Author(s): Plaza-Florido, Abel; Esteban-Cornejo, Irene; Mora-Gonzalez, Jose; Torres-Lopez, Lucia, V.; Osuna-Prieto, Francisco, J.; Gil-Cosano, Jose, J.; Radom-Aizik, Shlomit; Labayen, Idoia; Ruiz, Jonatan, R.; Altmäe, Signe; Ortega, Francisco, B.

Title: Gene–exercise interaction on brain health in children with overweight/obesity : the ActiveBrains randomized controlled trial

Year: 2023

Version: Accepted version (Final draft)

Please cite the original version:

Plaza-Florido, A., Esteban-Cornejo, I., Mora-Gonzalez, J., Torres-Lopez, L., Osuna-Prieto, F., Gil-Cosano, J., Radom-Aizik, S., Labayen, I., Ruiz, J., Altmäe, S., & Ortega, F. (2023). Gene–exercise interaction on brain health in children with overweight/obesity : the ActiveBrains randomized controlled trial. Journal of Applied Physiology, 135, 775-785. https://doi.org/10.1152/japplphysiol.00435.2023

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

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

Abel Plaza-Florido[1,2*], Irene Esteban-Cornejo[1,3,4], Jose Mora-Gonzalez[1], Lucia V. Torres-Lopez[1], Francisco J Osuna-Prieto[1,5], Jose J. Gil-Cosano[1,6], Shlomit Radom-Aizik[2], Idoia Labayen[3,7,8], Jonatan R Ruiz[1,3,4], Signe Altmäe[4,9,10], Francisco B. Ortega[1,3,11*]

[1] Department of Physical Education and Sports, Faculty of Sport Sciences, Sport and Health University Research Institute (iMUDS), University of Granada, Granada, Spain

[2] Pediatric Exercise and Genomics Research Center, Department of Pediatrics, School of Medicine, University of California at Irvine, Irvine, CA, USA.

[3] CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Granada, Spain

[4] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain.

[5] Instituto de Investigación Sanitaria Pere Virgili, University Hospital of Tarragona Joan XXIII, 43007 Tarragona, Spain.

[6] Department of Communication and Education, Universidad Loyola Andalucía, Dos Hermanas, Sevilla, Spain.

[7] Research Institute for Sustainability & Food Chain Innovation (IS-FOOD), Public University of Navarre. Department of Health Sciences, Public University of Navarre, Pamplona, Spain.

[8] IdiSNA, Navarra Institute for Health Research, Pamplona, Spain.

[9] Department of Biochemistry and Molecular Biology, Faculty of Sciences, University of Granada, Granada, Spain

[10] Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institutet, and Division of Obstetrics and Gynecology, Karolinska Universitetssjukhuset, Stockholm, Sweden.

[11] Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland.

*** Corresponding authors**: Abel Plaza-Florido (abeladrian@ugr.es); Francisco B. Ortega (ortegaf@ugr.es)

List of all the authors exactly as they should appear for PubMed indexing:

Plaza-Florido A, Esteban-Cornejo I, Mora-Gonzalez J, Torres-Lopez LV, Osuna-Prieto FJ, Gil-Cosano JJ, Radom-Aizik S, Labayen I, Ruiz JR, Altmäe S, Ortega FB

ABSTRACT

We investigated the interaction between a genetic score and an exercise intervention on brain health in children with overweight/obesity. One hundred one children with overweight/obesity (10.0 ± 1.5 years, 59% girls) were randomized into a 20-week combined exercise intervention or a control group. Several cognitive and academic outcomes were measured with validated tests. Hippocampal volume was quantified using magnetic resonance imaging. Six brain health-related polymorphisms (rs6265 [*BDNF*], rs2253206 [*CREB1*], rs2289656 [*NTRK2*], rs4680 [*COMT*], rs429358, and rs7412 [*APOE*]) were genotyped. Cognitive flexibility and academic skills improved significantly more in the exercise than in the control group only in the children with a "favorable" genetic profile (mean z score, 0.41-0.67 [95% CI 0.11 to 1.18], yet not in those with "less favorable" genetic profile. An individual response analysis showed that children responded to exercise in cognitive flexibility only in the "genetically favorable" group (i.e. 62% of them had a meaningful [≥0.2 Cohen *d*] increase in the exercise group compared with only 25% in the control group). This finding was consistent in per-protocol and intention-to-treat analyses (P=0.01 and P=0.03, respectively). The results were not significant or not consistent for the rest of outcomes studied. Our findings suggest that having a more favorable genetic profile makes children with overweight-obesity more responsive to exercise, particularly for cognitive flexibility.

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

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

1. Introduction

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

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

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

2. Methods

2.1 Study design and participants

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

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

2.2 Exercise intervention

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

2.3 Blood sampling and molecular analyses

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

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

2.3.1 Genotyping

Six brain health-related SNPs (rs6265, rs2253206, rs2289656, rs4680, rs429358, rs7412) located in 5 genes (*BDNF*, *CREB1*, *NTRK2*, *COMT* and *APOE*) were genotyped. DNA genotyping was performed using TaqMan® Genotyping Master Mix (Applied Biosystems, USA) for real-time polymerase chain reaction (RT-PCR). Assay ID for each SNP were: rs6265 no. C__11592758_10, rs2253206 no. C___2859107_10, rs2289656 no. C__15882271_20, rs4680 no. C__25746809_50, rs429358 no. C___3084793_20, rs7412 no. C____904973_10. Allelic discrimination assays were carried out in a QuantiStudio 6 Flex Fast Real-Time PCR System (Applied Biosystems, USA). Results were read using QuantStudio™ Real-Time PCR Software, Version 1.3 (Life Technologies, USA). The six SNPs (rs6265, rs2253206, rs2289656, rs4680, rs429358, rs7412) were considered for computing the genetic score. Each SNP was coded as follows: the low-response allele homozygote was assigned 0, heterozygote received 1, and homozygote for the high-response allele was assigned 2 (29). The number was assigned based on information provided by the scientific literature, for details see **Table S1**. The theoretical range of the score was from 0 (no beneficial alleles) to 12 (two copies of the beneficial alleles) (29). In order to have a balanced sample size in different subgroups, the median value of this genetic score, i.e., 6 beneficial alleles, was used to classify participants into two subgroups, “favorable” (≥6 beneficial alleles) or “unfavorable” (<6 beneficial alleles) genetic profile.

2.4 Intelligence

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

2.5 Executive function

2.5.1 Cognitive flexibility

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

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

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

2.5.2 Inhibition

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

2.5.3 Working memory

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

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

2.6 Academic performance

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

2.7 Hippocampal volume

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

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).

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

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

3. Results

The baseline characteristics of the participants stratified by genetic profile (i.e., "favorable" or "unfavorable" genetic profile) and group (i.e., exercise or control) are presented in **Table 1**. The genotype frequencies for each SNP were in Hardy-Weinberg equilibrium (**Table 2**). Per-protocol analyses showed an interaction of the genetic score with cognitive flexibility, as measured by Trail Making Test and the composite score, 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 signficant 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

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

4. Discussion

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

5. Conclusion

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

SUPPLEMENTAL MATERIAL: The supplementary tables and figures were deposit to a public access data repository (figshare, https://doi.org/10.6084/m9.figshare.23884197)

Supplemental Table S1
Supplemental Table S2
Supplemental Figure S1
Supplemental Figure S2
Supplemental Figure S3
Supplemental Figure S4

DATA AND RESOURCE AVAILABILITY

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

ACKNOWLEDGMENTS

We would like to thank the participants for their dedication and for making such a valuable contribution to science and space exploration

GRANTS

The project was mainly funded by the Spanish Ministry of Economy and Competitiveness (Reference DEP2013-47540, DEP2016-79512-R, and DEP2017-91544-EXP) and by the Andalusian Operational Programme supported with European Regional Development Funds (ERDF in English, FEDER in Spanish, projects ref: B-CTS-355-UGR18 and A-CTS-614-UGR20) and Junta de Andalucia (P20_00158). Grant Endo-Map PID2021-12728OB-100 funded by MCIN/AEI/10.13039/501100011033 and ERFD A way of making Europe; Grant RYC-2016-21199, funded by MCIN/AEI/10.13039/501100011033 and by ESF Investing in your future. This study was supported in part by the Pediatric Exercise and Genomics Research Center (PERC) Systems Biology Fund. A.P.F. is supported in part by NIH grant #: U01 TR002004 (REACH project). IE-C is supported by the Spanish Ministries of Economy and Competitiveness (RTI2018-095284-J-100), and Science and Innovation (RYC2019-027287-I). FJOP is supported by Margarita Salas program (Programa de recualificación del profesorado Universitario, Ministerio de Universidades). Additional support was obtained from the Unit of Excellence on EXERNET Research Network on Exercise and Health (DEP2005- 00046/ACTI; 09/UPB/19; 45/UPB/20; 27/UPB/21); Alicia Koplowitz Foundation. This study has been partially funded by the University of Granada, Plan Propio de Investigación 2016, Excellence actions: Units of Excellence; Unit of Excellence on Exercise and Health (UCEES), and by the Junta de Andalucía, Consejería de Conocimiento, Investigación y Universidades and European Regional Development Fund (ERDF), ref. SOMM17/6107/UGR.

DISCLOSURES

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

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

References

1. World Health Organization. Obesity and overweight: key facts. Accessed February 8, 2023 [Online]. [date unknown]. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.

2. **Bauer CCC**, **Moreno B**, **González-Santos L**, **Concha L**, **Barquera S**, **Barrios FA**. Child overweight and obesity are associated with reduced executive cognitive performance and brain alterations: A magnetic resonance imaging study in Mexican children. *Pediatric Obesity* 10: 196–204, 2015. doi: 10.1111/ijpo.241.

3. **Esteban-Cornejo I**, **Ortega FB**, **Catena**. Neural perspectives on cognitive control development during childhood and adolescence should take into account how the obesity affects brain development. *Acta Paediatrica* 107: 720–721, 2018. doi: 10.1111/ijlh.12426.

4. **Ou X**, **Andres A**, **Pivik RT**, **Cleves MA**, **Badger TM**. Brain gray and white matter differences in healthy normal weight and obese children. *Journal of Magnetic Resonance Imaging* 42: 1205–1213, 2015. doi: 10.1002/jmri.24912.

5. **Schaeffer DJ**, **Krafft CE**, **Schwarz NF**, **Chi L**, **Rodrigue AL**, **Pierce JE**, **Allison JD**, **Yanasak NE**, **Liu T**, **Davis CL**, **McDowell JE**. An 8-month exercise intervention alters frontotemporal white matter integrity in overweight children. *Psychophysiology* 51: 728–733, 2014. doi: 10.1111/psyp.12227.

6. **Krafft CE**, **Schwarz NF**, **Chi L**, **Weinberger AL**, **Schaeffer DJ**, **Pierce JE**, **Rodrigue AL**, **Yanasak NE**, **Miller PH**, **Tomporowski PD**, **Davis CL**, **McDowell JE**. An 8-month randomized controlled exercise trial alters brain activation during cognitive tasks in overweight children. *Obesity* 22: 232–242, 2014. doi: 10.1002/oby.20518.

7. **Davis CL**, **Tomporowski PD**, **McDowell JE**, **Austin BP**, **Miller PH**, **Yanasak NE**, **Allison JD**, **Naglieri JA**. Exercise Improves Executive Function and Achievement and Alters Brain Activation in Overweight Children: A Randomized, Controlled Trial. *Health Psychology* 30: 91–98, 2011. doi: 10.1037/a0021766.

8. **Krafft CE**, **Pierce JE**, **Schwarz NF**, **Chi L**, **Weinberger AL**, **Schaeffer DJ**, **Rodrigue AL**, **Camchong J**, **Allison JD**, **Yanasak NE**, **Liu T**, **Davis CL**, **McDowell JE**. An eight month randomized controlled exercise intervention alters resting state synchrony in overweight children. *Neuroscience* 256: 445–455, 2014. doi: 10.1016/j.neuroscience.2013.09.052.

9. **Ortega FB**, **Mora-Gonzalez J**, **Cadenas-Sanchez C**, **Esteban-Cornejo I**, **Migueles JH**, **Solis-Urra P**, **Verdejo-Román J**, **Rodriguez-Ayllon M**, **Molina-Garcia P**, **Ruiz JR**, **Martinez-Vizcaino V**, **Hillman CH**, **Erickson KI**, **Kramer AF**, **Labayen I**, **Catena A**. Effects

561 of an Exercise Program on Brain Health Outcomes for Children with Overweight or
562 Obesity: The ActiveBrains Randomized Clinical Trial. *JAMA Netw Open*,
563 1;5(8):e2227893, 2022. doi: 10.1001/jamanetworkopen.2022.27893

564 10. **Ross R**, **Goodpaster BH**, **Koch LG**, **Sarzynski MA**, **Kohrt WM**, **Johannsen NM**, **Skinner JS**,
565 **Castro A**, **Irving BA**, **Noland RC**, **Sparks LM**, **Spielmann G**, **Day AG**, **Pitsch W**, **Hopkins**
566 **WG**, **Bouchard C**. Precision exercise medicine: Understanding exercise response
567 variability. *British Journal of Sports Medicine* 53: 1141–1153, 2019. doi:
568 10.1136/bjsports-2018-100328.

569 11. **Yu F**, **Salisbury D**, **Mathiason MA**. Inter-individual differences in the responses to
570 aerobic exercise in Alzheimer's disease: Findings from the FIT-AD trial. *Journal of Sport*
571 *and Health Science* 10: 65–72, 2021. doi: 10.1016/j.jshs.2020.05.007.

572 12. **Sarzynski MA**, **Rice TK**, **Després JP**, **Pérusse L**, **Tremblay A**, **Stanforth PR**, **Tchernof A**,
573 **Barber JL**, **Falciani F**, **Clish C**, **Robbins JM**, **Ghosh S**, **Gerszten RE**, **Leon AS**, **Skinner JS**,
574 **Rao DC**, **Bouchard C**. The HERITAGE Family Study: A Review of the Effects of Exercise
575 Training on Cardiometabolic Health, with Insights into Molecular Transducers. 2022.

576 13. **Stroth S**, **Reinhardt RK**, **Thöne J**, **Hille K**, **Schneider M**, **Härtel S**, **Weidemann W**, **Bös K**,
577 **Spitzer M**. Impact of aerobic exercise training on cognitive functions and affect
578 associated to the COMT polymorphism in young adults. *Neurobiology of Learning and*
579 *Memory* 94: 364–372, 2010. doi: 10.1016/j.nlm.2010.08.003.

580 14. **Cramer SC**, **See J**, **Liu B**, **Edwardson M**, **Wang X**, **Radom-Aizik S**, **Haddad F**, **Shahbaba B**,
581 **Wolf SL**, **Dromerick AW**, **Winstein CJ**. Genetic Factors, Brain Atrophy, and Response to
582 Rehabilitation Therapy After Stroke. *Neurorehabilitation and Neural Repair* 36: 131–
583 139, 2022. doi: 10.1177/15459683211062899.

584 15. **Trampush JW**, **Yang MLZ**, **Yu J**, **Knowles E**, **Davies G**, **Liewald DC**, **Starr JM**, **Djurovic S**,
585 **Melle I**, **Sundet K**, **Christoforou A**, **Reinvang I**, **Derosse P**, **Lundervold AJ**, **Steen VM**,
586 **Espeseth T**, **Räikkönen K**, **Widen E**, **Palotie A**, **Eriksson JG**, **Giegling I**, **Konte B**, **Roussos**
587 **P**, **Giakoumaki S**, **Burdick KE**, **Payton A**, **Ollier W**, **Horan M**, **Chiba-Falek O**, **Attix DK**,
588 **Need AC**, **Cirulli ET**, **Voineskos AN**, **Stefanis NC**, **Avramopoulos D**, **Hatzimanolis A**,
589 **Arking DE**, **Smyrnis N**, **Bilder RM**, **Freimer NA**, **Cannon TD**, **London E**, **Poldrack RA**,
590 **Sabb FW**, **Congdon E**, **Conley ED**, **Scult MA**, **Dickinson D**, **Straub RE**, **Donohoe G**, **Morris**
591 **D**, **Corvin A**, **Gill M**, **Hariri AR**, **Weinberger DR**, **Pendleton N**, **Bitsios P**, **Rujescu D**, **Lahti**
592 **J**, **Le Hellard S**, **Keller MC**, **Andreassen OA**, **Deary IJ**, **Glahn DC**, **Malhotra AK**, **Lencz T**.
593 GWAS meta-analysis reveals novel loci and genetic correlates for general cognitive
594 function: A report from the COGENT consortium. *Molecular Psychiatry* 22: 336–345,
595 2017. doi: 10.1038/mp.2016.244.

596 16. **Wang CS**, **Kavalali ET**, **Monteggia LM**. BDNF signaling in context: From synaptic
597 regulation to psychiatric disorders. *Cell* 185: 62–76, 2022. doi:
598 10.1016/j.cell.2021.12.003.

599 17. **Miranda M**, **Morici JF**, **Zanoni MB**, **Bekinschtein P**. Brain-Derived Neurotrophic Factor:
600 A Key Molecule for Memory in the Healthy and the Pathological Brain. *Frontiers in*
601 *Cellular Neuroscience* 13: 1–25, 2019. doi: 10.3389/fncel.2019.00363.

602 18. **Ruiz-González D**, **Hernández-Martínez A**, **Valenzuela PL**, **Morales JS**, **Soriano-**
603 **Maldonado A**. Effects of physical exercise on plasma brain-derived neurotrophic factor

604 in neurodegenerative disorders: A systematic review and meta-analysis of randomized
605 controlled trials. *Neuroscience and Biobehavioral Reviews* 128: 394–405, 2021. doi:
606 10.1016/j.neubiorev.2021.05.025.

607 19. **Sandrini L**, **Di Minno A**, **Amadio P**, **Ieraci A**, **Tremoli E**, **Barbieri SS**. Association between
608 obesity and circulating brain-derived neurotrophic factor (BDNF) levels: Systematic
609 review of literature and meta-analysis. *International Journal of Molecular Sciences* 19,
610 2018. doi: 10.3390/ijms19082281.

611 20. **de Menezes-Junior FJ**, **Jesus ÍC**, **Brand C**, **Mota J**, **Leite N**. Physical Exercise and Brain-
612 Derived Neurotrophic Factor Concentration in Children and Adolescents: A Systematic
613 Review With Meta-Analysis. *Pediatric exercise science* 34: 44–53, 2022. doi:
614 10.1123/pes.2020-0207.

615 21. **Esvald EE**, **Tuvikene J**, **Sirp A**, **Patil S**, **Bramham CR**, **Timmusk T**. CREB family
616 transcription factors are major mediators of BDNF transcriptional autoregulation in
617 cortical neurons. *Journal of Neuroscience* 40: 1405–1426, 2020. doi:
618 10.1523/JNEUROSCI.0367-19.2019.

619 22. **Husain MA**, **Laurent B**, **Plourde M**. APOE and Alzheimer's Disease: From Lipid Transport
620 to Physiopathology and Therapeutics. *Frontiers in Neuroscience* 15: 1–15, 2021. doi:
621 10.3389/fnins.2021.630502.

622 23. **Vitolo O V.**, **Sant'Angelo A**, **Costanzo V**, **Battaglia F**, **Arancio O**, **Shelanski M**. Amyloid β-
623 peptide inhibition of the PKA/CREB pathway and long-term potentiation: Reversibility
624 by drugs that enhance cAMP signaling. *Proceedings of the National Academy of*
625 *Sciences of the United States of America* 99: 13217–13221, 2002. doi:
626 10.1073/pnas.172504199.

627 24. **Moriguchi Y**, **Shinohara I**. Effect of the COMT Val158Met genotype on lateral prefrontal
628 activations in young children. *Developmental Science* 21: 1–9, 2018. doi:
629 10.1111/desc.12649.

630 25. **Williams SN**, **Undieh AS**. Dopamine D1-like receptor activation induces brain-derived
631 neurotrophic factor protein expression. *NeuroReport* 20: 606–610, 2009. doi:
632 10.1097/WNR.0b013e32832a0a98.

633 26. **Cadenas-Sánchez C**, **Mora-González J**, **Migueles JH**, **Martín-Matillas M**, **Gómez-Vida J**,
634 **Escolano-Margarit MV**, **Maldonado J**, **Enriquez GM**, **Pastor-Villaescusa B**, **de Teresa C**,
635 **Navarrete S**, **Lozano RM**, **de Dios Beas-Jiménez J**, **Estévez-López F**, **Mena-Molina A**,
636 **Heras MJ**, **Chillón P**, **Campoy C**, **Muñoz-Hernández V**, **Martínez-Ávila WD**, **Merchan**
637 **ME**, **Perales JC**, **Gil Á**, **Verdejo-García A**, **Aguilera CM**, **Ruiz JR**, **Labayen I**, **Catena A**,
638 **Ortega FB**. An exercise-based randomized controlled trial on brain, cognition, physical
639 health and mental health in overweight/obese children (ActiveBrains project):
640 Rationale, design and methods. *Contemporary Clinical Trials* 47: 315–324, 2016. doi:
641 10.1016/j.cct.2016.02.007.

642 27. **Cole TJ**, **Lobstein T**. Extended international (IOTF) body mass index cut-offs for thinness,
643 overweight and obesity. *Pediatric Obesity* 7: 284–294, 2012. doi: 10.1111/j.2047-
644 6310.2012.Y00064.x.

28. **Moore SA**, **McKay HA**, **Macdonald H**, **Nettlefold L**, **Baxter-Jones ADG**, **Cameron N**, **Brasher PMA**. Enhancing a somatic maturity prediction model. *Medicine and Science in Sports and Exercise* 47: 1755–1764, 2015. doi: 10.1249/MSS.0000000000000588.

29. **Sarzynski MA**, **Ghosh S**, **Bouchard C**. Genomic and transcriptomic predictors of response levels to endurance exercise training. *The Journal of Physiology* 595: 2931–2939, 2017. doi: 10.1113/JP272559.

30. **Reitan RM**, **Wolfson D**. The Trail Making Test as an initial screening procedure for neuropsychological impairment in older children. *Archives of Clinical Neuropsychology* 19: 281–288, 2004. doi: 10.1016/S0887-6177(03)00042-8.

31. **Andrews Espy, K; Cwik M**. The Development of a Trail Making Test in Young children: The TRAILS-P. 18: 411–422, 2009. doi: 10.1080/138540409052416.The.

32. **Amodeo DA**, **Grospe G**, **Zang H**, **Dwivedi Y**, **Ragozzino ME**. Cognitive flexibility impairment and reduced frontal cortex BDNF expression in the ouabain model of mania. *Neuroscience* 345: 229–242, 2017. doi: 10.1016/j.neuroscience.2016.05.058.

33. **Hwang J**, **Brothers RM**, **Castelli DM**, **Glowacki EM**, **Chen YT**, **Salinas MM**, **Kim J**, **Jung Y**, **Calvert H**. Acute high-intensity exercise-induced cognitive enhancement and brain-derived neurotrophic factor in young, healthy adults. *Neuroscience Letters* 630: 247–253, 2016. doi: 10.1016/j.neulet.2016.07.033.

34. **Lee LC**, **Su MT**. Multiple epigenetic biomarkers for evaluation of students ' academic performance. 18: 1–10, 2019. doi: 10.1111/gbb.12559.

35. **Chen J**, **Lipska BK**, **Halim N**, **Ma QD**, **Matsumoto M**, **Melhem S**, **Kolachana BS**, **Hyde TM**, **Herman MM**, **Apud J**, **Egan MF**, **Kleinman JE**, **Weinberger DR**. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): Effects on mrna, protein, and enzyme activity in postmortem human brain. *American Journal of Human Genetics* 75: 807–821, 2004. doi: 10.1086/425589.

36. **Cools R**. Dopaminergic modulation of cognitive function-implications for L-DOPA treatment in Parkinson's disease. *Neuroscience and Biobehavioral Reviews* 30: 1–23, 2006. doi: 10.1016/j.neubiorev.2005.03.024.

37. **Matthews N**, **Vance A**, **Cummins TDR**, **Wagner J**, **Connolly A**, **Yamada J**, **Lockhart PJ**, **Panwar A**, **Wallace RH**, **Bellgrove MA**. The COMT Val158 allele is associated with impaired delayed-match-to-sample performance in ADHD. *Behavioral and Brain Functions* 8: 1–9, 2012. doi: 10.1186/1744-9081-8-25.

682

683

684

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.

716

717

718

719

720

721

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 ± 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 frecuencies	Allele frecuencies	X^2 Hardy-Weinberg equilibrium	Computation of the genotype to calculate the genetic score (for more details see **Table S1**)
rs6265	*BDNF*	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	*CREB1*	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	*NTRK2*	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	*COMT*	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	*APOE*	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	*APOE*	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		
	Intervention group	Control group	Intervention group	Control group	Genetic favourable	Genetic unfavourable	Gene *x* exercise interaction (p-value)
Intelligence							
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
Executive function							
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
Academic performance							
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

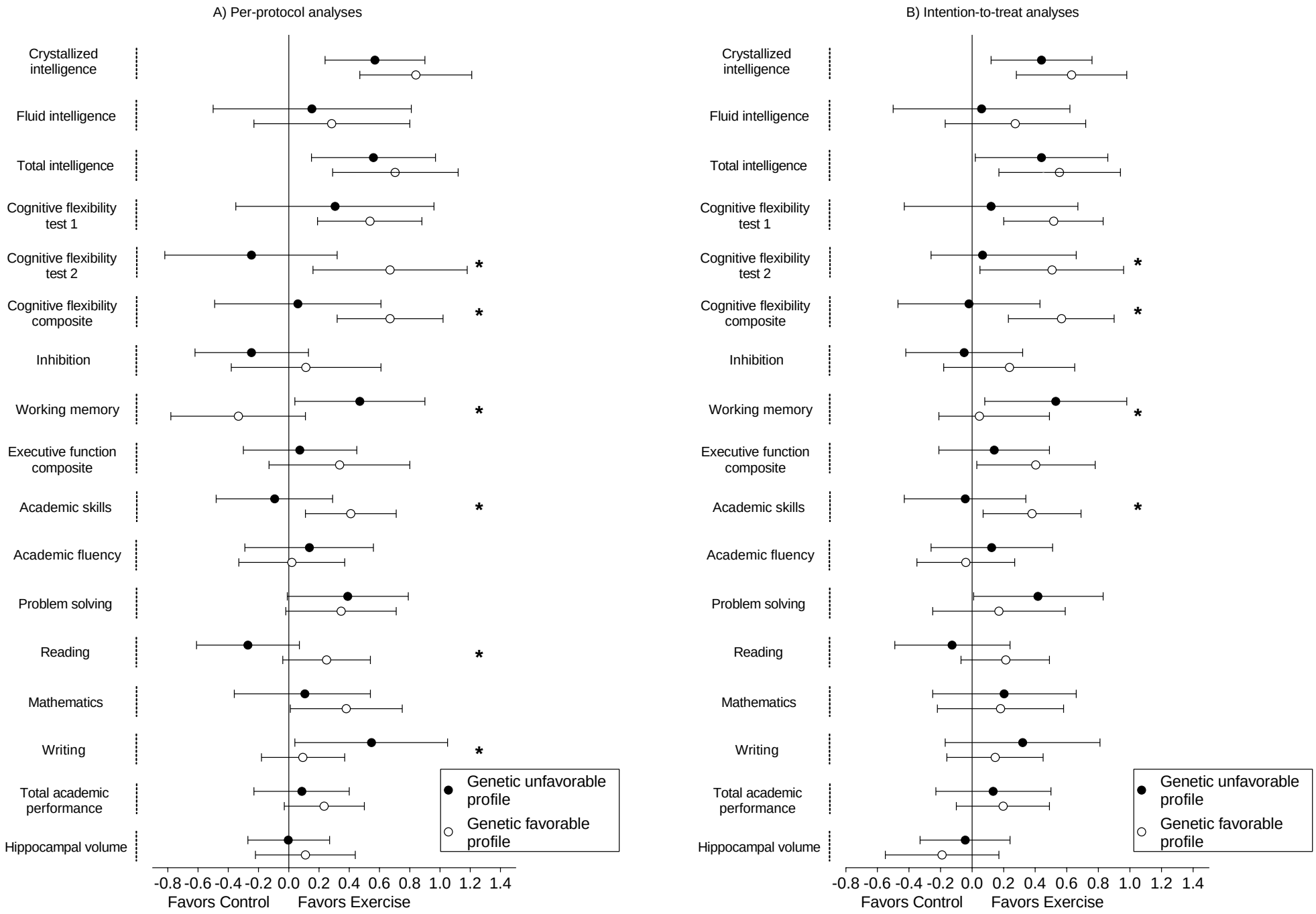
Brain structure

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

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 *x* 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 *x* 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" [21exercise 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
Crystallized intelligence
Fluid intelligence
Total intelligence
Cognitive flexibility test 1
Cognitive flexibility test 2
Cognitive flexibility composite
Inhibition
Working memory
Executive function composite
Academic skills
Academic fluency
Problem solving
Reading
Mathematics
Writing
Total academic performance
Hippocampal volume
*
Genetic unfavorable profile
Genetic favorable profile
-0.8 -0.6 -0.4 -0.2 0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4
Favors Control
Favors Exercise

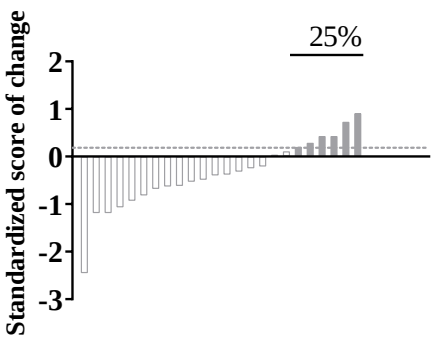
Individual responses on cognitive flexibility

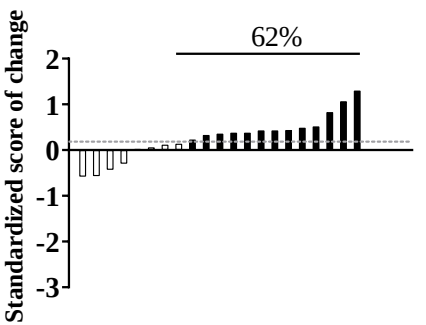
Favorable genetic profile

Unfavorable genetic profile

A) Per-protocol

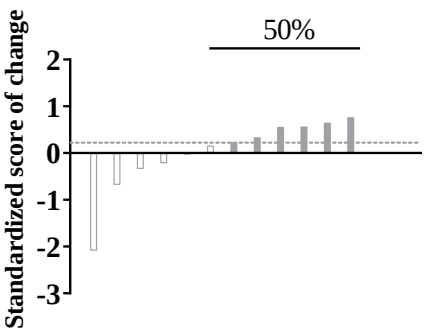
***P* = 0.01**

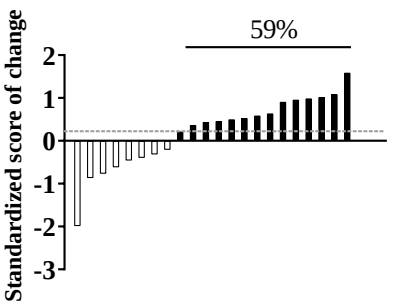




B) Per-protocol

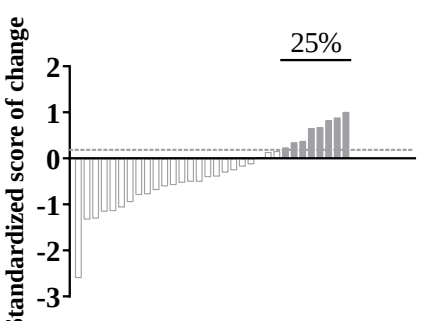
P = 0.72

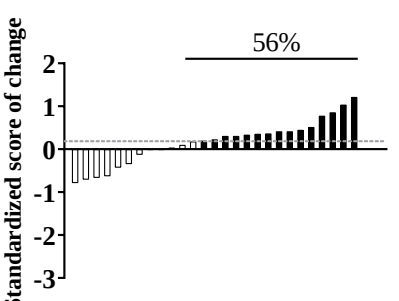




C) Intention-to-treat

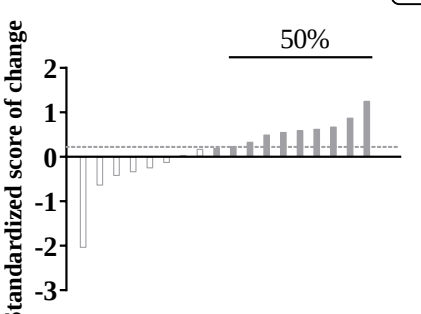
***P* = 0.03**

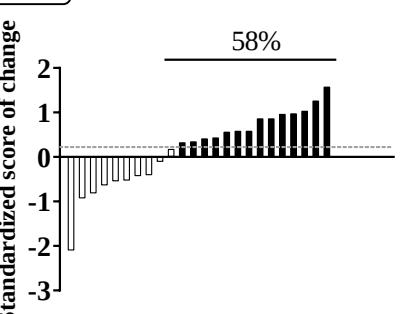




D) Intention-to-treat

P = 0.76





Control **Exercise** **Control** **Exercise**

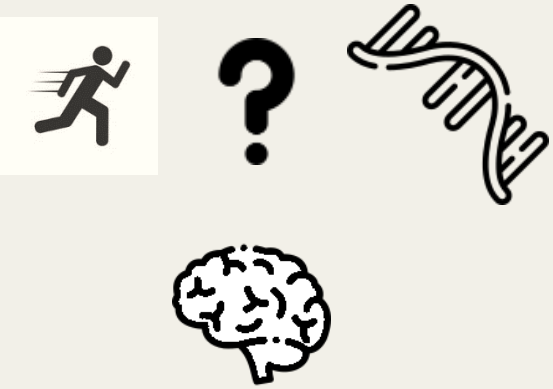
Meaningful increase (Cohen's $d \geq 0.2$)

Non-meaningful change (Cohen's $d < 0.2$)

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

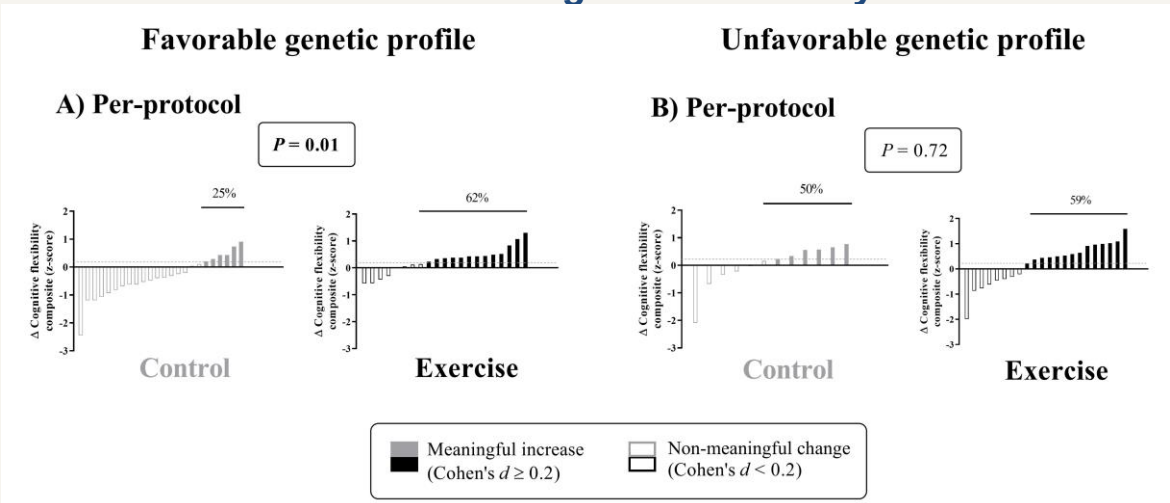
METHODS

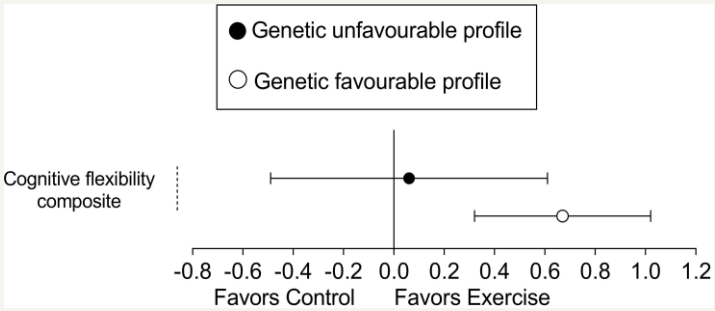
101 children with overweight/obesity (8-11 years old) were randomly allocated to the exercise and control groups



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.