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1 RUNNING HEAD: Gene-exercise interaction and brain health in children

2 **Gene-exercise interaction on brain health in children with**
3 **overweight/obesity: The ActiveBrains randomized controlled**
4 **trial**

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65 **ABSTRACT**

66 We investigated the interaction between a genetic score and an exercise intervention on
67 brain health in children with overweight/obesity. One hundred one children with
68 overweight/obesity (10.0 ± 1.5 years, 59% girls) were randomized into a 20-week
69 combined exercise intervention or a control group. Several cognitive and academic
70 outcomes were measured with validated tests. Hippocampal volume was quantified
71 using magnetic resonance imaging. Six brain health-related polymorphisms (rs6265
72 [*BDNF*], rs2253206 [*CREBI*], rs2289656 [*NTRK2*], rs4680 [*COMT*], rs429358, and
73 rs7412 [*APOE*]) were genotyped. Cognitive flexibility and academic skills improved
74 significantly more in the exercise than in the control group only in the children with a
75 “favorable” genetic profile (mean z score, 0.41-0.67 [95% CI 0.11 to 1.18], yet not in
76 those with “less favorable” genetic profile. An individual response analysis showed that
77 children responded to exercise in cognitive flexibility only in the “genetically
78 favorable” group (i.e. 62% of them had a meaningful ≥ 0.2 Cohen *d*] increase in the
79 exercise group compared with only 25% in the control group). This finding was
80 consistent in per-protocol and intention-to-treat analyses (P=0.01 and P=0.03,
81 respectively). The results were not significant or not consistent for the rest of outcomes
82 studied. Our findings suggest that having a more favorable genetic profile makes
83 children with overweight-obesity more responsive to exercise, particularly for cognitive
84 flexibility.

85 **Keywords:** Genetics; cognition; pediatrics; physical activity; fitness

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91 **NEW & NOTEWORTHY:** Inter-individual differences have been reported in brain
92 health-related outcomes in response to exercise interventions in adults, which could be
93 partially explained by genetic background differences. However, the role of genetic
94 polymorphisms on brain health-related outcomes in response to exercise interventions
95 remains unexplored in pediatric population. The current study in children with
96 overweight/obesity showed that a genetic score composed of six brain health-related
97 polymorphisms (*BDNF*, *CREB1*, *NTRK2*, *COMT*, and *APOE*) regulated the exercise-
98 induced response on several brain health comes, yet mainly and more consistently on
99 cognitive flexibility.

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113 **1. Introduction**

114 The prevalence of childhood obesity worldwide increased from 4 to 18%
115 between 1975 and 2016 (1). Excess of body weight/adiposity during childhood is
116 inversely associated with children's brain health indicators, including a poor cognitive
117 and academic performance, and reduced gray matter volume (2–4). Several randomized
118 controlled trials (RCTs) reported that regular exercise counteracts the negative impact
119 of childhood obesity on brain health (5–8). Indeed, our recent RCT showed the positive
120 effects of a 20-week combined exercise intervention on a broad set of brain health
121 indicators such as intelligence, cognitive flexibility, and academic performance in
122 children with overweight/obesity (OW/OB) (9).

123 A consensus statement reported considerable interindividual differences in the
124 response to exercise interventions on cardiometabolic risk factors and cardiorespiratory
125 fitness (10). Nevertheless, less is known on the interindividual response to exercise
126 interventions on brain health. Yu *et al.* reported interindividual differences brain health
127 indicators in response to an aerobic exercise intervention in older adults (11). Genetic
128 background, including single nucleotide polymorphisms (SNPs), could partially explain
129 these interindividual differences in response to exercise interventions (10, 12). In this
130 regard, Stroth *et al.* reported that 17 weeks of running training improved cognitive
131 function in young adults presenting the Val/Val genotype for the SNPs rs4680 in
132 Catechol-O-methyltransferase (*COMT*) compared to peers with the Met/Met genotype
133 (13). On the other hand, SNPs (rs6265, rs429358, and rs7412) in brain-derived
134 neurotrophic factor (*BDNF*) and apolipoprotein E (*APOE*) did not explained inter-
135 individual differences in motor status and brain volume in response to motor

136 rehabilitation therapy after stroke (14). Several genes have an impact on complex
137 phenotypes and they show small effect sizes, which when combined may have an
138 impact (12, 15). Thus, the use of genetic scores could be more powerful than individual
139 SNPs to reveal the influence of genetic constitution on brain health in response to
140 exercise interventions. However, to our knowledge, the interaction between a genetic
141 score based on SNPs genes related to brain health and the effects of an exercise
142 intervention on brain health remains unexplored in pediatric population.

143 Previous literature has identified several genes that encode proteins related to
144 cognition or brain outcomes (**Table S1**). For instance, BDNF is one of the most
145 investigated neurotrophic factor involved in neuronal physiology and cognition (16, 17).
146 Circulating BDNF levels are reduced in patients with neurodegenerative diseases and
147 obesity (18, 19), while RCTs reported that exercise might increase circulating BDNF
148 protein levels (18, 20). BDNF binds to the neurotrophic tyrosine kinase receptor 2
149 (NTRK2; also called TrkB) on neurons inducing the activation of downstream pathways
150 such as PI3K-AKT and Ras-MAPK, modulating the neurotransmitter release in
151 hippocampal neurons(16). Importantly, cAMP-responsive element binding proteins
152 (CREB) are a transcription factor family considered the main regulator of BDNF
153 expression at the transcriptional level in cortical neurons (21). Also, APOE plays an
154 important role through the transport of cholesterol and peptides with involved in
155 cognitive function, such as amyloid beta(22), which can inactivate the PKA/CREB
156 pathway (23). COMT is an enzyme that regulates dopamine levels in different brain
157 regions, such as the prefrontal cortex (24). Indeed, dopamine can increase BDNF
158 production in a concentration-dependent manner in hippocampal tissue and may
159 influence cognition (25).

160 This study aimed to examine the interaction between genetic background,
161 specifically brain health-related SNPs in a set of candidate genes (*BDNF*, *CREB1*,
162 *NTRK2*, *COMT* and *APOE*) and the effects of a 20-week exercise intervention on
163 primary brain health outcomes (i.e., intelligence, executive function [cognitive
164 flexibility, inhibition, and working memory], academic performance, hippocampal
165 volume) in children with OW/OB from the ActiveBrains RCT. Thus, we hypothesized
166 that the brain health-related genetic background would play a role in the effect of the
167 ActiveBrains exercise intervention on brain health outcomes.

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169 **2. Methods**

170 *2.1 Study design and participants*

171 From a total of 109 participants randomized in the ActiveBrains RCT (9, 26), 101
172 children with valid and complete genetic data were included in this study. The primary
173 aim of this project was to study the effects of the exercise program on brain health
174 outcomes (9). The inclusion criteria were: (i) to be children with OW/OB according to
175 the age- and sex-specific World Obesity Federation cut-off points (27); (ii) to be 8 to 11
176 years old; (iii) not to present neurological disorders or physical disabilities; (iv) for
177 girls, not to have started the menstruation at the beginning of the study; (v) for this
178 specific study to have blood samples and valid genotype data. The exclusion criteria
179 were: (i) to take medications that affect the function of the central nervous system; (ii)
180 to have any physical disabilities or neurological disorders that may limit exercise
181 performance; (iii) to be left-handed (due to brain differences in neuroimaging); (iv) to
182 report an attention-deficit/hyperactivity disorder. Body mass index (kg/m^2) was
183 calculated using body weight and height assessed with an electronic scale and a

184 stadiometer (Seca instruments, Germany, Ltd), while peak height velocity was
185 computed as an indicator of maturational status (28). The children's parents or guardians
186 gave their written informed consent for them to take part in the trial. The ActiveBrains
187 trial received approval from the University of Granada's ethics committee and was
188 registered on ClinicalTrials.gov (NCT02295072). This trial adhered to the CONSORT
189 (Consolidated Standards of Reporting Trials) guidelines (9). The entire pre- and post-
190 exercise data was gathered between November 21, 2014, and June 30, 2016. The
191 evaluation of all the brain health outcomes was described in detail previously (9, 26).

192 *2.2 Exercise intervention*

193 The exercise group was instructed to perform at least three of the five provided
194 supervised exercise sessions each week (in total 20 weeks). Sessions lasted 90 minutes
195 (including 60 minutes of aerobic exercise and 30 minutes of resistance training).
196 Exercise sessions were built around games and other enjoyable activities to increase
197 motivation and adherence. The adherence was recorded as the number of sessions
198 attended vs. the total number of sessions recommended in the program and expressed in
199 percentage (6 participants were removed from the per-protocol analyses for the low
200 attendance to the exercise program, i.e., <70% of the 3 recommended sessions/week)
201 (9). The participants in the control group carried on with their regular activities of daily
202 life. At the beginning of the study, information on healthy eating and physical activity
203 recommendations were given to both the control and exercise groups. The 20-week
204 exercise intervention has been described in detail in our previous study (9).

205 *2.3 Blood sampling and molecular analyses*

206 Blood samples were obtained after an overnight fast of 12 h between 8:30- 10:30
207 AM. at the hospital. Ethylenediamine tetraacetic acid-filled (EDTA) tubes were used to

208 collect blood, which was subsequently centrifuged for 10 minutes at 4°C with 1000 g of
209 force. Leukocytes were isolated, aliquoted, and kept at -80°C for SNPs analyses.

210 2.3.1 Genotyping

211 Six brain health-related SNPs (rs6265, rs2253206, rs2289656, rs4680, rs429358,
212 rs7412) located in 5 genes (*BDNF*, *CREB1*, *NTRK2*, *COMT* and *APOE*) were
213 genotyped. DNA genotyping was performed using TaqMan® Genotyping Master Mix
214 (Applied Biosystems, USA) for real-time polymerase chain reaction (RT-PCR). Assay
215 ID for each SNP were: rs6265 no. C__11592758_10, rs2253206 no. C__2859107_10,
216 rs2289656 no. C__15882271_20, rs4680 no. C__25746809_50, rs429358 no.
217 C__3084793_20, rs7412 no. C__904973_10. Allelic discrimination assays were
218 carried out in a QuantiStudio 6 Flex Fast Real-Time PCR System (Applied Biosystems,
219 USA). Results were read using QuantStudio™ Real-Time PCR Software, Version 1.3
220 (Life Technologies, USA). The six SNPs (rs6265, rs2253206, rs2289656, rs4680,
221 rs429358, rs7412) were considered for computing the genetic score. Each SNP was
222 coded as follows: the low-response allele homozygote was assigned 0, heterozygote
223 received 1, and homozygote for the high-response allele was assigned 2 (29). The
224 number was assigned based on information provided by the scientific literature, for
225 details see **Table S1**. The theoretical range of the score was from 0 (no beneficial
226 alleles) to 12 (two copies of the beneficial alleles) (29). In order to have a balanced
227 sample size in different subgroups, the median value of this genetic score, i.e., 6
228 beneficial alleles, was used to classify participants into two subgroups, “favorable” (≥ 6
229 beneficial alleles) or “unfavorable” (< 6 beneficial alleles) genetic profile.

230 2.4 Intelligence

231 Intelligence was measured using the Spanish version of the Kaufman Brief
232 Intelligence Test (K-BIT) (9). Experienced evaluators individually administered the K-
233 BIT, and the different scores were calculated. K-BIT includes vocabulary (assess word
234 knowledge using pictures that answer a question or illustrate a word) and matrices sub-
235 tests (evaluate the kid's ability to make visual analogies spatial relationships).
236 Crystallized intelligence score was obtained from the vocabulary sub-tests, while fluid
237 intelligence score was estimated from the matrices sub-tests. A total intelligence score
238 was calculated using crystallized and fluid intelligence scores.

239 *2.5 Executive function*

240 *2.5.1 Cognitive flexibility*

241 Cognitive flexibility was measured using the Design Fluency Test and The Trail
242 Making Test (9, 30, 31). The Design Fluency Test is composed of three different
243 conditions (filled dots, empty dots, and switching), each lasting 1 minute (in total 3
244 minutes). Children were instructed to connect dots using four straight lines to design as
245 many novel shapes/designs as possible during the abovementioned period. The total
246 number of correct designs in the three conditions was computed in one single variable,
247 so that higher values indicate a better cognitive flexibility performance.

248 The Trail Making Test includes five different conditions, but in the current study
249 only the condition 2 and condition 4 were used (hereinafter called Part A and Part B,
250 respectively). Regarding Part A, children had to draw lines to connect numbers 1–25
251 following an ascending order and try to be as fast as possible (no more than 2.5 minutes
252 to finish the part A). In Part B, children had to draw a line to connect the numbers
253 (numerically) and the letters (alphabetically), switching each time from a number to a
254 letter in consecutive order (e.g., 1–A–2–B–3–C, and so on). The maximum time to

255 complete the part B was 4 minutes. A smaller part B – part A difference (seconds)
256 indicated better cognitive flexibility. We computed a composite z-score for cognitive
257 flexibility which was calculated as the re-normalized mean of the z-scores for the
258 Design Fluency Test and Trail Making Test.

259 *2.5.2 Inhibition*

260 Inhibition was assessed using a modified version of the Stroop test (9). The
261 condition 1 and condition 3 were used in this study. Condition 1 consisted of naming
262 the color of filled rectangles. Regarding condition 3, color-words were printed in a color
263 that differs from their meaning (e.g., the word “orange” printed in blue), while the task
264 consisted of avoiding reading the word and naming the color of the word (i.e., blue is
265 the correct answer in the above example). The variable inhibition was computed as the
266 difference between the completion time (seconds) in condition 3 and condition 1 (i.e.,
267 completion time in condition 3 – condition 1) (9). For analytical purposes, the variable
268 inhibition was reverted (i.e., it was multiplied by -1), so that higher values were related
269 to better cognitive performance.

270 *2.5.3 Working memory*

271 A modified version of the Delayed NonMatch-to-Sample (DNMS) computerized
272 task was used to evaluate working memory (9). A total of 16 practice trials were
273 presented on a computer screen using E-Prime software (Psychology Software Tools,
274 Pittsburgh, PA), followed by 140 experimental trials in 5 different blocks. Each trial had
275 two phases (choice and sample) and high and low memory loads. The high working
276 memory load (100 trials) was used for the current study. Participants were required to
277 memorize a set of four different sequential stimuli (Pokémon cartoons) as part of the
278 pre-target phase. During the selection phase following the last stimulus, two distinct

279 Pokémon were presented. Participants were instructed to choose the one that had not
280 been exhibited before. Working memory was measured using response accuracy (%)
281 under high load. Higher response accuracy denoted improved working memory
282 capabilities.

283 *2.6 Academic performance*

284 Academic performance was reported using the Spanish version of the
285 Woodcock-Johnson III Tests of Achievement (9). Different academic tests (reading,
286 mathematics, oral language, written language, social sciences, and humanities) were
287 individually performed by children in a session of 100-120 min. All academic tests
288 included in the Woodcock-Johnson III battery were corrected by two independent
289 researchers and processed in the Compuscore and profile software version 3.1
290 (Riverside Publishing Company, Itasca, IL, USA). Academic performance scores for
291 reading, mathematics, writing, academic skills, academic fluency, problem solving, and
292 total academic performance were computed. For more detailed information about
293 different academic components and scores calculations, please see <https://n9.cl/zsikj> and
294 Ortega *et al.*, (9).

295 *2.7 Hippocampal volume*

296 Hippocampal volume was measured with the FMRIB's Integrated Registration
297 and Segmentation Tool (FIRST) in FMRIB's Software Library (FSL) version 5.0.7. The
298 tool FIRST uses a Bayesian framework from morphological brain models obtained from
299 the Center for Morphometric Analysis, Massachusetts General Hospital, Boston, MA,
300 USA. Brain volume analyses were reported in detail elsewhere (9).

301 *2.8 Statistical analyses*

302 Statistical analyses were carried out using the SPSS software (Version 22.0,
303 IBM Corp., Armonk, NY, USA). Since we were more interested in the efficacy than the
304 effectiveness of our exercise intervention (i.e., in the effects on brain health
305 outcomes when exercise was actually done, that was to attend to at least 70% of
306 program'sd sessions), the main findings were derived from the per-protocol analyses. In
307 addition, we report the results using the intention-to-treat principle which include all
308 participants initially randomized in the analysis. For this purpose, multiple imputation
309 of missing values was applied using the predictive mean matching approach, for more
310 details see our previous publication (9). Overall, dropouts and non-dropouts did not
311 differ in the primary study outcomes (as described in the main article of the trial (9)).

312 Two-way analysis of covariance (ANCOVA) was performed to explore the
313 interaction between the genetic score and the effects of the exercise intervention on
314 brain health indicators. The model included: factor 1, genetic score (0 = unfavorable
315 genetic profile; 1 = favorable genetic profile); factor 2, group (0 = control; 1 =
316 intervention); outcome (post-intervention values); covariable (baseline values of the
317 outcome studied). P-value <0.1 was considered indicative of a potential gene x exercise
318 interaction, which was further explored in separate analyses by genetic sub-groups. This
319 study was powered to test the effects of the intervention (control vs. exercise) in the
320 whole sample; therefore, these gene-group interaction analyses were exploratory, and
321 we considered that there was certain evidence of interaction when $P < 0.1$.

322 Subsequently, one-way ANCOVA (factor: group [0 = control; 1 = intervention];
323 outcome [post-intervention values]; covariable [baseline values of the outcome studied])
324 was performed to report mean differences of post-intervention values (adjusted by
325 baseline values) of brain outcomes between exercise and control groups (9), separately
326 for each specific genetic profile (i.e., “favorable” and “unfavorable” genetic profile).

327 For intervention effects, we kept the standard 5% alpha error (i.e. P-value <0.05) for
328 consistency with the reporting of the intervention effects in this trial (9), yet we are
329 aware that by splitting the sample into two genetic groups the power was markedly
330 reduced and only relatively large effect sizes will be flagged as significant. In addition,
331 due to the high number of outcomes, we performed multiple hypothesis testing
332 corrections, i.e. false discovery rate [FDR] Benajmini-Hochberg procedure, in line with
333 the primary paper (9). The standardized effects of the exercise intervention on brain
334 health outcomes were presented using Z-scores of change (9). It shows how many
335 standard deviations (SDs) of the postexercise program values changed from the baseline
336 mean and SD values. This effect size can be interpreted as follows: a small effect size
337 (0.2 SDs), a medium effect size (0.5 SDs), and a large effect size (0.8 SDs) (9).

338 In addition, we explored the individual changes in brain health outcomes that
339 showed an interaction $P < 0.1$. We reported the % of children that showed a meaningful
340 change (> 0.2 Cohen's d) for brain health outcomes with statistically significant
341 differences of % between subgroups (chi-square test). We performed exploratory sex
342 interaction analyses.

343

344 **3. Results**

345 The baseline characteristics of the participants stratified by genetic profile (i.e.,
346 “favorable” or “unfavorable” genetic profile) and group (i.e., exercise or control) are
347 presented in **Table 1**. The genotype frequencies for each SNP were in Hardy-Weinberg
348 equilibrium (**Table 2**). Per-protocol analyses showed an interaction of the genetic score
349 with cognitive flexibility, as measured by Trail Making Test and the composite score,
350 working memory, academic skills, reading and writing (all $P < 0.1$) (**Table 3**). Exercise

351 only increased cognitive flexibility and academic skills in children presenting a
352 “favorable” genetic profile (mean z score, 0.41-0.67 [95% CI 0.11 to 1.18]; **Figure 1**
353 **[Panel A]** and **Table 3**), yet not in the rest of brain health outcomes. Among children
354 presenting an “unfavorable” genetic profile, exercise only improved working memory
355 and writing (mean z score, 0.47 [95% CI 0.04 to 0.90] and mean z score, 0.55 [95% CI
356 0.04 to 1.05]; **Figure 1 [Panel A]** and **Table 3**). An interaction effect was reported for
357 reading ($P < 0.1$; **Table 3**), but the effect of exercise intervention was not statistically
358 significant in both subgroups computed using the genetic score ($P > 0.05$; **Table 3**).
359 Reading showed a trend to improve in the “favorable” genetic profile but not in the
360 “unfavorable” group (**Figure 1 [Panel A]** and **Table 3**). All the results described before
361 were consistent when using intention-to-treat instead of per-protocol analyses (**Figure 1**
362 **[Panel B]**; **Table S2**, except for the no interaction of the genetic score with writing and
363 reading ($P > 0.1$). The significant effects on cognitive flexibility and academic skills
364 remained consistent after correction for multiple comparisons ($FDR < 0.05$), but the
365 effects on working memory and writing became non-significant ($FDR > 0.05$).

366 Regarding the individual changes in brain health outcomes, only cognitive
367 flexibility showed statistically significant differences in the % of participants that
368 reported a meaningful change among subgroups (**Figure 2**). An individual response
369 analysis among the children with a “favorable” genetic profile showed that 62% of them
370 had a meaningful (≥ 0.2 Cohen *d*) increase in cognitive flexibility in the exercise group
371 compared with only 25% in the control group (**Figure 2**). This result was consistent in
372 per-protocol and intention-to-treat analyses ($P = 0.01$, $P = 0.03$ respectively) (**Figure 2**).
373 We did not find differences at the individual response level for the other variables that
374 showed a gene*exercise interaction P -value < 0.1 (**Figures S1-4**). For exploratory
375 purposes, we tested whether the most consistent and robust gene*exercise interaction

376 observed in cognitive flexibility was also consistent in boys and girls, so we analyzed
377 the sex*gene*exercise interaction on this primary outcome and observed no evidence of
378 sex having a moderating effect (sex interaction P=0.31, P=0.15 for per-protocol and
379 intention-to-treat analyses, respectively). We also explored the sex interactions for the
380 rest of the outcomes studied, finding no interactions, except for a few academic
381 outcomes. However, separate analyses by sex would not be meaningful since the sample
382 would be stratified too much, i.e., in 8 groups (2 sexes * 2 genetic groups * 2
383 intervention groups) having many of them less than 10 participants per group.

384

385 **4. Discussion**

386 This study showed, for the first time, the role of a genetic background, namely a
387 combination score of polymorphisms in brain health-related candidate genes, on the
388 response to a 20-week exercise intervention in a broad set of brain health indicators in
389 children with OW/OB. The genetic score composed of 6 candidate (selected based on
390 evidence) SNPs located in genes that encode proteins with important role in the brain
391 health [*BDNF*, *CREB1*, *NTRK2*, *COMT*, and *APOE*] modulated the exercise-induced
392 response on cognitive flexibility, working memory, and academic performance. The
393 significant effect persisted after correction for multiple comparisons for cognitive
394 flexibility and academic skills, but not for working memory or writing. Our findings
395 suggest that the differential response to exercise according to the genetic predisposition
396 was especially consistent and robust for cognitive flexibility in the analyses conducted
397 both at group and individual level, as well as in per-protocol and intention-to-treat
398 analyses. The beneficial effect of the genetic profile on the response to exercise in
399 cognitive flexibility seems to be also consistent in boys and girls, as evidenced by the
400 non-interaction by sex observed.

401 The ActiveBrains RCT reported an improvement on cognitive flexibility after a
402 20-week exercise intervention in children with OW/OB (9). The current study adds that
403 the abovementioned improvements were observed only in children with a "favorable"
404 genetic profile. Interestingly, studies performed in animal models demonstrated that
405 cognitive flexibility impairment was related to decreased BDNF production in the
406 frontal cortex (32). In this regard, exercise training increases BDNF protein levels in the
407 plasma of children and adults, benefiting brain health (18, 20). Also, a single bout of
408 high-intensity exercise improved cognitive flexibility in healthy young adults,
409 specifically the performance on the Trail Making test, in parallel to an improvement of
410 BDNF protein levels in circulation(33). Also, the expression levels of other proteins
411 such as CREB1 and NTRK2 (BDNF receptor) influenced by SNPs included in our
412 genetic score can regulate BDNF protein expression and function (16, 21), affecting
413 cognitive flexibility. Furthermore, academic skills that are regulated by BDNF (34)
414 improved only after the exercise intervention in children with a “favorable” genetic
415 profile. We can hypothesize that the genetic score can modulate the expression of the
416 abovementioned proteins in response to exercise, contributing to improve cognitive
417 flexibility. Importantly, crystallized intelligence was the brain outcome with the largest
418 effect size in the ActiveBrains RCT (9). In the current study, we did not observe an
419 interaction between the genetic score and crystallized intelligence; however, the effect
420 size was larger in the “favorable” compared to the “unfavorable” genetic subgroup
421 **(Table 3)**.

422 The ActiveBrains RCT has shown that working memory and writing did not
423 change after a 20-week exercise intervention (9). Interestingly, in the current study, we
424 observed that exercise improved working memory and writing in children classified as
425 having “unfavorable” genetic profiles. However, no significant effects were observed

426 after correction for multiple comparisons, nor significant differences were observed in
427 the individual response to exercise in both genetic groups for these two outcomes,
428 which suggest these findings are not consistent or robust, and not much attention should
429 be paid on them. Importantly, another aspect that elucidates the complex relationship
430 between genetic variants, exercise, and cognition is that a given allele for the same
431 candidate SNP could be beneficial for some aspects of cognition and hampering for
432 others. As an illustration, the *COMT* gene encodes an enzyme (Catechol-O-
433 methyltransferase) that regulates dopamine levels and time of action in the prefrontal
434 cortex. The change of Met for Val allele [*COMT* rs4680] results in a three- to four-fold
435 decreased activity of *COMT* activity that contribute to an extended dopamine action in
436 the prefrontal cortex (24, 35). Interestingly, high levels of dopamine in prefrontal regions
437 (observed in carriers of the Met allele in *COMT* rs4680) may be beneficial for working
438 memory but a disadvantage for cognitive flexibility (24, 36, 37).

439 Our study has some limitations that should be acknowledged. Complex
440 phenotypes such as brain health indicators are influenced by several genes with small
441 effect sizes, which in concert may exert an effect (12, 15). Thus, our target approach
442 would need to be tested by whole genome-wide analyses and in larger RCTs.
443 Importantly, we carefully selected six SNPs based on the scientific literature, although
444 most of the studies relating these genetic variants to brain health indicators were
445 performed in adults, older adults and patients with neurological diseases. Therefore,
446 there was almost inexistent evidence on relevant genes derived from studies in pediatric
447 populations. Future RCTs should explore the genome unbiasedly by performing whole
448 genome-wide analyses integrated with proteomics data in larger cohorts of children.
449 Furthermore, studies with larger sample size and power should confirm or contrast our
450 findings.

451 **5. Conclusion**

452 Our findings revealed that the studied genetic score using brain health-related
453 polymorphisms (selected based on previous scientific literature) influenced the
454 response to exercise in cognitive flexibility and academic skills. Notably, the impact of
455 the genetic score was more consistent and pronounced on cognitive flexibility compared
456 to other outcomes, showing that children with a more favorable genetic profile
457 improved more their cognitive flexibility as a result of the exercise intervention than
458 their peers with a “less favorable” genetic profile. To further enhance our understanding
459 of the genetic factors influencing brain health in response to exercise training, future
460 randomized controlled trials should employ whole genome analyses without bias, yet
461 that requires very large sample size and power, or alternatively aggregation of data from
462 different trials.

463

464 **SUPPLEMENTAL MATERIAL:** The supplementary tables and figures were deposited
465 to a public access data repository
466 (figshare, <https://doi.org/10.6084/m9.figshare.23884197>)

467 *Supplemental Table S1*

468 *Supplemental Table S2*

469 *Supplemental Figure S1*

470 *Supplemental Figure S2*

471 *Supplemental Figure S3*

472 *Supplemental Figure S4*

473

474 **DATA AND RESOURCE AVAILABILITY**

475 We did not obtain children’s parents consent to widely share the data nor was it
476 included in the IRB protocol.

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502 **DISCLOSURES**

503 The authors declare that they have no competing interests.

504 **AUTHORS' CONTRIBUTIONS**

505 APF, IEC, JMG, LVT, FJO, SA, FBO participated in the manuscript design/conception;
506 APF, IEC, JMG, LVT, JGC, SA, FBO data acquisition, APF and FBO data analysis,
507 APF, JMG and JGC figure generation, APF first drafting of the manuscript; APF, IEC,
508 JMG, LVT, FJOP, JGC, SRA, IL, JRR, SA, FBO critically revised the manuscript for
509 important intellectual content. All authors have read and approved the final version of
510 the manuscript, and agree with the order of presentation of the authors.

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685 **FIGURE LEGENDS**

686 **Figure 1.** Per-protocol (A) and intention-to-treat (B) effects of the ActiveBrains
687 exercise intervention on the intelligence, executive function, academic performance, and
688 brain structure by genetic profile. To simplify the interpretation of the results, the
689 Stroop Color-Word Test and Trail Making Test were inverted, i.e., a higher value
690 indicates a better performance. * indicates a significant gene x exercise interaction (p-
691 value < 0.1). Number of participants with valid data for each variable pre-and post-
692 intervention in the per-protocol analyses: Intelligence outcomes, Cognitive flexibility 1,
693 Inhibition (N=84; genetic “favorable” [21exercise and 28 control] and genetic
694 “unfavorable” [22 exercise and 13 control]), Cognitive flexibility 2, Cognitive
695 flexibility composite z-score (N=79; genetic “favorable” [21 exercise and 24 control]
696 and genetic “unfavorable” [22 exercise and 12 control]), working memory (N=81;
697 genetic “favorable” [19 exercise and 27 control] and genetic “unfavorable” [22 exercise
698 and 13 control]), Executive function composite z-score (N=77; genetic “favorable” [19
699 exercise and 24 control] and genetic “unfavorable” [22 exercise and 12 control]),
700 academic performance outcomes (N=83, genetic “favorable” [21 exercise and 27
701 control] and genetic “unfavorable” [22 exercise and 13 control]), hippocampal volume
702 (N=77; genetic “favorable” [20 exercise and 23 control] and genetic “unfavorable” [21
703 exercise and 13 control]).

704

705 **Figure 2.** Individual change distribution in cognitive flexibility (Panels A and B per-
706 protocol analyses and panels C and D intention-to-treat analyses) for both control and
707 exercise groups, and by genetic profile. Dashed lines indicate a meaningful increase
708 regarding baseline levels. P value from the chi-squared test. Number of participants
709 with valid data for cognitive flexibility composite z-score pre-and post-intervention in
710 the per-protocol analyses (N=79; genetic “favorable” [21 exercise and 24 control] and
711 genetic “unfavorable” [22 exercise and 12 control]). The standardized score of change
712 indicates how many standard deviations have the post-intervention values changed with
713 respect to the baseline mean and standard deviation. E.g., a 0.70 Z-score means that the
714 value at post-intervention is 0.70 standard deviations higher than the mean value at
715 baseline, indicating a positive change, with negative values indicating the opposite.

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Table 1. Descriptive baseline characteristics of the *ActiveBrains* participants by genetic profile and type of intervention.

	All		Favorable genetic profile		Unfavorable genetic profile		Control group		Exercise group	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Age (years)	101	10.03 ± 1.51	59	9.91 ± 1.24	42	10.19 ± 1.00	50	10.09 ± 1.16	51	9.96 ± 1.16
Sex										
Girls (n %)	41	59%	26	44%	15	36%	23	46%	18	35%
Boys (n %)	60	41%	33	56%	27	64%	27	54%	33	65%
Weight (kg)	101	55.93 ± 10.99	59	55.61 ± 10.72	42	55.38 ± 11.47	50	55.96 ± 9.42	51	55.90 ± 12.43
Height (cm)	101	143.91 ± 8.55	59	143.37 ± 8.55	42	144.68 ± 8.59	50	145.22 ± 7.99	51	142.63 ± 8.94
Body mass index (kg/m ²)	101	26.79 ± 3.51	59	26.83 ± 3.24	42	26.74 ± 3.89	50	26.42 ± 2.96	51	27.16 ± 3.97
Peak height velocity (years)	101	-2.28 ± 0.99	59	-2.32 ± 1.09	42	-2.23 ± 0.85	50	-2.13 ± 1.07	51	-2.43 ± 0.91
Wave of participation (%)										
First (n %)	16	15%	7	12%	9	21%	7	14%	9	18%
Second (n %)	45	45%	32	54%	13	31%	23	46%	22	43%
Third (n %)	40	40%	20	34%	20	48%	20	40%	20	39%
Intelligence										
Crystallized intelligence (typical punctuation)	101	103.15 ± 13.26	59	103.64 ± 14.07	42	102.45 ± 12.15	50	102.58 ± 12.00	51	103.71 ± 14.48
Fluid intelligence (typical punctuation)	101	97.87 ± 13.17	59	97.08 ± 13.57	42	98.98 ± 12.65	50	98.84 ± 12.23	51	96.92 ± 14.08

Total intelligence (typical punctuation)	101	98.22 ± 12.65	59	98.10 ± 13.56	42	98.38 ± 11.39	50	98.42 ± 11.85	51	98.02 ± 11.49
Executive function										
Cognitive flexibility 1 (total correct designs)	101	19.75 ± 6.47	59	19.53 ± 6.03	42	20.07 ± 7.10	50	20.08 ± 6.95	51	19.43 ± 6.01
Cognitive flexibility 2 (sec)	101	90.99 ± 43.36	59	91.19 ± 39.46	42	90.72 ± 48.83	50	94.78 ± 44.91	51	87.27 ± 41.91
Cognitive flexibility composite z-score	101	0.01 ± 1.00	59	-0.04 ± 0.93	42	0.02 ± 1.11	50	-0.03 ± 1.06	51	0.00 ± 0.96
Inhibition (sec)	101	40.81 ± 17.41	59	43.46 ± 19.51	42	37.10 ± 13.27	50	41.16 ± 19.60	51	40.48 ± 15.15
Working memory (% response accuracy)	101	65.43 ± 16.58	59	63.35 ± 16.22	42	69.75 ± 16.30	50	62.50 ± 18.09	51	68.30 ± 14.56
Executive function composite z-score	101	0.01 ± 1.00	59	-0.14 ± 0.97	42	0.18 ± 1.101	50	-0.09 ± 1.13	51	0.07 ± 0.85
Academic performance (standard score)										
Academic skills	101	118.65 ± 15.31	59	115.07 ± 14.17	42	123.67 ± 15.59	50	116.86 ± 14.70	51	120.39 ± 15.83
Academic fluency	101	103.38 ± 11.42	59	102.41 ± 11.42	42	104.74 ± 11.41	50	102.52 ± 12.81	51	104.22 ± 9.92
Problem solving	101	99.56 ± 9.37	59	99.10 ± 10.06	42	100.21 ± 8.38	50	97.29 ± 9.04	51	101.78 ± 9.23
Reading	101	107.94 ± 12.58	59	106.14 ± 11.44	42	110.46 ± 13.77	50	105.71 ± 11.69	51	110.12 ± 13.14
Mathematics	101	101.80 ± 10.86	59	101.14 ± 11.32	42	102.73 ± 10.25	50	99.49 ± 10.51	51	104.06 ± 10.83
Writing	101	113.89 ± 12.31	59	110.91 ± 12.56	42	118.09 ± 10.75	50	113.45 ± 13.51	51	114.33 ± 11.13
Total academic performance	101	109.16 ± 11.66	59	107.17 ± 11.32	42	111.96 ± 11.70	50	107.19 ± 11.71	51	111.10 ± 11.40
Hippocampal volume (mm ³)	101	6997.62 ± 619.27	59	7015.80 ± 643.85	42	6972.07 ± 589.73	50	6967.46 ± 661.74	51	7027.18 ± 579.70

Values are expressed as means \pm standard deviations (SD), unless otherwise indicated. BDNF = Brain-derived neurotrophic factor. Intelligence outcomes (i.e., Crystallized, Fluid, and Total Intelligence) were measured by the Kaufman Brief Intelligence Test. Cognitive flexibility 1 was measured by the Design Fluency Test and expressed as number of total correct designs of the three conditions. Cognitive flexibility 2 was measured by the Trail Making Test and expressed as the total completion time (sec) of Part A subtracted from the total completion time (sec) of Part B. A smaller B – A difference score (sec) indicated better cognitive flexibility. Cognitive flexibility composite z-score was calculated as the re-normalized mean of the z-scores for Cognitive flexibility 1 and Cognitive flexibility 2. Inhibition was measured by the Stroop Color-Word Test. The inhibition score was obtained by subtracting condition 3 completion time – condition 1 completion time (sec). The lower the difference between tests' times, the better the performance was considered. Working memory was measured by the Delayed Non-Match-to sample task. Executive function composite z-score was calculated as the re-normalized mean of the z-scores for Cognitive flexibility, Inhibition, and Working memory. Academic performance was measured by the Spanish version of the Woodcock Johnson III Test of Achievement. Academic skills are the sum of components based on basic skills such as reading decoding, mathematics calculation, and spelling. Academic fluency is the sum of the components based on reading, calculation, and writing fluency. Problem solving is the sum of the components based on solving academic problems in reading, mathematics, and writing. Total academic performance is the overall measure of the academic performance based on reading, mathematics, and writing.

Table 2. Genotype and allele frequencies in genes analyzed in 101 children with overweight/obesity.

SNP	Gene	Genotype frequencies	Allele frequencies	X^2 Hardy-Weinberg equilibrium	Computation of the genotype to calculate the genetic score (for more details see Table S1)
rs6265	<i>BDNF</i>	CC (62; 61%) CT (32; 32%) TT (7; 7%)	p (C allele; 0.77) q (T allele; 0.23)	0.99	0 = TT; 1 = CT; 2 = CC
rs2253206	<i>CREB1</i>	GG (36; 36%) AG (53; 52%) AA (12; 12%)	p (G allele; 0.62) q (A allele; 0.38)	1.27	0 = GG; 1 = AG; 2 = AA
rs2289656	<i>NTRK2</i>	GG (69; 36%) AG (26; 52%) AA (6; 12%)	p (G allele; 0.81) q (A allele; 0.19)	2.49	0 = GG; 1 = AG; 2 = AA
rs4680	<i>COMT</i>	GG (31; 31%) AG (49; 48%) AA (21; 21%)	p (G allele; 0.55) q (A allele; 0.45)	0.04	0 = AA; 1 = AG; 2 = GG
rs429358	<i>APOE</i>	CC (0; 0%) CT (15; 15%) TT (86; 85%)	p (T allele; 0.93) q (C allele; 0.07)	0.65	0 = CC; 1 = CT; 2 = TT
rs7412	<i>APOE</i>	TT (1; 1%) CT (14; 14%) CC (86; 85%)	p (C allele; 0.93) q (T allele; 0.07)	0.25	0 = CC; 1 = CT; 2 = TT

BDNF, Brain-derived neurotrophic factor; CREB1, cAMP responsive element binding protein 1; NTRK2, tyrosine kinase receptor 2; COMT, Catechol-Omethyltransferase; APOE, apolipoprotein E; SNP, Single nucleotide polymorphism. The term p represents the frequency of the homozygous dominant genotype, while the term q indicates the frequency of the homozygous recessive genotype. $X^2 > 0.05$ shows that genotype distributions in children with overweight/obesity were in Hardy-Weinberg equilibrium

Table 3 Effects of the ActiveBrains exercise intervention (per-protocol analyses) on z-score post-intervention outcomes (Z-score of change from baseline) by genetic favourable/unfavourable profiles.

	Favorable genetic profile		Unfavorable genetic profile		Intervention vs. Control differences by genetic profile		Gene <i>x</i> exercise interaction (p-value)
	Intervention group	Control group	Intervention group	Control group	Genetic favourable	Genetic unfavourable	
<i>Intelligence</i>							
Crystallized intelligence	0.74 (0.46, 1.02)	-0.10 (-0.34, 0.15)	0.42 (0.21, 0.62)	-0.15 (-0.42, 0.11)	0.84 (0.47, 1.21)	0.57 (0.24, 0.90)	0.31
Fluid intelligence	0.40 (0.13, 0.79)	0.12 (-0.22, 0.45)	0.39 (0.01, 0.78)	0.24 (-0.27, 0.75)	0.28 (-0.23, 0.80)	0.15 (-0.50, 0.81)	0.73
Total intelligence	0.71 (0.40, 1.02)	0.01 (-0.27, 0.27)	0.57 (0.32, 0.82)	0.01 (-0.31, 0.34)	0.70 (0.29, 1.12)	0.56 (0.15, 0.97)	0.53
<i>Executive function</i>							
Cognitive flexibility 1	0.63 (0.38, 0.89)	0.09 (-0.13, 0.32)	0.70 (0.31, 1.09)	0.39 (-0.12, 0.91)	0.54 (0.19, 0.88)	0.31 (-0.35, 0.96)	0.44
Cognitive flexibility 2	0.55 (0.18, 0.92)	-0.12 (-0.47, 0.22)	0.37 (0.03, 0.70)	0.61 (0.16, 1.07)	0.67 (0.16, 1.18)	-0.24 (-0.82, 0.32)	0.02*
Cognitive flexibility composite z-score	0.30 (0.04, 0.55)	-0.37 (-0.61, -0.14)	0.24 (-0.09, 0.56)	0.18 (-0.26, 0.62)	0.67 (0.32, 1.02)	0.06 (-0.49, 0.61)	0.05*

Inhibition	0.38 (0.01, 0.76)	0.27 (-0.05, 0.59)	0.64 (0.42, 0.87)	0.89 (0.60, 1.19)	0.11 (-0.38, 0.61)	-0.25 (-0.62, 0.13)	0.28
Working memory	-0.31 (-0.66, 0.02)	0.02 (-0.27, 0.30)	0.35 (0.09, 0.62)	-0.12 (-0.46, 0.23)	-0.33 (-0.78, 0.11)	0.47 (0.04, 0.90)	0.01*
Executive function composite z-score	0.01 (-0.34, 0.36)	-0.33 (-0.64, -0.02)	0.31 (0.08, 0.53)	0.24 (-0.06, 0.54)	0.34 (-0.13, 0.80)	0.07 (-0.30, 0.45)	0.40
<i>Academic performance</i>							
Academic skills	0.26 (0.04, 0.48)	-0.15 (-0.34, 0.05)	0.37 (0.14, 0.61)	0.46 (0.16, 0.77)	0.41 (0.11, 0.71)	-0.09 (-0.48, 0.29)	0.05*
Academic fluency	0.24 (-0.02, 0.49)	0.22 (-0.01, 0.44)	0.26 (0.01, 0.51)	0.12 (-0.21, 0.46)	0.02 (-0.33, 0.37)	0.14 (-0.29, 0.56)	0.58
Problem solving	0.29 (0.02, 0.56)	-0.06 (-0.29, 0.18)	0.48 (0.24, 0.72)	0.09 (-0.22, 0.41)	0.35 (-0.02, 0.71)	0.39 (-0.01, 0.79)	0.65
Reading	0.14 (-0.08, 0.35)	-0.11 (-0.30, 0.08)	0.32 (0.12, 0.53)	0.59 (0.33, 0.86)	0.25 (-0.04, 0.54)	-0.27 (-0.61, 0.07)	0.04*
Mathematics	0.24 (-0.04, 0.51)	-0.14 (-0.38, 0.10)	0.47 (0.17, 0.78)	0.33 (-0.07, 0.73)	0.38 (0.01, 0.75)	0.14 (-0.36, 0.54)	0.41
Writing	0.36 (0.16, 0.57)	0.27 (0.09, 0.45)	0.42 (0.11, 0.72)	-0.13 (-0.53, 0.27)	0.09 (-0.18, 0.37)	0.55 (0.04, 1.05)	0.09*
Total academic performance	0.24 (0.05, 0.43)	0.01 (-0.17, 0.17)	0.46 (0.26, 0.65)	0.37 (0.12, 0.62)	0.23 (-0.03, 0.50)	0.09 (-0.23, 0.40)	0.62

Brain structure

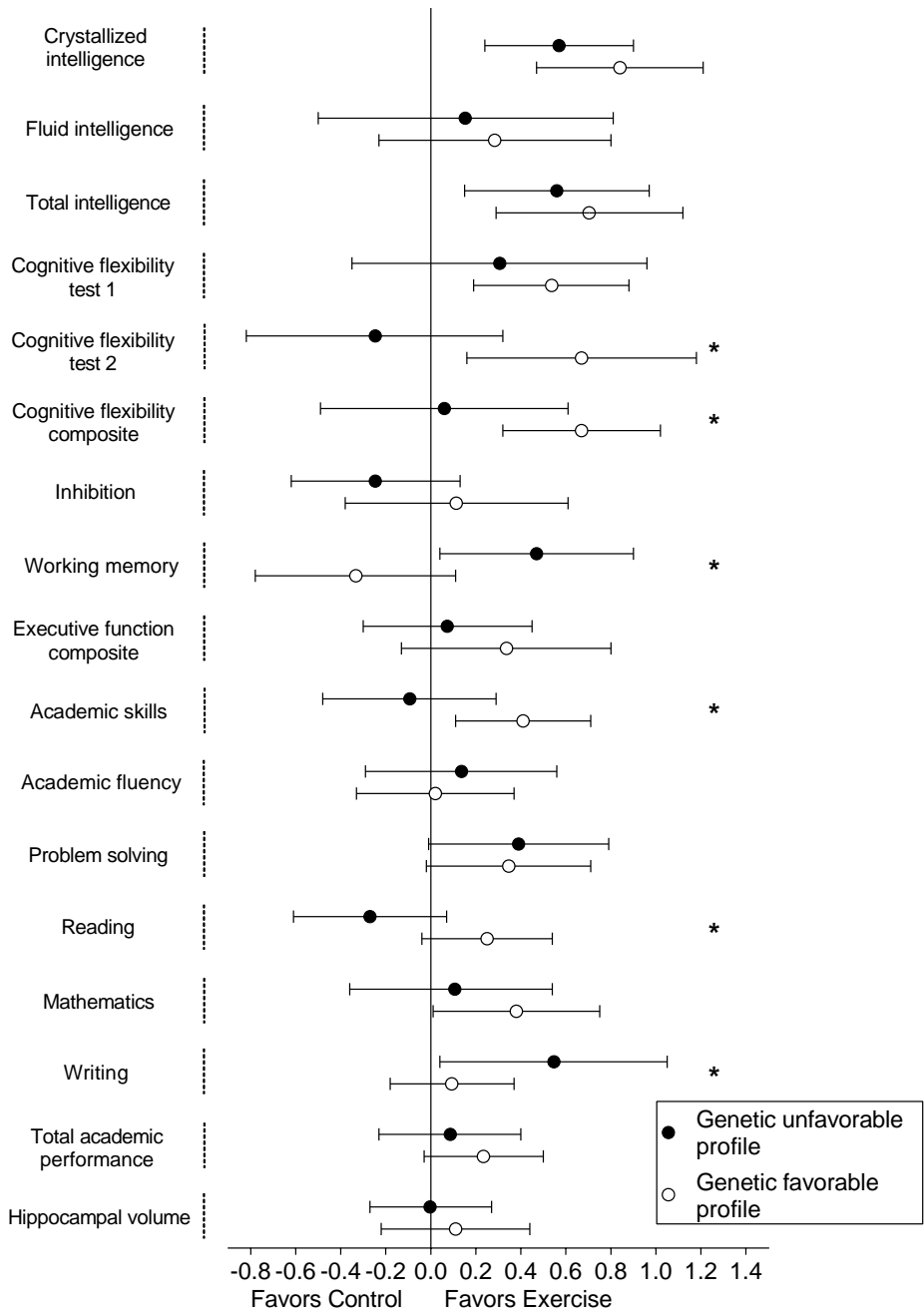
Hippocampal volume	0.37 (0.13, 0.61)	0.26 (0.04, 0.49)	0.11 (-0.05, 0.29)	0.12 (-0.09, 0.33)	0.11 (-0.22, 0.44)	-0.01 (-0.27, 0.27)	0.62
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Z-score values indicate how many standard deviations have the post-intervention values changed with respect to the baseline mean and standard deviation. E.g., a 0.70 Z-score means that the mean value at post-intervention is 0.70 standard deviations higher than the mean value at baseline, indicating a positive change, with negative values indicating the opposite. Values are expressed as mean (95% CI). Analyses were adjusted for baseline values. Gene \times exercise interaction p-value indicates the interaction between genetic predisposition profile and the effects induced by exercise intervention (ANCOVA analyses, factor 1: genotype profile [0 unfavorable genetic profile; 1 favorable genetic profile]; factor 2: group [0 control; 1 intervention]; outcome: post-intervention values; covariable: baseline outcomes). An asterisk (*) indicates a significant gene \times exercise interaction (p-value < 0.1). Bold numbers indicate $P < 0.05$ for the difference between intervention and control group for a specific genetic profile.

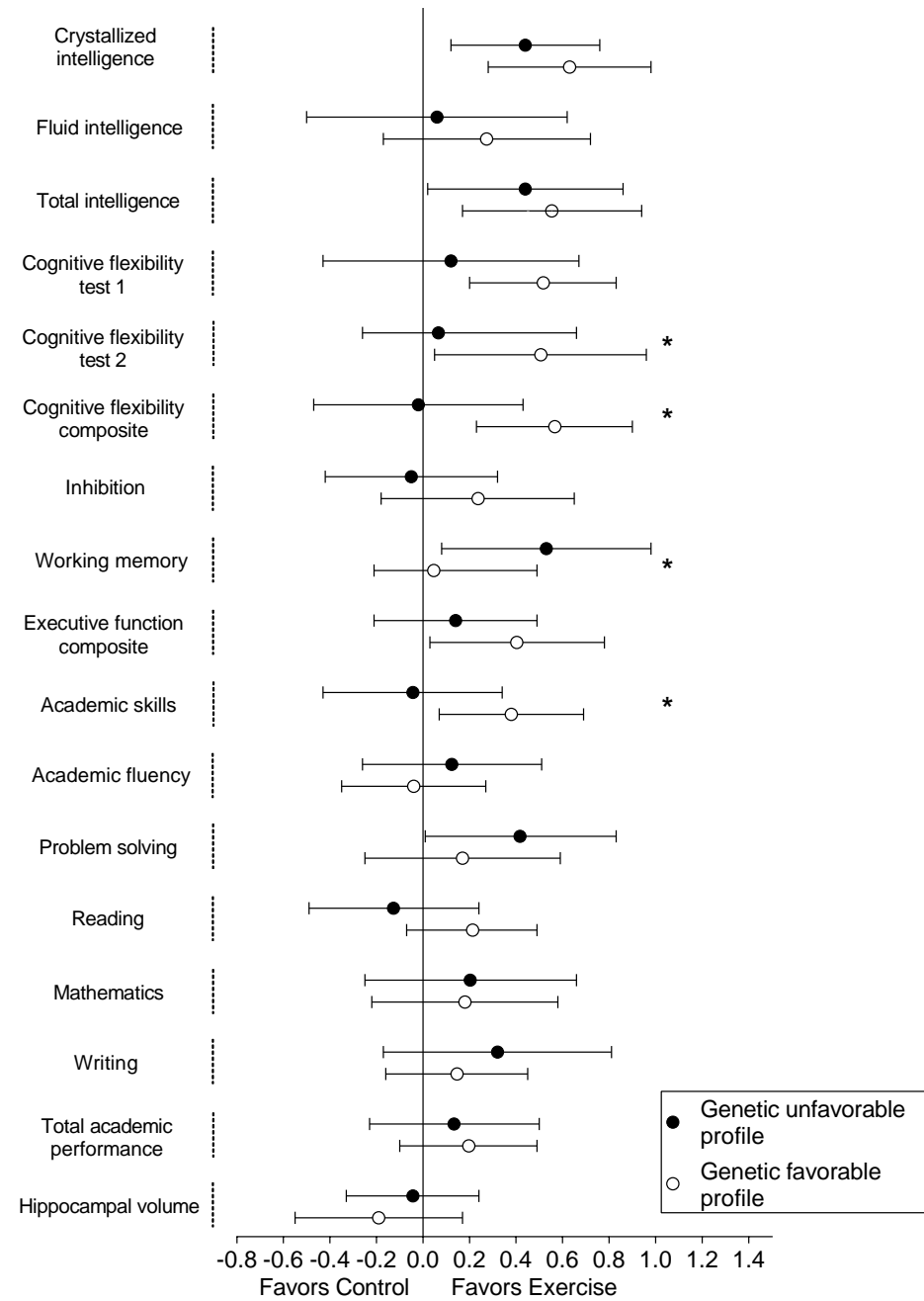
Intelligence outcomes (i.e., Crystallized, Fluid, and Total Intelligence) were measured by the Kaufman Brief Intelligence Test. Cognitive flexibility 1 was measured by the Design Fluency Test and expressed as number of total correct designs of the three conditions. Cognitive flexibility 2 was measured by the Trail Making Test and expressed as the total completion time (sec) of Part A subtracted from the total completion time (sec) of Part B. A smaller B – A difference score (sec) indicated better cognitive flexibility (to simplify the interpretation of the results, the Trail Making Test was inverted, i.e., a higher value indicates a better performance). Cognitive flexibility composite z-score was calculated as the re-normalized mean of the z-scores for Cognitive flexibility 1 and Cognitive flexibility 2. Inhibition was measured by the Stroop Color-Word Test. The inhibition score was obtained by subtracting condition 3 completion time – condition 1 completion time (sec). The lower the difference between tests' times, the better the performance was considered (to simplify the interpretation of the results, the Stroop Color-Word Test was inverted, i.e., a higher value indicates a better performance). Working memory was measured by the Delayed Non-Match-to sample task. Executive function composite z-score was calculated as the re-normalized mean of the z-scores for Cognitive flexibility, Inhibition, and Working memory. Academic performance was measured by the Spanish version of the Woodcock Johnson III Test of Achievement. Academic skills are the sum of components based on basic skills such as reading decoding, mathematics calculation, and spelling. Academic fluency is the sum of the components based on reading, calculation, and writing fluency. Problem solving is the sum of the components based on solving academic problems in reading, mathematics, and writing. Total academic performance is the overall measure of the academic performance based on reading, mathematics, and writing. Number of participants with valid data for each variable pre-and post-intervention: Intelligence outcomes, Cognitive flexibility 1, Inhibition (N=84; genetic “favorable” [21 exercise and 28 control] and genetic “unfavorable” [22 exercise and 13 control]), Cognitive flexibility 2, Cognitive flexibility composite z-score (N=79; genetic

“favorable” [21 exercise and 24 control] and genetic “unfavorable” [22 exercise and 12 control]), working memory (N=81; genetic “favorable” [19 exercise and 27 control] and genetic “unfavorable” [22 exercise and 13 control]), Executive function composite z-score (N=77; genetic “favorable” [19 exercise and 24 control] and genetic “unfavorable” [22 exercise and 12 control]), academic performance outcomes (N=83, genetic “favorable” [21 exercise and 27 control] and genetic “unfavorable” [22 exercise and 13 control]), hippocampal volume (N=77; genetic “favorable” [20 exercise and 23 control] and genetic “unfavorable” [21 exercise and 13 control]).

A) Per-protocol analyses



B) Intention-to-treat analyses



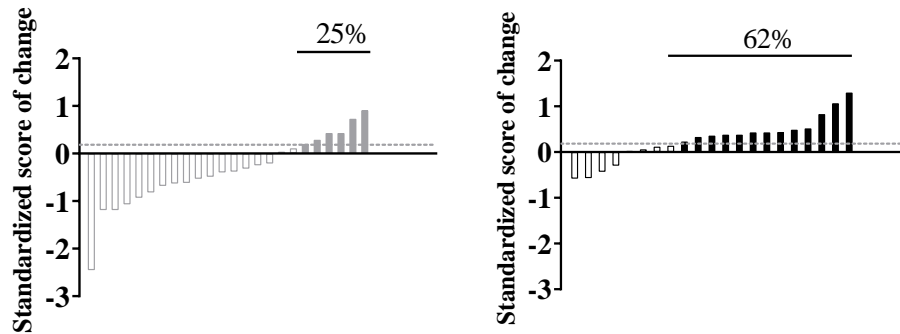
Individual responses on cognitive flexibility

Favorable genetic profile

Unfavorable genetic profile

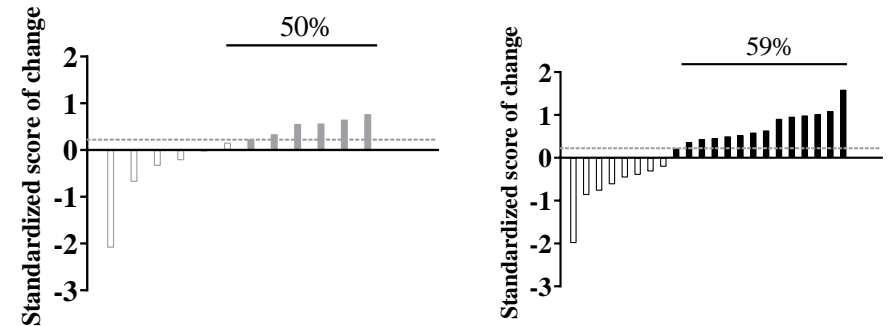
A) Per-protocol

$P = 0.01$



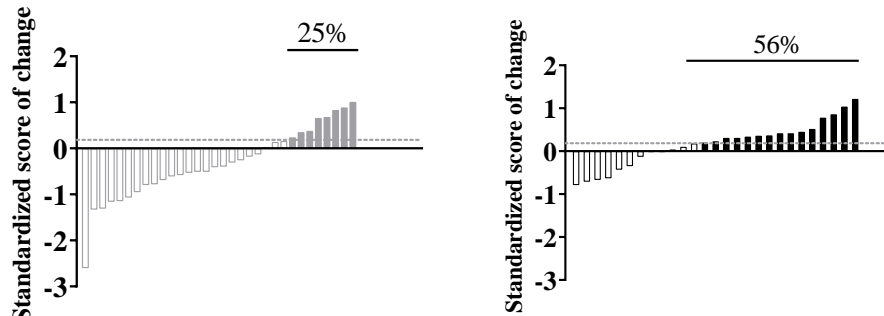
B) Per-protocol

$P = 0.72$



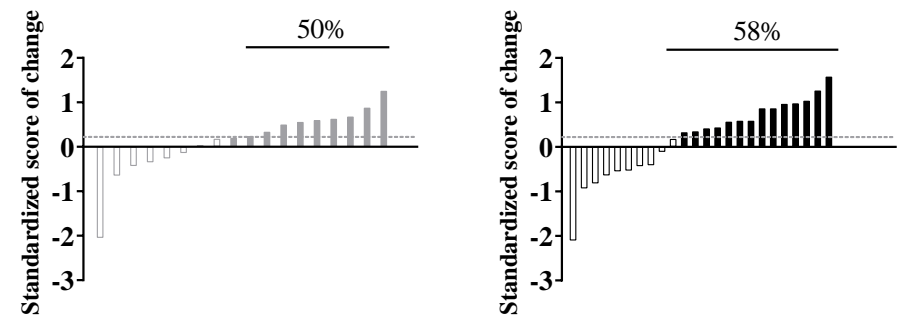
C) Intention-to-treat

$P = 0.03$



D) Intention-to-treat

$P = 0.76$



Control

Exercise

Control

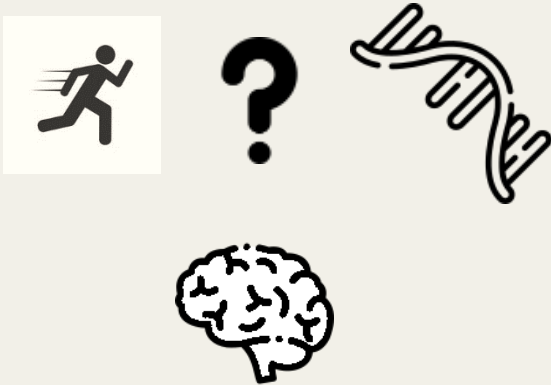
Exercise

■ Meaningful increase (Cohen's $d \geq 0.2$) □ Non-meaningful change (Cohen's $d < 0.2$)

Gene-exercise interaction on brain health in children with overweight/obesity: The ActiveBrains randomized controlled trial

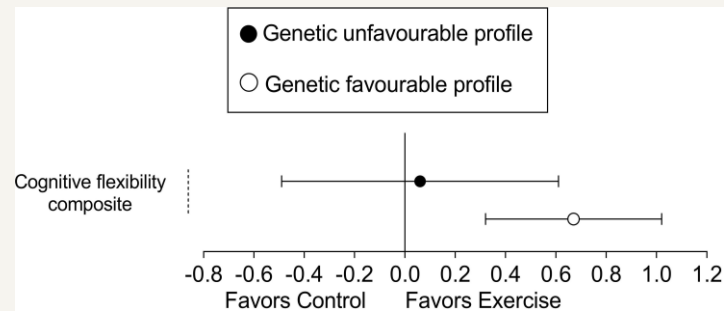
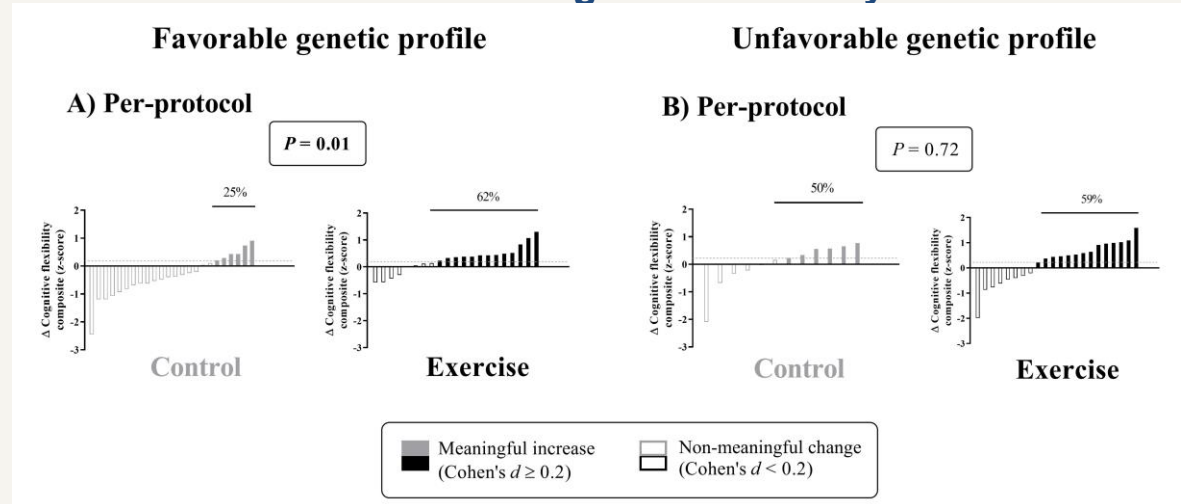
METHODS

101 children with overweight/obesity (8-11 years old) were randomly allocated to the exercise and control groups



We aimed to study the interaction between a genetic score and the effects of a 20-week exercise intervention on brain health outcomes

OUTCOME: Cognitive flexibility



CONCLUSION Our findings suggest that having a more “favorable” genetic profile makes children with overweight-obesity more responsive to exercise, particularly for cognitive flexibility.