neurobiol Aging 2009;30(10):1657–76.

36. Patenaude B. Bayesian Statistical Models of Shape and Appearance for Subcortical Brain Segmentation. Dep Clin Neurol2007;

37. Thompson PM, Hayashi KM, De Zubicaray GI, Janke AL, Rose SE, Semple J, et al. Mapping hippocampal and ventricular change in Alzheimer disease. Neuroimage 2004;22(4):1754–66.

38. Blair RC, Karniski W. An alternative method for significance testing of waveform difference potentials. Psychophysiology 1993;30(5):518–24.

39. Pantazis D, Nichols TE, Baillet S, Leahy RM. A comparison of random field theory and

permutation methods for the statistical analysis of MEG data. Neuroimage 2005;25(2):383–94.

40. Thompson PM, Hayashi KM, de Zubicaray G, Janke AL, Rose SE, Semple J, et al. Dynamics of gray matter loss in Alzheimer's disease. J Neurosci 2003;23(3):994–1005.

41. Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum Brain Mapp 2002;15(1):1–25.

42. Nakagawa S, Cuthill IC. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol Rev Camb Philos Soc [Internet] 2007 [cited 2013 May 21];82(4):591–605. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17944619

43. Voss MW, Vivar C, Kramer AF, van Praag H. Bridging animal and human models of exercise-induced brain plasticity. Trends Cogn Sci 2013;17(10):525–44.

44. Cotman CW, Berchtold NC, Christie LA. Exercise builds brain health: key roles of growth factor cascades and inflammation. Trends Neurosci 2007;30(9):464–72.

45. Aguiar ASJ, Speck AE, Prediger RDS, Kapczinski F, Pinho RA. Downhill training upregulates mice hippocampal and striatal brain-derived neurotrophic factor levels. J Neural Transm 2008;115(9):1251–5.

46. Marais L, Stein DJ, Daniels WMU. Exercise increases BDNF levels in the striatum and decreases depressive-like behavior in chronically stressed rats. Metab Brain Dis 2009;24(4):587–97.

47. Huang T, Larsen KT, Ried-Larsen M, Møller NC, Andersen LB. The effects of physical activity and exercise on brain-derived neurotrophic factor in healthy humans: a review. Scand J Med Sci Sports [Internet] 2014;24(1):1–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23600729

48. Szuhany KL, Bugatti M, Otto MW. A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. J Psychiatr Res [Internet] 2014 [cited 2014 Oct 31];Available from: http://linkinghub.elsevier.com/retrieve/pii/S0022395614002933

49. Liu-Ambrose T, Nagamatsu LS, Voss MW, Khan KM, Handy TC. Resistance training and functional plasticity of the aging brain: a 12-month randomized controlled trial. NeurobiolAging 2012;33(8):1690–8.

50. Rojas Vega S, Knicker a, Hollmann W, Bloch W, Strüder HK. Effect of resistance exercise on serum levels of growth factors in humans. Horm Metab Res 2010;42(13):982–6.

51. Ekelund U, Franks PW, Wareham NJ, Aman J. Oxygen uptakes adjusted for body composition in normal-weight and obese adolescents. Obes Res 2004;12(3):513–20.

52. Gracia-Marco L, Ortega FB, Jiménez-Pavón D, Rodríguez G, Castillo MJ, Vicente-Rodríguez G, et al. Adiposity and bone health in Spanish adolescents. The HELENA study. Osteoporos Int [Internet] 2012 [cited 2013 Jun 11];23(3):937–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21562873

53. Artero EG, Espana-Romero V, Ortega FB, Jimenez-Pavon D, Ruiz JR, Vicente-Rodriguez G, et al. Health-related fitness in adolescents: underweight, and not only overweight, as an influencing factor. The AVENA study. Scand J Med Sci Sport [Internet] 2010 [cited 2013

May 24];20(3):418–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19558383

54. Ortega FB, Sanchez-Lopez M, Solera-Martinez M, Fernandez-Sanchez A, Sjostrom M, Martinez-Vizcaino V. Self-reported and measured cardiorespiratory fitness similarly predict cardiovascular disease risk in young adults. Scand J Med Sci Sport 2012;23(6):749–57.

55. Janz KF, Dawson JD, Mahoney LT. Tracking physical fitness and physical activity from childhood to adolescence: the muscatine study. Med Sci Sports Exerc [Internet] 2000;32(7):1250–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10912890

Table 1. Descriptive characteristics of the study sample.

	All (N=44)		Boys (n=24)		Girls (n=20)	
	Mean	SD	Mean	SD	Mean	SD
Age (y) *	9.7	0.2	9.7	0.2	9.7	0.2
Socioeconomic status †	4.6	0.8	4.4	1.0	4.8	0.6
Weight (kg)	41.1	10.4	42.7	11.8	39.3	8.4
Height (cm)	145.3	7.6	145.5	7.7	145.0	7.6
Body mass index (kg/m^2)	19.3	3.6	19.9	4.0	18.5	3.0
Cardiorespiratory fitness: 20m shuttle run test (VO_2max,ml/kg/min)	43.0	4.2	44.2	4.7	41.6	3.0
Upper-limbs muscular strength: Handgrip (kg)	16.5	3.3	17.1	3.0	15.7	3.5
Lower-limbs muscular strength: Standing long jump (cm)	123.5	25.9	132.4	23.7	112.9	24.9
Speed-agility: 4x10m shuttle run test (s) ‡	15.4	2.6	14.5	2.1	16.4	2.8
Grey matter volume (mm^3)	690.7	54.0	716.4	49.7	659.9	42.0
White matter volume (mm^3)	463.3	40.1	480.8	39.5	442.4	30.1
Cerebrospinal fluid (mm^3)	270.3	21.7	279.4	19.2	259.5	19.7
Total intracranial volume (mm^3)	1424.4	112.8	1476.6	105.7	1361.8	87.6

SD: standard deviation

* The age presented in the table is the one that the children had at the first evaluation visit, i.e. the day of the magnetic resonance imaging assessment.

† Socioeconomic status of the child is a score index based on the Hollingshead Scale (1975), which includes parental marital status, education and occupation; it ranged from 2.1 to 6.6, with higher values indicating higher socioeconomic status.

‡ The lower the score in the 4x10m shuttle run test (i.e. less seconds to cover a fixed distance) the higher the performance (i.e. the faster and more agile the child is).

Table 2. Pearson correlations between hemisphere volumes (in mm³) of subcortical brain structures.

	Left		Right		Left-Right correlation
Nucleus	Mean	SD	Mean	SD	r
Accumbens	509	111	431	99	.632
Amygdala	1017	134	979	173	.513
Caudate	3817	401	3913	415	.859
Hippocampus	3602	390	3643	455	.468
Pallidum	1622	135	1683	149	.575
Putamen	4940	542	5087	594	.802
Thalamus	7728	691	7623	642	.841

SD: standard deviation; r: linear correlation.

Table 3.Correlations between fitness variables and subcortical brain shape.

		Cardiorespiratory fitness: VO$_2$max			Strength: Handgrip			Strength: Standing long jump			Speed-agility: 4x10m shuttle run*		
		k	Peak part r		k	Peak part r		k	Peak part r		k	Peak part r	
Nucleus			Expan	Contr		Expan	Contr		Expan	Contr		Expan	Contr
Accumbens	Right	0/0	NS	NS	0/0	NS	NS	0/0	NS	NS	0/0	NS	NS
	Left	**7/14**	**0.318**	**-0.304**	**0/10**	NS	**-0.426**	0/0	NS	NS	0/0	NS	NS
Amygdala	Right	**14/0**	**0.378**	NS	NS	NS	NS	0/0	NS	NS	0/0	NS	NS
	Left	0/0	NS	NS	**0/19**	NS	**-0.408**	0/0	NS	NS	0/0	NS	NS
Caudate	Right	0/0	NS	NS	0/0	NS	NS	0/0	NS	NS	0/0	NS	NS
	Left	0/0	NS	NS	**7/0**	**0.367**	NS	0/0	NS	NS	7/**0**	**0.312**	NS
Hippocampus	Right	**13/4**	**0.290**	**-0.346**	**0/23**	NS	**-0.326**	0/0	NS	NS	0/0	NS	NS
	Left	0/0	NS	NS	**0/51**	NS	**-0.463**	0/0	NS	NS	**6/0**	**0.294**	NS
Pallidum	Right	0/0	NS	NS	0/0	NS	NS	0/0	NS	NS	**0/13**	NS	**-0.342**
	Left	**13/0**	**0.409**	NS	0/0	NS	NS	**23/0**	**0.466**	NS	0/0	NS	NS
Putamen	Right	**14/0**	**0.391**	NS	**0/40**	NS	**-0.487**	0/0	NS	NS	**0/24**	NS	**-0.474**
	Left	**10/0**	**0.370**	NS	NS	NS	NS	0/0	NS	NS	0/0	NS	NS
Thalamus	Right	0/0	NS	NS	NS	NS	NS	**0/24**	NS	**-0.413**	0/0	NS	NS
	Left	0/0	NS	NS	**8/0**	**0.402**	NS	0/0	NS	NS	0/0	NS	NS

NS indicates non-significant correlation, otherwise the correlation coefficients shown were significant at $P<0.05$. Data shown are from the permutation tests for shape analysis, showing significant partial correlations between radial distances and fitness variables. Positive correlations indicating that higher fitness was associated with expansions (larger radial distance) whereas negative correlations are indicating that higher fitness was associated with contractions (shorter radial distance) in the subcortical nuclei studied. Number of significant vertices (k) includes both positive/negative (data presented in this order) significant correlations. All the analyses were controlled for age at first evaluation visit, the time difference between MRI and fitness assessments, sex, socioeconomic status of the family, birth weight, body mass index and total intracranial volume.

* The lower the score in the 4x10m shuttle run test (i.e. less seconds to cover a fixed distance) the higher the performance (i.e. the faster and more agile the child is).

Figure 1.An illustration of radial distances (light green) to the medial line (blue) in a section of the left caudate nucleus. The medial line is independent of the pose of the nucleus.

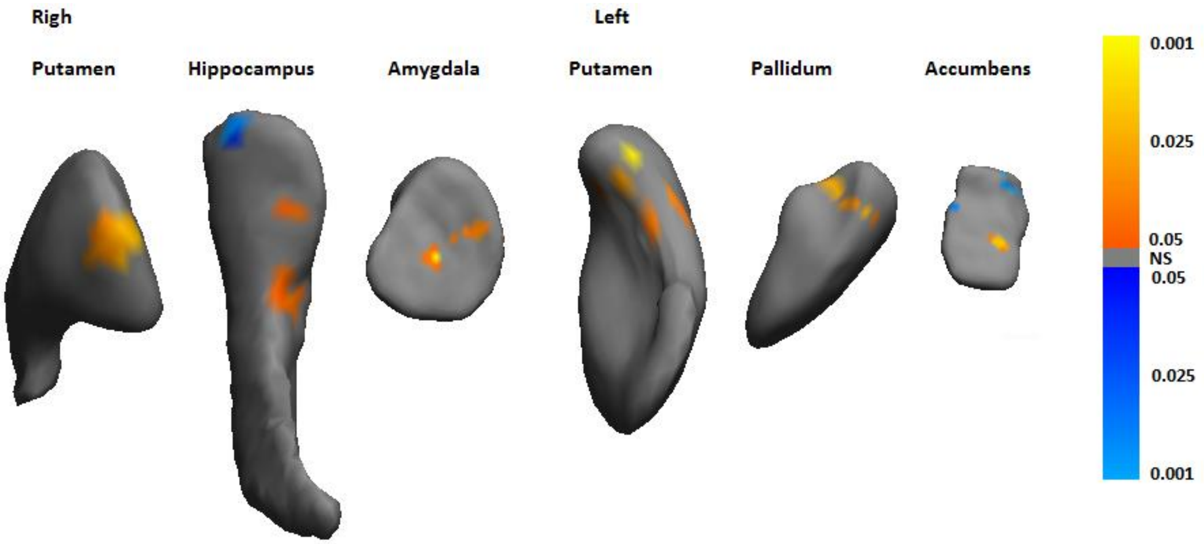

Figure 2. Mappings of significant subcortical nuclei expansions/contractions related to cardiorespiratory fitness (maximal oxygen consumption, VO_2max). The color bar indicated the significance corrected p-values, with blue indicating significant negative associations between predictor and outcome, yellow indicating significant positive associations and grey indicating no association. All the analyses were controlled for age at first evaluation visit, the time difference between MRI and fitness assessments, sex, socioeconomic status of the family, birth weight, body mass index, and total intracranial volume.

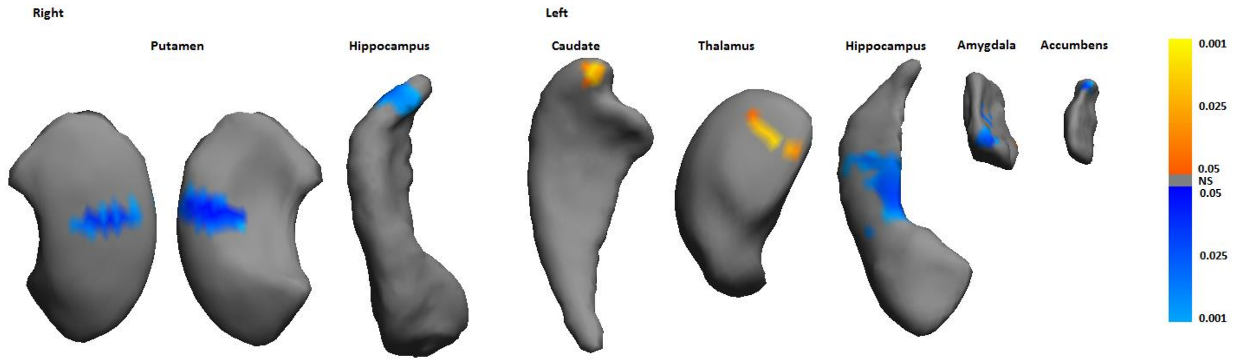

Figure 3. Mappings of significant subcortical nuclei expansions/contraction related to handgrip strength. The color bar indicated the significance corrected p-values, with blue indicating significant negative associations between predictor and outcome, yellow indicating significant positive associations and grey indicating no association. All the analyses were controlled for age at first evaluation visit, the time difference between MRI and fitness assessments, sex, socioeconomic status of the family, birth weight, body mass index, and total intracranial volume.

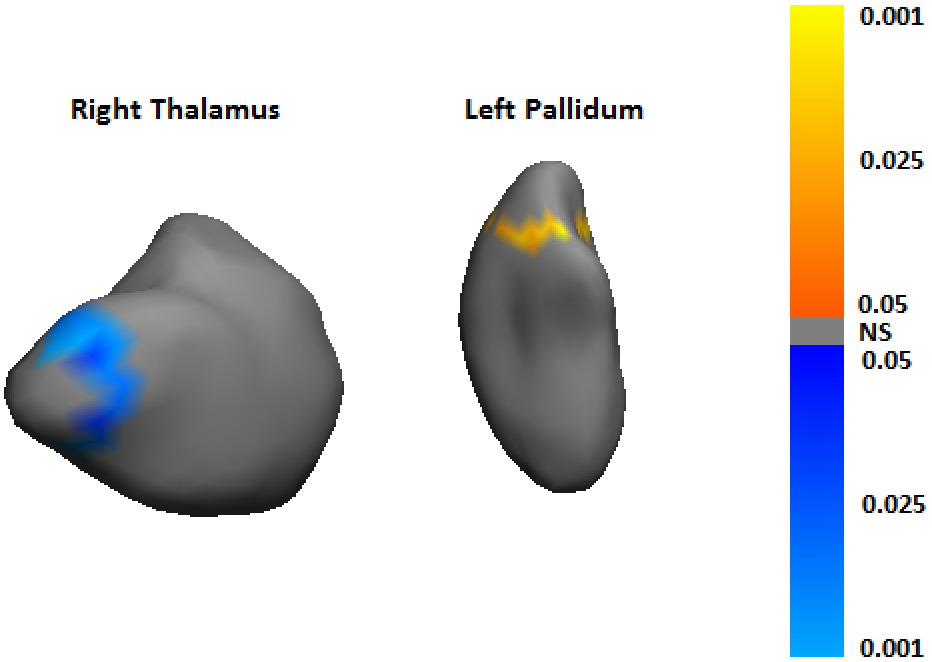

Figure 4. Mappings of significant subcortical nuclei expansions/contraction related to lower-body muscular strength as measured by the standing long jump. Only the most significant sides of the nuclei are displayed. The color bar indicated the significance corrected p-values, with blue indicating significant negative associations between predictor and outcome, yellow indicating significant positive associations and grey indicating no association. All the analyses were controlled for age at first evaluation visit, the time difference between MRI and fitness assessments, sex, socioeconomic status of the family, birth weight, body mass index, and total intracranial volume.

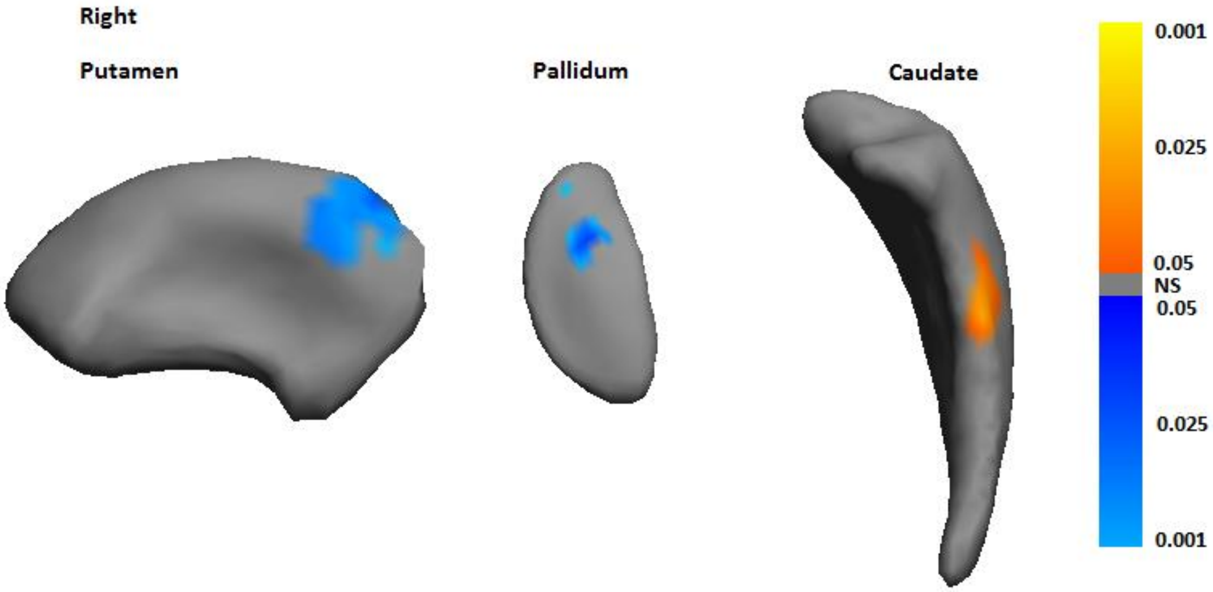

Figure 5. Mappings of significant subcortical nuclei expansions/contraction related to speed-agility. Only the most significant sides of the nuclei are displayed. The color bar indicated the significance corrected p-values, with blue indicating significant negative associations between predictor and outcome, yellow indicating significant positive associations and grey indicating no association. The lower the score in the 4x10m shuttle run test (i.e. less seconds to cover a fixed distance) the higher the performance (i.e. the faster and more agile the child is). Consequently, negative association actually means positive associations between speed-agility performance and enlargements of subcortical nuclei. All the analyses were controlled for age at first evaluation visit, the time difference between MRI and fitness assessments, sex, socioeconomic status of the family, birth weight, body mass index, and total intracranial volume.