



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

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

3 *Fitness, physical activity and brain activity*

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1  
2  
3 **19 Abstract**  
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5 20 The aim of the present study was to examine the associations of physical fitness, sedentary time  
6  
7 21 and physical activity (PA) with working memory and neuroelectric activity in children with  
8  
9 22 overweight/obesity. Seventy-nine children with overweight/obesity ( $10.2 \pm 1.1$  years old)  
10  
11 23 participated in this cross-sectional study. We assessed physical fitness components (i.e., muscular  
12  
13 24 strength, speed-agility and cardiorespiratory fitness) using the ALPHA battery. Sedentary time  
14  
15 25 and PA were assessed by GT3X+ accelerometers (ActiGraph, Pensacola, FL, USA). Working  
16  
17 26 memory was assessed using the Delayed Non-Matched-to-Sample task; mean reaction time (RT)  
18  
19 27 and response accuracy were registered. Neuroelectric activity (i.e., P3 amplitude and latency) was  
20  
21 28 registered using the ActiveTwo System of BioSemi electroencephalogram. Higher upper-limbs  
22  
23 29 absolute strength was associated to lower response accuracy ( $P=0.023$ ), while higher lower-limbs  
24  
25 30 relative-to-weight strength was associated with larger P3 amplitude ( $P<0.05$ ). Higher speed-  
26  
27 31 agility and cardiorespiratory fitness levels were associated with shorter mean RT and larger P3  
28  
29 32 amplitude, and speed-agility was also associated with shorter P3 latency (all  $P<0.05$ ). Vigorous  
30  
31 33 PA was associated with larger P3 amplitude ( $P<0.05$ ). No associations were found for sedentary  
32  
33 34 time or the rest of PA intensities ( $P\geq 0.05$ ). In addition to cardiorespiratory fitness, muscular  
34  
35 35 strength and speed agility are also associated with working memory and neuroelectric activity in  
36  
37 36 children with overweight/obesity. The association between PA and working memory is intensity-  
38  
39 37 dependent, as significant findings were only observed for vigorous PA. Randomized controlled  
40  
41 38 trials in this population would help to better understand whether improvements in different  
42  
43 39 components of fitness and PA lead to better working memory and underlying brain function.  
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46

47 **40 Keywords**  
48

49 41 Aerobic fitness; Brain function; Cognition; EEG; Executive function; Health; P3; Youth  
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## 1. Introduction

Apart from the well-known benefits of physical fitness and physical activity (PA) on youth's physical health,<sup>1,2</sup> low levels of both fitness and PA might be further related to poorer executive function and brain health in children.<sup>3</sup> These detrimental associations have been also found in individuals with obesity.<sup>4-6</sup> In fact, childhood obesity has been negatively associated with the structure and function of several brain regions that underlie executive function,<sup>6,7</sup> as well as with impairments in executive function processes per se, particularly working memory.<sup>8</sup> 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.<sup>9</sup> There are only three studies examining the cross-sectional association between physical fitness components and working memory in children with normal weight.<sup>10-12</sup> 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.<sup>12</sup> 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,<sup>13</sup> whereas total daily PA and moderate-to-vigorous PA (MVPA) were not associated with working memory.<sup>14</sup> However, no studies included speed-agility, a key component for executive function,<sup>4,15</sup> 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.<sup>16,17</sup>

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.<sup>18</sup> Specifically, previous studies have shown that higher-fit children, in term of cardiorespiratory fitness, have larger amplitude (i.e., increased attentional resource allocation

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3 71 during stimulus engagement) and shorter latency (i.e., faster processing speed) of the P3  
4  
5 72 component than their lower-fit peers while performing an attentional inhibition task.<sup>19,20</sup>  
6  
7 73 However, no previous studies examined other physical fitness components (i.e., muscular strength  
8  
9 74 or speed-agility) or sedentary time and PA in relation to the neuroelectric activity underlying  
10  
11 75 working memory in children.  
12  
13 76 Importantly, all the aforementioned studies have focused on healthy children with normal weight.  
14  
15 77 Based on previous research declaring the negative influence of childhood obesity on executive  
16  
17 78 function,<sup>7,8</sup> the aim of the present study was to investigate the association of different physical  
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19 79 fitness components (i.e., muscular strength, speed-agility, and cardiorespiratory fitness),  
20  
21 80 sedentary time and PA with working memory and neuroelectric activity in a sample of children  
22  
23 81 with overweight/obesity. Given previous research on the association between physical fitness,  
24  
25 82 physical activity, and executive functions,<sup>3</sup> we hypothesized that physical fitness components and  
26  
27 83 PA, but not sedentary time, would positively relate to working memory and neuroelectric activity.  
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## 30 84 **2. Materials and Methods**

### 31 85 **Participants**

32  
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34 86 Participants in this study were recruited from the ActiveBrains project  
35  
36 87 (<http://profith.ugr.es/activebrains>). The complete methodology, procedures and  
37  
38 88 inclusion/exclusion criteria for the project has been described elsewhere.<sup>21</sup> Briefly, the study was  
39  
40 89 conducted in three waves of participation and, initially, a total of 110 children with  
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42 90 overweight/obesity (i.e., defined as such according to sex-and-age specific international World  
43  
44 91 Obesity Federation cut-off points) aged 8–11 years were recruited from Granada (Spain). The  
45  
46 92 present study focuses only upon the baseline assessment data prior to randomization. A final  
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48 93 sample of 79 children with overweight/obesity ( $10.2 \pm 1.1$  years old; 64.6% boys) with complete  
49  
50 94 baseline data for physical fitness, sedentary time, PA, working memory (i.e., >15 trials completed  
51  
52 95 per task condition), and ERPs (i.e., neuroelectric activity non-artifacted) were included in this  
53  
54 96 study.  
55  
56 97 Description and characteristics of the study were given to parents or legal guardian and a written  
57  
58 98 informed consent was provided by them. The ActiveBrains project was approved by the Ethics  
59  
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3 99 Committee on Human Research of the University of Granada, and was registered in  
4  
5 100 ClinicalTrials.gov (identifier: NCT02295072).  
6

### 7 101 **Physical fitness components**

8  
9 102 Components of physical fitness (i.e., muscular strength, speed-agility and  
10  
11 103 cardiorespiratory fitness) were assessed using the ALPHA (Assessing Levels of Physical fitness  
12  
13 104 and Health in Adolescents) health-related physical fitness test battery for children and adolescents  
14  
15 105 which has been shown to be valid, reliable and feasible for the assessment of physical fitness in  
16  
17 106 youth.<sup>22</sup>

18  
19  
20 107 Briefly, upper-limbs muscular strength and lower-limbs muscular strength were assessed using  
21  
22 108 the maximum handgrip strength test and the standing long jump test, respectively. A digital hand  
23  
24 109 dynamometer with an adjustable grip (TKK 5101 Grip D, Takei, Tokyo, Japan) was used to assess  
25  
26 110 the handgrip strength. Each child performed the test twice, and the mean score of the maximum  
27  
28 111 score of left and right hands was calculated as an absolute measurement of upper-limbs muscular  
29  
30 112 strength (kg). The standing long jump test was performed three times and the longest jump was  
31  
32 113 recorded in centimeters (cm) as a relative measurement of lower-limbs muscular strength. For  
33  
34 114 exploratory analyses, we computed a relative-to-body weight measurement from upper-limbs  
35  
36 115 muscular strength (kg / body weight) and an absolute measurement from lower-limbs muscular  
37  
38 116 strength (cm \* kg).

39  
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41 117 Speed-agility was assessed using the 4×10-meter shuttle-run test (4×10m SRT). The test was  
42  
43 118 performed twice and the fastest time was recorded in seconds. Since a longer completion time  
44  
45 119 indicates a lower fitness level, for analyses purposes we inverted this variable by multiplying test  
46  
47 120 completion time (sec) by -1. Thus, higher scores indicated higher speed-agility levels.

48  
49 121 Cardiorespiratory fitness was assessed through the 20-meter shuttle-run test (20m SRT).<sup>23</sup> This  
50  
51 122 test was performed once and always at the end of the fitness battery testing session. The total  
52  
53 123 number of completed laps was registered.

### 54 124 **Sedentary time and physical activity**

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56  
57 125 Sedentary time and PA were assessed by accelerometer (GT3X+, ActiGraph, Pensacola,  
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59 126 FL, USA). Children wore simultaneously two accelerometers located on the right hip and non-

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3 127 dominant wrist during 7 consecutive days (24h/day), and they were instructed to remove them  
4  
5 128 only for water activities (i.e., bathing or swimming). Data was presented from the hip-location  
6  
7 129 and also from non-dominant wrist as supplementary. Further information about the whole data  
8  
9 130 processing criteria is shown in **Supplementary material 1**. In brief, total minutes per day of  
10  
11 131 sedentary time, light PA, moderate PA, vigorous PA, and MVPA were calculated using the GGIR  
12  
13 132 package in R (v. 1.5-18, <https://cran.r-project.org/web/packages/GGIR/>)<sup>24</sup> and the previously  
14  
15 133 published cut-points by Hildebrand et al.<sup>25,26</sup>

### 134 **Working memory**

135 All participants completed a modified version of the Delayed Non-Match-to-Sample  
136 (DNMS) computerized task to assess working memory.<sup>27</sup> All trials were presented focally on a  
137 computer screen using E-Prime software (Psychology Software Tools, Pittsburgh, PA). Each trial  
138 consisted of two phases: sample and choice. The sample phase included a memory set of four  
139 sequential stimuli. We adapted stimuli for children, and thus *Pokemon* cartoons were presented  
140 on a blue background. Participants were asked to remember 4 stimuli displayed for 500 ms with  
141 a 1000 ms inter-stimulus interval between them. After the presentation of the 4 stimuli and after  
142 a 4000 ms delay interval, a target consisting in 2 different cartoons presented together was shown  
143 during the choice phase for 1800 ms (**Figure 1**). During this phase, participants were asked to  
144 select the cartoon that was not shown on the 4 previous stimuli.

145 A total of 16 practice trials plus 140 experimental trials were presented. The practice phase was  
146 carried out before the presentation of experimental trials to make sure that all participants were  
147 familiarized with the cartoons and started the experimental task in equal conditions. Then, the  
148 140 total trials were shown in 4 blocks of 35 trials each in a randomized order. For the low  
149 memory load condition (i.e., 40 trials), the 4 stimuli presented during the sample phase were  
150 repeated, whereas for the high memory load condition (i.e., 100 trials) 4 different stimuli were  
151 presented before the choice phase demanding greater working memory capacity. Duration of the  
152 task ranged from 35 to 45 min. Mean reaction time (RT) and response accuracy (%) were  
153 registered.

## 154 **Neuroelectric activity**

155 Neuroelectric activity was recorded from 64 electrode sites (Fpz, AFz, Fz, FCz, Cz, CPz,  
156 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,  
157 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  
158 based on the International 10–10 system<sup>28</sup> using the ActiveTwo System of BioSemi (24-bit  
159 resolution, biopotential measurement system with Active Electrodes; Biosemi, Amsterdam,  
160 Netherlands). Prior to electroencephalography (EEG) recordings, inter-electrodes impedance was  
161 <10k $\Omega$  with CMS and DRL sites serving as online (active and passive) ground electrodes. Details  
162 of the online BioSemi reference method can be found at  
163 <http://www.biosemi.com/faq/cms&drl.htm>. The EEG was conducted by using a 64-channel  
164 Active Two BioSemi EEG recording system (BioSemi; Amsterdam, Holland) using a sampling  
165 rate of 1024 Hz and a 100-Hz low-pass filter.

166 Information about EEG data pre-processing and processing is shown in **Supplementary material**  
167 **2**. Briefly, the P3 component was defined as the largest positive-going peak within a 300 to 800  
168 ms latency window. Data were then averaged across a 9-electrode site region of interest over the  
169 parietal and occipital regions (P1/Z/2, PO3/Z/4, O1/Z/2). Amplitude was measured as the  
170 difference between the mean pre-stimulus baseline and mean peak-interval amplitude; while peak  
171 latency was defined as the time point corresponding to the maximum peak amplitude.

## 172 **Potential confounders**

173 Sex, age, peak height velocity (PHV), body mass index (BMI), wave of participation,  
174 parental educational level and intelligence quotient (IQ) were used as potential confounders in  
175 the analyses. PHV is an indicator of maturity during childhood and adolescence and we used age  
176 and anthropometric variables to calculate it following Moore's equations.<sup>29</sup> Wave of participation  
177 was a categorical variable according to the moment of participation (wave 1, 2, or 3) of each child  
178 in the study. Parental educational level was assessed by a self-report questionnaire filled by the  
179 parents and we combined responses of both parents as: neither of them had a university degree;  
180 one of them had a university degree; both of them had a university degree.<sup>30</sup> The total composite



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3 181 IQ was assessed by the Spanish version of a valid and reliable tool named The Kaufman Brief  
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5 182 Intelligence Test (K-BIT).<sup>31</sup>  
6

7 183 **Statistical analysis**  
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9 184 The characteristics of the study sample are presented as means and standard deviations  
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11 185 (SD) or percentages. Prior to all analyses, the extreme values were winsorized to limit their  
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13 186 influence; this method allows replacing extreme high/low values for the closest (highest/lowest)  
14  
15 187 valid value.<sup>32</sup> After checking for normal distribution, response accuracy from the low working  
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17 188 memory load was normalized since it showed skewed distribution. Interaction analyses were  
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19 189 performed between sex and physical fitness, sedentary time and PA on the outcomes. No  
20  
21 190 significant interactions with sex were found (all  $P_s \geq 0.10$ ) so analyses were carried out for the  
22  
23 191 whole sample. Paired sample t-test was used to analyze differences in working memory (i.e.,  
24  
25 192 mean RT and response accuracy) and neuroelectric (i.e., P3 amplitude and latency) outcomes  
26  
27 193 between low and high working memory loads. Bivariate Pearson correlations were performed to  
28  
29 194 test the associations between potential confounders and working memory and neuroelectric  
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31 195 outcomes. Statistical summary of these correlations is provided in **Table S1**.  
32

33 196 Hierarchical linear stepwise regression analyses were performed to examine the associations of  
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35 197 physical fitness, sedentary time and PA (i.e., data from both hip and wrist locations) with working  
36  
37 198 memory and neuroelectric measurements. Stepwise method was used and all potential  
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39 199 confounders (i.e., sex, age, PHV, BMI, wave of participation, parental educational level and IQ)  
40  
41 200 were included into step 1 to test their association to the outcomes (working memory or  
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43 201 neuroelectric activity). This step was performed to select the potential confounders that explain  
44  
45 202 the higher amount of variance of their association with working memory and neuroelectric  
46  
47 203 outcomes (see tables footnotes). Subsequently, hierarchical regressions were carried out entering  
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49 204 each physical fitness, sedentary time and PA variable into step 2 in separate regression analyses  
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51 205 after the inclusion of confounders previously found significantly associated to the outcomes in  
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53 206 step 1.  
54

55 207 Additionally, computation of the median for all the predictors was performed in order to visually  
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57 208 represent the relationship of physical fitness, sedentary time and PA with P3 amplitude and  
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3 209 latency. A significance level of  $P < 0.05$  was set. Additionally, multiple comparisons correction  
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5 210 was performed by independent variables (i.e., physical fitness, and sedentary time and PA) using  
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7 211 the Benjamini-Hochberg method.<sup>33</sup> All the statistical procedures were performed using the SPSS  
8  
9 212 software for Mac (version 22.0, IBM Corporation).

### 213 3. Results

#### 214 Working memory: Reaction time and response accuracy

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

#### 220 Physical fitness

221 The descriptive characteristics of the study sample can be found in **Table 1**. Higher upper-  
222 limbs absolute strength was associated with lower response accuracy in the high load ( $\beta = -0.270$ ,  
223  $P = 0.023$ ) (**Table 2**). This association remained significant after exploratory analysis with relative-  
224 to-body weight upper-limbs strength fitness ( $\beta = -0.223$ ,  $P = 0.032$ ). Higher speed-agility was  
225 associated with shorter mean RT in both the low and high loads ( $\beta = -0.231$  and  $\beta = -0.251$ ,  
226 respectively; both  $P < 0.05$ ). Higher cardiorespiratory fitness was also associated with a shorter  
227 mean RT in the high load ( $\beta = -0.243$ ,  $P = 0.031$ ). No significant association was observed between  
228 lower-limbs relative or absolute strength and working memory ( $P \geq 0.05$ ).

#### 229 Sedentary time and physical activity

230 Higher vigorous PA was associated with higher response accuracy in the high load  
231 ( $\beta = 0.219$ ,  $P = 0.028$ ). However, the significance disappeared after correcting for multiple  
232 comparisons. No associations were observed for sedentary time and the rest of PA intensities  
233 ( $P \geq 0.05$ ). Furthermore, when performing sedentary time and PA analyses from the non-dominant  
234 wrist-placement, no associations were found with working memory ( $P \geq 0.05$ ) (**Table S2**).

#### 235 Neuroelectric activity: P3 amplitude

236 Paired sample t-test did not show a significant difference between low and high working  
237 memory loads with respect to P3 amplitude ( $10.07 \pm 4.33 \mu\text{V}$  and  $10.14 \pm 4.16 \mu\text{V}$ , respectively;  
238  $P=0.822$ ).

### 239 *Physical fitness*

240 Higher lower-limbs relative strength, speed-agility and cardiorespiratory fitness were  
241 associated with larger P3 amplitude in both low and high working memory loads with  $\beta$  ranging  
242 from 0.251 to 0.337 (all  $P<0.05$ ) (**Table 3**). However, the associations found for lower-limbs  
243 relative strength disappeared when it was expressed in absolute terms ( $\beta=0.102$  and  $\beta=0.115$  for  
244 low and high P3 amplitude;  $P>0.05$ ). No significant associations were found between upper-limbs  
245 absolute or relative strength and P3 amplitude ( $P\geq 0.05$ ). These relationships can be visually  
246 observed in **Figure 2**.

### 247 *Sedentary time and physical activity*

248 After correcting for multiple comparisons, only higher vigorous PA from hip was  
249 associated with larger P3 amplitude in the low working memory load ( $\beta=0.390$ ;  $P<0.001$ ) (**Table**  
250 **3**). No associations were observed for sedentary time and the rest of PA intensities ( $P\geq 0.05$ ).  
251 These relationships can be graphically observed in **Figure 3**. Furthermore, when using data from  
252 the non-dominant wrist-placement (**Table S3**) and after correcting for multiple comparisons, only  
253 higher vigorous PA was associated with larger P3 amplitude in the low load ( $\beta=0.289$ ,  $P=0.008$ ).

### 254 **Neuroelectric activity: P3 latency**

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

258 Associations of physical fitness, sedentary time and PA with P3 latency can be observed  
259 in **Table S4**, and in **Table S5** for the PA data from non-dominant wrist.

### 260 *Physical fitness*

261 Briefly, higher speed-agility was associated with shorter P3 latency in both low and high  
262 loads ( $\beta=-0.252$  and  $\beta=-0.277$ , respectively; both  $P<0.05$ ), as well as cardiorespiratory fitness  
263 but only in the low load ( $\beta=-0.314$ ,  $P=0.008$ ).

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3 264 *Sedentary time and physical activity*  
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5 265 No associations were found between sedentary time, PA, and P3 latency using either the  
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7 266 hip or wrist data ( $P \geq 0.05$ ).  
8

9 267 **4. Discussion**

10  
11 268 **Main findings**

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13 269 Our findings contribute to the existent literature by suggesting that: 1) Not only  
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15 270 cardiorespiratory fitness, as shown in previous research, but also speed-agility were consistently  
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17 271 associated with working memory (i.e., shorter mean RT) and the P3 component (i.e., larger  
18  
19 272 amplitude and shorter latency). However, inconsistent findings were observed for muscular  
20  
21 273 strength, with upper-limbs absolute strength associated with lower response accuracy, and lower-  
22  
23 274 limbs relative strength associated with larger P3 amplitude; 2) The relationship of PA with  
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25 275 working memory and neuroelectric activity was intensity-dependent and seemed consistent across  
26  
27 276 accelerometer locations and cut-points (i.e., hip and wrist). Thus, only vigorous PA related to a  
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29 277 higher response accuracy and to a larger P3 amplitude regardless of the accelerometer location.  
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32 278 **Physical fitness components and working memory**

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34 279 In the present study, speed-agility and cardiorespiratory fitness were associated with  
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36 280 shorter RT observed in the high working memory load. Despite the lack of studies in children  
37  
38 281 with overweight/obesity, our results regarding cardiorespiratory fitness are in line with several  
39  
40 282 previous studies carried out in children with normal weight.<sup>10,11</sup> In these studies, higher-fit  
41  
42 283 children had a higher response accuracy than their lower-fit peers during a working memory task.  
43  
44 284 Similarly, in a randomized-controlled trial, increases in  $VO_2$ max resulting from a PA intervention  
45  
46 285 consisting in 70 min of daily MVPA were associated with improvements in children's working  
47  
48 286 memory.<sup>34</sup> A recent systematic review showed that complex motor skills (i.e., speed-agility) are  
49  
50 287 strongly related to higher-order cognitive skills, which may include working memory.<sup>15</sup> Whereas  
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52 288 our findings on fitness and working memory performance were observed for mean RT, the  
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54 289 majority of previous research found these associations for response accuracy.<sup>9,10</sup> However,  
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56 290 previous studies included a sample of children with normal weight, and since our study was  
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58 291 carried out in children with overweight/obesity, it could be that in the present study, RT was a  
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3 292 more sensitive index to detect fitness associations in this population. Thus, it may be that children  
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5 293 in the present study carried out more proactive cognitive strategies (i.e., early selection, shorter  
6  
7 294 RT) in order to achieve optimal completion of task goals via regulation of attentional  
8  
9 295 engagement.<sup>20</sup> Collectively, as our results strengthen the existent evidence suggesting that  
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11 296 different components of physical fitness may positively affects working memory,<sup>3,34</sup> future  
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13 297 studies should focus on conducting intervention programs to gain causal evidence for the role of  
14  
15 298 different fitness components on working memory in children with overweight/obesity.

16  
17 299 In regards with muscular strength, we found inconsistencies in the associations between upper-  
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19 300 limbs absolute strength and lower-limbs relative strength with respect to working memory. To  
20  
21 301 our knowledge, only one study analyzed this relationship in children with normal weight,  
22  
23 302 reporting contradictory findings with respect to ours with upper-limbs absolute strength in  
24  
25 303 overweight/obese.<sup>12</sup> In that study, overall muscular strength was associated with higher response  
26  
27 304 accuracy during high working memory load. Possible explanations for the discrepancies between  
28  
29 305 studies include the use of different types of strength (i.e., absolute versus relative), different types  
30  
31 306 of musculature involve (i.e., upper- versus lower-limbs versus overall muscular strength), or  
32  
33 307 different types of working memory task (i.e., DNMS versus n-back). In particular, the reason for  
34  
35 308 our negative association between upper-limbs absolute strength and response accuracy may be  
36  
37 309 the overweight/obese status of our sample as it has been speculated previously,<sup>35</sup> as well as the  
38  
39 310 higher levels of upper-limbs absolute strength of these individuals in our study (i.e., there was a  
40  
41 311 significant correlation between body weight and upper-limbs absolute strength of  $r=0.542$ ,  
42  
43 312  $P<0.001$ ). In fact, this negative association remained significant after exploratory analysis using  
44  
45 313 a relative-to-body weight measurement of upper-limbs strength. In this context, a recent  
46  
47 314 systematic review reported shorter RT but more commission errors (i.e., less response accuracy)  
48  
49 315 among children with higher BMI.<sup>35</sup> This fact may derive from a higher impulsivity of children  
50  
51 316 with overweight/obesity<sup>36</sup> that are also the strongest ones, which may in turn explain the lower  
52  
53 317 response accuracy observed in our study.

54  
55 318 In general, the association found between cardiorespiratory fitness and mean RT was observed in  
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57 319 the high working memory load. This indicates that higher-fit children were faster in responding

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3 320 and that the relationship of cardiorespiratory fitness may be selective for task loads engendering  
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5 321 greater amounts of working memory. In fact, these findings are consistent with prior studies in  
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7 322 children with normal weight and support the idea that cardiorespiratory fitness may be particularly  
8  
9 323 beneficial for more cognitively demanding processes.<sup>10,12</sup> Further, when comparing RTs between  
10  
11 324 high and low loads, higher RTs were observed in the high load compared with the low load,  
12  
13 325 although this difference did not reach the significance. On the other hand, speed-agility was  
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15 326 associated with shorter RT in both loads, indicating faster information processing speed  
16  
17 327 regardless of the working memory demands. A recent study using the same sample as in the  
18  
19 328 present research found that higher speed-agility was associated with better movement  
20  
21 329 competency.<sup>37</sup> Proper movement competency is partly determined by a neuromuscular/motor  
22  
23 330 control network,<sup>38</sup> which may also relate to shorter RTs. Considering that a prior study showed  
24  
25 331 that children with overweight/obesity exhibited larger RT than their normal weight peers,<sup>39</sup> it may  
26  
27 332 be that children with overweight/obesity have more room for improvement from speed-agility  
28  
29 333 which may be reflected by shorter RTs. However, further studies comparing children with  
30  
31 334 overweight/obesity and normal weight peers with respect to speed-agility and processing speed  
32  
33 335 are needed to draw firm conclusions.

### 336 **Physical fitness components and neuroelectric activity**

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

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3 347 with cardiorespiratory fitness as a continuous outcome by including the full range of this variable  
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5 348 (i.e., as has been previously recommended).<sup>3</sup>  
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7 349 Apart from cardiorespiratory fitness, this study also provides novel data on the positive  
8  
9 350 association of muscular strength and speed-agility with P3 amplitude and latency during a  
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11 351 working memory task in children with overweight/obesity. Although direct comparisons cannot  
12  
13 352 be made, our findings with lower-body muscular strength may be explained by the fact that  
14  
15 353 muscular fitness is associated with a variety of health benefits in children<sup>40</sup> which have been  
16  
17 354 associated with enhanced working memory.<sup>41,42</sup> However, this must be interpreted with caution  
18  
19 355 since the associations for lower-limbs relative strength disappeared when it was expressed in  
20  
21 356 absolute terms. Across all fitness components, speed-agility and cardiorespiratory fitness had the  
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23 357 highest number of significant associations with working memory performance and P3 amplitude  
24  
25 358 and latency, which may be explained by previous findings in the same sample showing that these  
26  
27 359 two fitness components were also the main ones associated to brain volumes.<sup>4</sup> Specifically, these  
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29 360 components were related to increased volume of cortical and subcortical brain structures<sup>4</sup> that  
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31 361 have been shown to directly influence memory in children (e.g., hippocampus, premotor cortex,  
32  
33 362 etc.).<sup>43</sup> These key neural structures subserving working memory processes are still developing  
34  
35 363 throughout childhood,<sup>44</sup> suggesting that some brain structures might be highly susceptible to  
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37 364 environmental factors such as engagement in aerobic exercise and motor tasks during  
38  
39 365 development.<sup>45</sup> However, further randomized-controlled trials should confirm these findings.  
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42  
43 366 The positive associations found for each fitness component with P3 amplitude were observed  
44  
45 367 regardless of working memory load. This is supported by a prior study using an inhibitory control  
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47 368 task, which showed that higher-fit children exhibited larger P3 amplitude than their lower-fit peers  
48  
49 369 across task's conditions.<sup>19</sup> However, the majority of literature has declared that the association  
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51 370 between physical fitness and P3 amplitude appears when task demands increase.<sup>3,20</sup> Despite this,  
52  
53 371 the associations found across conditions in the present study may be due to different arguments.  
54  
55 372 For instance, the characteristics and design of the DNMS task may be a limitation itself to detect  
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57 373 selective associations of fitness with attentional resources allocation during high working memory  
58  
59 374 processes.  
60

### 375 **Sedentary time, physical activity, working memory and neuroelectric activity**

376 In regards to the relationship between sedentary time, PA, and working memory  
377 performance, we found that vigorous PA (from hip data) was associated with higher response  
378 accuracy in the high memory load. However, this finding should be interpreted with caution since  
379 the significant association disappeared after controlling for multiple comparisons. Two previous  
380 cross-sectional studies investigated this relationship in children with normal weight.<sup>13,14</sup> They  
381 showed non-significant associations of objectively-measured sedentary time, total PA, MVPA,  
382 with the Visual Memory Span task,<sup>14</sup> and the Spatial Span task.<sup>13</sup> None of these studies assessed  
383 other PA intensities (i.e., light, moderate or vigorous PA). Vigorous PA was also related to a  
384 larger P3 amplitude and it was consistent across accelerometer locations (i.e., hip or non-dominant  
385 wrist). Despite no previous study has analyzed the relation between PA and neuroelectric activity  
386 during working memory task, our association between vigorous PA and P3 amplitude may be due  
387 to an intensity-dependent relation of PA with working memory. This is supported by an  
388 intervention study where two different types of PA intensity-based physical education classes  
389 were delivered to an intervention and a control group.<sup>46</sup> In this study, a significant PA intensity  
390 effect was found since the children from the intervention group (i.e., higher PA intensity classes)  
391 had better performance after the program on a working memory test battery in comparison with  
392 their peers in the control group (i.e., regular physical education lessons). Another important  
393 finding from our study was the consistency between accelerometer's locations (i.e., hip and wrist)  
394 with respect to the relation of sedentary time and PA with working memory and neuroelectric  
395 activity. However, the lack of evidence in this respect indicate that these findings are preliminary  
396 and call for more studies investigating the association of different PA intensities and  
397 accelerometer-locations with the neuroelectric system in children.

### 398 **Limitations and strengths**

399 Several limitations of the present study must be highlighted. First, the cross-sectional  
400 design does not allow us to draw causal interpretations. Second, PA such as bicycling and  
401 swimming cannot be captured by the accelerometers, and our identification of sedentary time was  
402 not sensitive to postures, so we cannot differ between different sedentary behaviors and, therefore,



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3 403 some standing activity could be classified as sedentary time. On the other hand, the main strength  
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5 404 of this study was that, to the best of our knowledge, this was the first study to investigate the  
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7 405 relationship between different physical fitness components, not only cardiorespiratory fitness, and  
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9 406 objectively sedentary time and PA with working memory and the neuroelectric activity  
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11 407 underlying it in a sample of children with overweight/obesity. Other strengths were the objective  
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13 408 and standardized assessment of physical fitness, PA and the cognitive variables; the analysis of  
14  
15 409 different intensities of PA; the use of all predictors as continuous variables; and the availability  
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17 410 of sedentary time and PA data from two different accelerometer locations (i.e., hip and non-  
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19 411 dominant wrist).

## 22 412 **5. Perspective**

23  
24 413 Our results add to the literature on physical fitness and cognition by providing support not  
25  
26 414 only for cardiorespiratory fitness relation, but also muscular strength and speed agility relation to  
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28 415 working memory and the neuroelectric activity (i.e., P3 component) in children with  
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30 416 overweight/obesity. Speed-agility and cardiorespiratory fitness were the fitness components more  
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32 417 consistently related to both working memory performance and neuroelectric activity. The  
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34 418 association of PA with working memory and neuroelectric activity was intensity-dependent, since  
35  
36 419 only vigorous PA demonstrated a consistent (i.e., for both hip and wrist's locations) relationship.  
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38 420 From a public health perspective, promoting physical activity that enhances speed-agility and  
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40 421 cardiorespiratory fitness, and also reaches high intensity PA levels, may be important not only for  
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42 422 the physical health, but also for working memory and underlying neuroelectric activity in children  
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44 423 with overweight/obesity. However, our observational findings need to be supported with exercise-  
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46 424 based randomized controlled trials inducing improvements in different fitness and PA  
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48 425 components to test whether such improvements lead to better working memory in  
49  
50 426 overweight/obese youth.

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PROOF



**Table 1.** Descriptive characteristics of the study sample.

	All (n=79)	Boys (n=51)	Girls (n=28)
<i>Sociodemographic characteristics</i>			
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 <sup>2</sup> )	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
<i>Physical fitness</i>			
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
<i>Sedentary time and PA (min/day)†</i>			

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3	Sedentary time	827.2 ± 44.5	821.4 ± 45.9	837.7 ± 40.3
4				
5	Light PA	65.8 ± 15.6	67.3 ± 15.7	63.2 ± 15.3
6				
7	Moderate PA	34.3 ± 14.8	38.5 ± 15.8	26.8 ± 9.2
8				
9	Vigorous PA	3.3 ± 2.8	4.0 ± 3.1	2.1 ± 1.5
10				
11	MVPA	37.6 ± 16.8	42.5 ± 17.9	28.9 ± 10.1
12				
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14				
15	<i>Working memory task</i>			
16				
17	Low working memory load			
18				
19	Mean reaction time (ms)*	920.3 ± 143.3	934.4 ± 156.9	894.6 ± 112.4
20				
21	Response accuracy (%)	83.0 ± 12.2	81.6 ± 13.5	85.6 ± 9.0
22				
23				
24	High working memory load			
25				
26	Mean reaction time (ms)*	907.8 ± 142.8	912.0 ± 153.7	900.2 ± 122.6
27				
28	Response accuracy (%)	68.9 ± 13.5	69.6 ± 15.1	67.7 ± 10.0
29				
30				
31	<i>Neuroelectric measurements</i>			
32				
33	Low working memory load			
34				
35	P3 amplitude (µV)	10.1 ± 4.3	10.1 ± 4.6	10.0 ± 3.9
36				
37	P3 latency (ms)*	469.6 ± 76.7	417.6 ± 80.8	465.8 ± 69.4
38				
39				
40	High working memory load			
41				
42	P3 amplitude (µV)	10.1 ± 4.2	10.0 ± 4.3	10.5 ± 4.0
43				
44	P3 latency (ms)*	495.1 ± 81.5	503.5 ± 84.6	479.9 ± 74.5
45				

Values are expressed as means ± standard deviations, unless otherwise indicated.

PA=Physical activity; MVPA=Moderate-to-Vigorous Physical Activity. \*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

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3 measured by the 4×10-meter shuttle run test. Cardiorespiratory fitness was measured by  
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5 the 20-meter shuttle run test (20m SRT).  
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**Table 2.** 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 <sup>2</sup>	R <sup>2</sup>	β	P	R <sup>2</sup>	R <sup>2</sup>	β	P	R <sup>2</sup>	R <sup>2</sup>	β	P	R <sup>2</sup>	R <sup>2</sup>	β	P
	change				change				change				change			
<i>Physical fitness</i>																
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	<b>0.023*</b>
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	<b>0.037*</b>	0.105	0.011	0.107	0.327	0.063	0.063	-0.251	<b>0.026*</b>	0.278	0.000	-0.001	0.995

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4	Cardiorespiratory	0.066	0.012	-0.110	0.332	0.115	0.022	0.150	0.177	0.059	0.059	-0.243	<b>0.031*</b>	0.281	0.003	0.061	0.586
5																	
6	fitness (laps)																
7																	
8	<i>Sedentary time</i>																
9																	
10	<i>and PA (min/day)</i>																
11																	
12																	
13	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
14																	
15	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
16																	
17	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
18																	
19	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	<b>0.028</b>
20																	
21	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
22																	
23																	
24																	

ENMO=Euclidian norm minus one; PA=Physical activity; MVPA=Moderate-to-vigorous physical activity. 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 and 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 (20m SRT).

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

1  
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4 low working memory load: for mean reaction time and the response accuracy, the parental educational level was included as confounder  $\beta=$   
5  $0.234$  and  $\beta=0.305$ , respectively; both  $P<0.05$ ). Hierarchical stepwise regression models for high working memory load: for mean reaction time,  
6  
7 no confounder was included; for response accuracy, wave of participation, age, and intelligence quotient were included as confounders ( $\beta_{\text{wave}}=$   
8  $0.354$ ,  $\beta_{\text{age}}=0.254$ , and  $\beta_{\text{IQ}}=0.319$ ; all  $P<0.05$ ).  $\beta$  values are standardized. †This variable was inverted so that higher values indicate better  
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10 performance. ‡Normalized values were used in the analysis.  
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**Table 3.** 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 <sup>2</sup>	R <sup>2</sup> change	$\beta$	P	R <sup>2</sup>	R <sup>2</sup> change	$\beta$	P
<i>Physical fitness</i>								
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	<b>0.040*</b>	0.070	0.070	0.264	<b>0.019*</b>
Speed-agility (sec)*	0.157	0.105	0.325	<b>0.003*</b>	0.128	0.128	0.358	<b>0.001*</b>
Cardiorespiratory fitness (shuttles)	0.165	0.113	0.337	<b>0.002*</b>	0.063	0.063	0.251	<b>0.025*</b>
<i>Sedentary time and PA (min/day)</i>								
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	<b>&lt;0.001*</b>	0.075	0.075	0.274	<b>0.015</b>
MVPA	0.106	0.054	0.233	<b>0.035</b>	0.029	0.029	0.169	0.135

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ENMO=Euclidian norm minus one; PA=Physical activity; MVPA=Moderate-to-vigorous physical activity. 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 and 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 (20m SRT).

Potential confounders (i.e., sex, age, peak height velocity, body mass index, wave of participation, parental educational level and IQ) were

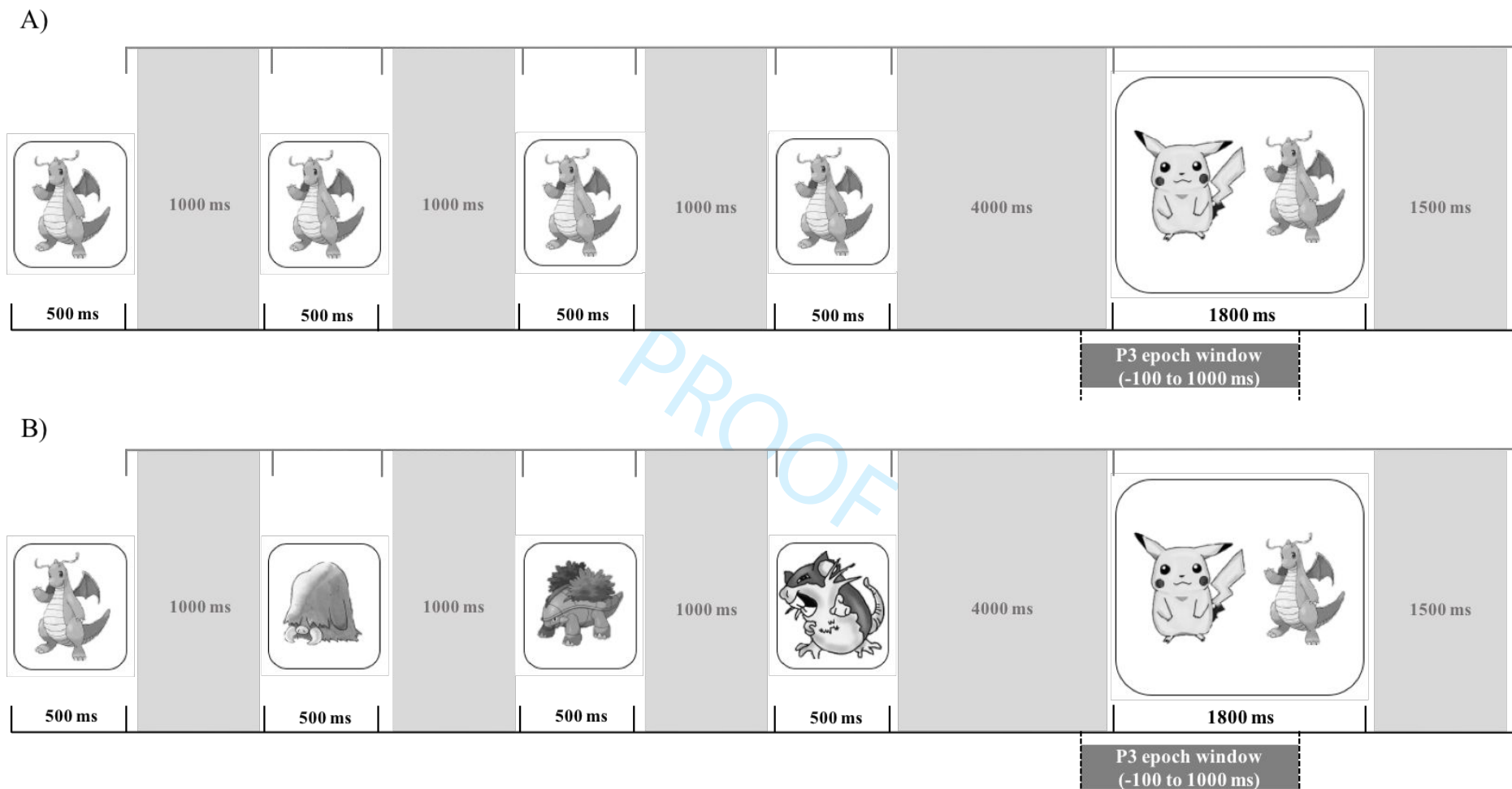
included into step 1 of the hierarchical stepwise regression to test their association to the outcomes. Hierarchical stepwise regression models for

the P3 amplitude from the low working memory load was adjusted by wave of participation ( $\beta=0.228$ ,  $P=0.043$ ). Hierarchical stepwise regression

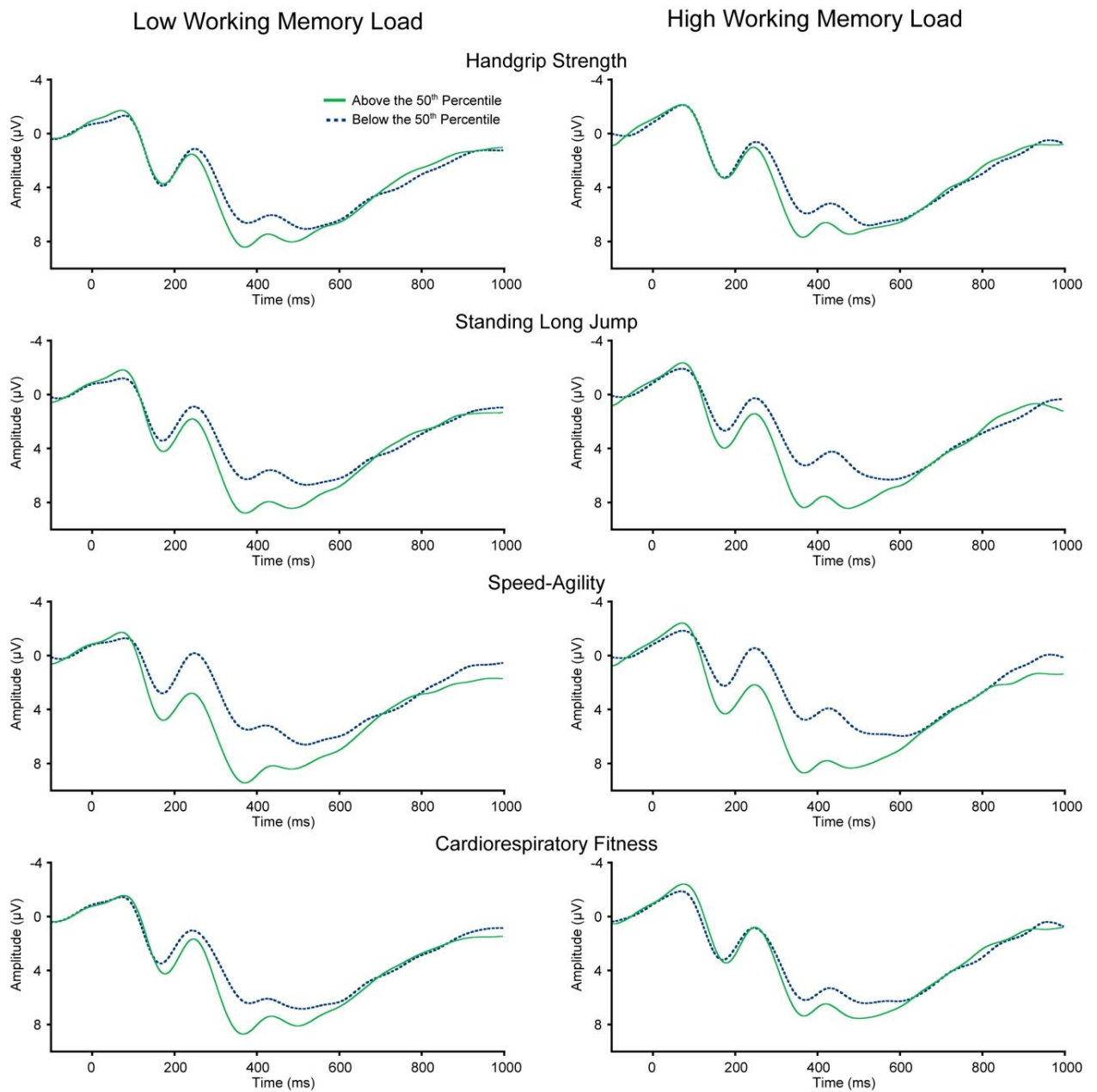
models for the P3 amplitude from the high working memory load was not adjusted by any confounder.  $\beta$  values are standardized. \*This variable

was inverted so that higher values indicate better performance.

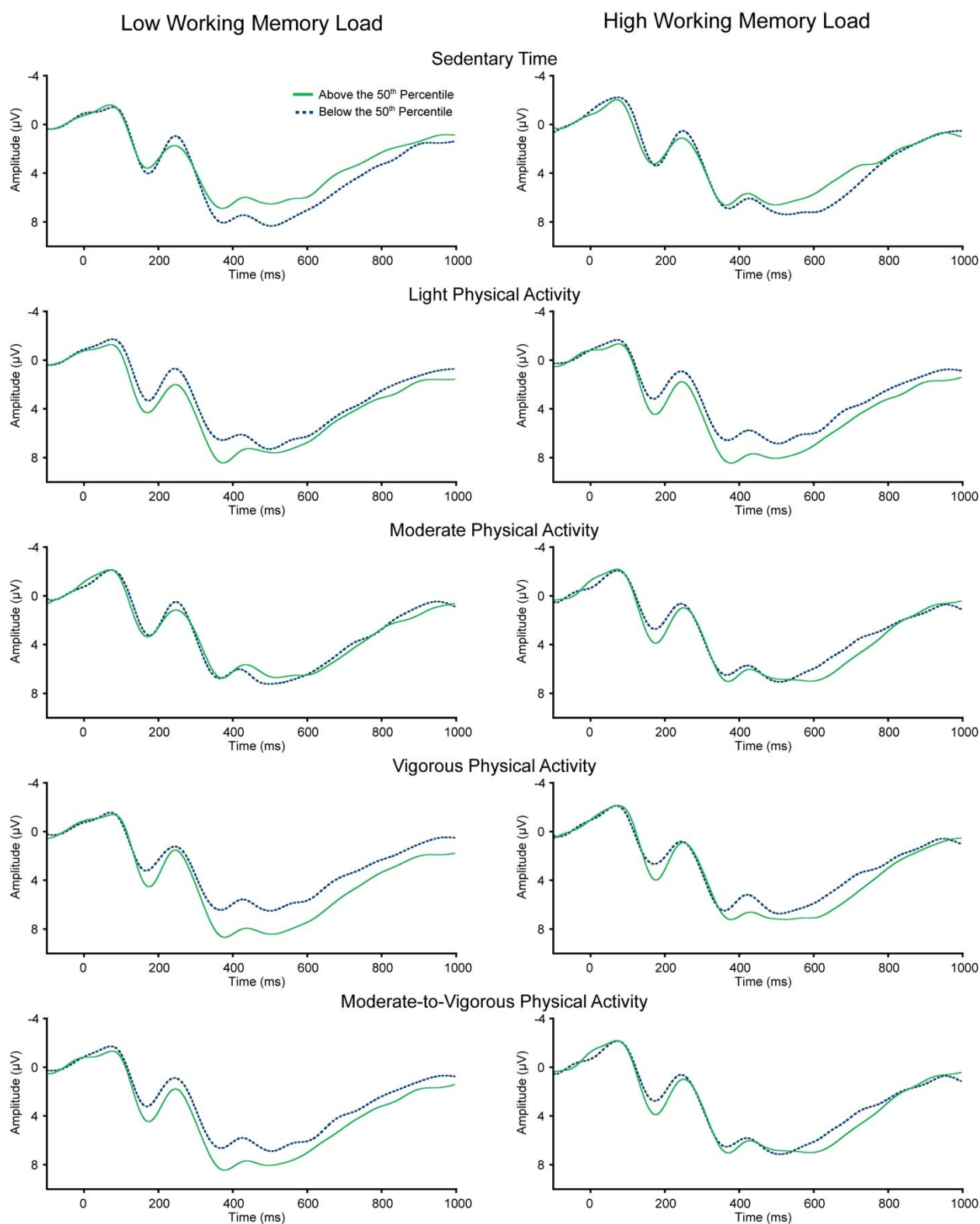




**Figure 1.** Low load (A) and high load (B) delayed-non-match to sample working memory task, and epoch window for the extraction of P3 amplitude and latency.



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



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