Author(s): Rodriguez-Ayllon, María; Plaza-Florido, Abel; Mendez-Gutierrez, Andrea; Altmäe, Signe; Solis-Urra, Patricio; Aguilera, Concepción M.; Catena, Andrés; Ortega, Francisco B.; Esteban-Cornejo, Irene

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Original article

The effects of a 20-week exercise program on blood-circulating biomarkers related to brain health in overweight or obese children: The ActiveBrains project

María Rodriguez-Ayllon [a,b], Abel Plaza-Florido [a], Andrea Mendez-Gutierrez [c], Signe Altmäe [d], Patricio Solis-Urra [a,e,f], Concepción M. Aguilera [c], Andrés Catena [g], Francisco B. Ortega [a,h,i,*], Irene Esteban-Cornejo [a]

[a] *PROFITH "PROmoting FITness and Health through physical activity" research group, Sport and Health University Research Institute (iMUDS), Department of Physical and Sports Education, Faculty of Sport Sciences, University of Granada, Granada 18071, Spain*
[b] *Department of Epidemiology, Erasmus University Medical Center, Rotterdam 3015 GD, the Netherlands*
[c] *Department of Biochemistry and Molecular Biology II, School of Pharmacy, University of Granada, Granada 18071, Spain*
[d] *Department of Biochemistry and Molecular Biology, Faculty of Sciences, University of Granada, Granada 18071, Spain*
[e] *Nuclear Medicine Services, Virgen de Las Nieves University Hospital, Granada 18014, Spain*
[f] *Faculty of Education and Social Sciences, Universidad Andres Bello, Viña del Mar 2531015, Chile*
[g] *Department of Experimental Psychology, School of Psychology, University of Granada, Granada 18011, Spain*
[h] *Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä 40014, Finland*
[i] *Department of Bioscience and Nutrition, Karolinska Institutet, Huddinge, SE 141 57, Sweden*

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Abstract

Background: Emerging research supports the idea that exercise positively affects neurodevelopment. However, the mechanisms linking exercise with brain health are largely unknown. We aimed to investigate the effect of exercise on (a) blood biomarkers selected based on previous evidence (brain-derived neurotrophic factor, β-hydroxybutyrate (BHB), cathepsin B (CTSB), kynurenine, fibroblast growth factor 21 (FGF21), soluble vascular cell adhesion molecule-1 (sVCAM-1)); and (b) a panel of 92 neurology-related proteins (discovery analysis). We also investigated whether changes in these biomarkers mediate the effects of exercise on brain health (hippocampal structure and function, cognitive performance, and mental health).

Methods: We randomized 81 overweight/obese children (10.1 ± 1.1 years, 41% girls) into 2 groups: either 20 weeks of aerobic plus resistance exercise or control. Candidate biomarkers were assessed using enzyme-linked immunosorbent assay (ELISA) for kynurenine, FGF21, and CTSB; colorimetry for β-hydroxybutyrate; and XMap for brain-derived neurotrophic factor and soluble vascular cell adhesion molecule-1. The 92 neurology-related proteins were analyzed by an antibody-based proteomic analysis.

Results: Our intervention had no significant effect on candidate biomarkers (all $p > 0.05$). In the discovery analysis, a reduction in circulating macrophage scavenger receptor type-I was observed (standardized differences between groups = −0.3, $p = 0.001$). This effect was validated using ELISA methods (standardized difference = −0.3, $p = 0.01$). None of the biomarkers mediated the effects of exercise on brain health.

Conclusions: Our study does not support a chronic effect of exercise on candidate biomarkers. We observed that while chronic exercise reduced the levels of macrophage scavenger receptor type-I, it did not mediate the effects of exercise on brain health. Future studies should explore the implications of this novel biomarker for overall health.

Keywords: Brain development; Childhood; MRI; Physical activity; Proteomic

1. Introduction

Childhood obesity is a major health concern and negatively associated with brain health.[1–3] Exercise positively affects neurodevelopment during childhood,[4–6] which in turn might

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* Corresponding author.
E-mail address: ortegaf@ugr.es (F.B. Ortega).

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protect the brain against the adverse effects of obesity at early stages in life. However, molecular mechanisms linking exercise with brain health indicators are still lacking. In this regard, brain-derived neurotrophic factor (BDNF) is a neurotrophin involved in the growth and healthy maintenance of neurons, and it exerts its major activity within the central nervous system.[7–9] A growing number of studies have indicated that decreased levels of circulating BDNF are associated with cognitive decline and reduced performance on learning and memory tasks[10] as well as with anxiety and depression in animals and adult humans.[11,12]

Exercise produces chronic benefits in the brain that enhance cognitive function and mental health.[13] For instance, chronic exercise leads to an increase in BDNF in the central nervous system, which promotes improvement in cognitive ability and anxiety and depressive-like behaviors in animal models.[14–16] In adults, previous exercise interventions showed improvement in hippocampal plasticity and memory associated with BDNF changes.[17,18] Though these results are widely recognized, we still lack an understanding of the molecular mechanisms that elicit the positive effects of chronic exercise on brain health indicators (i.e., BDNF, brain structure, brain function, cognition, and mental health), especially during childhood—a period of life when the brain is growing and developing.

Due to ethical considerations, blood is the primary tissue used to study the molecular response to exercise in children (muscle biopsy, for instance, is rarely used in research on children).[19] Recent studies in animal models have tested several hypotheses to identify novel circulating biomarkers influenced by exercise,[20–22] which could be helpful for understanding how chronic exercise induces changes in different brain health indicators.[23] First, the metabolite β-hydroxybutyrate (BHB) appears to increase chronically in the liver after exercise intervention.[21] Particularly, it crosses the blood–brain barrier (BBB) and activates BDNF promoters in the hippocampus. This induction, in turn, mediates the positive effects of chronic exercise on memory, cognition, and synaptic transmission.[21] Second, cathepsin B (CTSB) also appears to cross the BBB and increase BDNF levels,[23] which could affect hippocampal neurogenesis, hippocampal-dependent learning, memory, and depression.[22,23] Third, exercise increases the peroxisome proliferator-activated receptor gamma coactivator 1-alpha–dependent muscular expression of kynurenine aminotransferase enzymes.[24] Likewise, higher expression of kynurenine aminotransferase induces a beneficial shift in the balance between the neurotoxic kynurenine and the neuroprotective kynurenic acid.[25] The fact that kynurenic acid is not able to cross the BBB protects the brain from stress-induced kynurenine accumulation, neuroinflammation, changes in synaptic plasticity, and depressive symptoms.[24–26] Fourth, plasma fibroblast growth factor 21 (FGF21) can cross the BBB,[27] decrease oxidative stress and neuroinflammation, and improve cognition.[28,29] Meanwhile, a positive association between exercise and circulating FGF21 levels has been reported in some (but not all) studies in older people.[29] Lastly, elevated soluble vascular cell adhesion molecule-1 (sVCAM-1) appears to promote neuroinflammation,[30,31] which can have negative consequences on cognitive function and emotional regulation.[30] Although more research is needed to confirm this, literature suggests exercise could reduce sVCAM-1 in children.[32] Collectively, despite these interesting observations, the effects of chronic exercise on BDNF, BHB, CTSB, kynurenine, FGF21, and sVCAM-1, as well as its mediating role on brain health during childhood, remain unexplored.

Besides target molecules, the effects of exercise on brain health could also be related to a more complex group of proteins and their interactions. A more comprehensive set of plasma proteins would aid in understanding such complex patterns. In this context, high-throughput proteomic analysis methods permit the simultaneous quantification of several plasma proteins,[33] thereby allowing for the exploration of a broad set of blood-circulating biomarkers related to the beneficial effects of exercise on brain health during childhood. Therefore, the primary aims of this study were: (a) to examine whether a 20-week exercise program has an effect on selected blood-circulating biomarkers in children with overweight/obesity (OW/OB) (candidate analysis); and (b) to explore the effect of a 20-week exercise program on 92 neurology-related plasma proteins, which may reveal new candidate biomarkers related to exercise (discovery analysis). As a secondary aim, we investigated whether changes in these emerging blood-circulating biomarkers mediate the effects of exercise on brain health (i.e., hippocampal structure and function, cognition, and mental health) in children with OW/OB. Since, as we stated above, some blood-circulating biomarkers are expected to activate BDNF, we also explored whether changes in those biomarkers mediate the effect of exercise on BDNF levels. Overall, our main hypothesis was that chronic exercise induces changes in circulating blood levels of the selected biomarkers, which may have a beneficial effect on brain health during childhood.

2. Methods

2.1. Design and participants

Children from the ActiveBrains project (http://profith.ugr.es/activebrains) participated in this study. It is a randomized controlled trial designed to examine the effects of a 20-week physical exercise program on brain, cognitive, and academic performance, as well as on selected physical and mental health outcomes in children with OW/OB. All data were collected between November 21, 2014 and June 30, 2016. For feasibility reasons, the ActiveBrains project was conducted in 3 waves: the first academic year of the project (i.e., 2014–2015), the beginning of the following academic year (i.e., 2015–2016), and later on in the same academic year (i.e., 2015–2016). The full set of outcomes (e.g., blood collections and magnetic resonance imaging scans) was assessed twice, immediately before and after the 20-week exercise program.

The ActiveBrains project was approved by the Human Research Ethics Committee of the University of Granada. Additionally, it was registered with ClinicalTrials.gov (identifier: NCT02295072). The parents or legal guardians of the children provided written informed consent to participate in the trial.

Details of the methodology of the ActiveBrains project have been described elsewhere.[34] Briefly, to be eligible, participants must: (a) be 8 to 11.9 years old; (b) be OW/OB according to the World Obesity Federation cut-off points;[35,36] (c) not suffer from physical disabilities or neurological disorders that prohibit them from exercise; (d) in the case of girls, not have started menstruating at the moment of baseline assessments; (e) report no use of medications that influence central nervous system function; (f) be right-handed (as measured by the Edinburgh inventory);[37] and (g) not report an attention-deficit/hyperactivity disorder over the 85th percentile as measured by the attention-deficit/hyperactivity disorder rating scale.[38]

2.2. Intervention and control

The exercise group was asked to join at least 3 (of 5 offered) supervised exercise sessions per week. Sessions had a duration of 90 min (60 min of aerobic exercise and 30 min of resistance exercise). Sessions were based on playful activities and games involving coordinative exercises. Heart rate monitors (POLAR RS300X; Polar Electro Oy, Kempele, Finland) were used to recording participants' exercise intensity during sessions. Children in this group spent an average of 38 min per session above 80% of their maximum heart rate. Participants in the control group continued their usual routines.[39]

A posteriori power analysis showed that a sample size of 81 children is sufficient to detect small-to-medium effect sizes (i.e., 0.3 standard deviations (SDs)) assuming an error of 0.05 and 80% statistical power. Further details related to the randomization, power and sample size, physical exercise intervention, and control group condition have been described by Ortega et al.[39]

2.3. Blood-circulating biomarkers

Blood samples were obtained for biochemical and hematological screening tests between 8:30 a.m. and 10:30 a.m. Blood was collected into tubes containing ethylenediamine tetraacetic acid and then centrifuged (10 min at 4°C, 1000×*g*). Plasma was isolated, aliquoted, and stored at −80°C for further analyses.

2.3.1. Candidate blood-circulating biomarkers

First, the analysis of mature BDNF levels in plasma was performed using XMap technology (Luminex, Austin, TX, USA) and human monoclonal antibodies (EMD Milliplex Map Kit, Millipore, Billerica, MA, USA). For mature BDNF, we used the Human Neurodegenerative Disease Magnetic Bead Panel 3 (catalog HNDG3MAG-36K; EMD Millipore, Billerica, MA, USA) with a sensitivity of 0.23 pg/mL and an intra- and inter-assay precision coefficient of variation (CV) of <5.4% and <5.3%, respectively. Second, plasma kynurenine levels were quantified by enzyme-linked immunosorbent assay (ELISA) (BA E-2200, LDN, Nordhorn, Germany; CV = 12.9) according to the manufacturer's instructions. Third, serum levels of FGF21 and CTSB were measured by ELISA, using the kits RD191108200R (Biovendor, Brno, Czech Republic; CV = 7.62) and ab272205 (Abcam, Cambridge, UK; CV = 2.91), respectively. Fourth, serum BHB concentration was quantified by a colorimetric assay (ab83390, Abcam; CV = 4.27) and sVCAM-1 was determined with XMap technology (Luminex) using HNDG3MAG-36K (Millipore) (CV = 3.36). Candidate blood-circulating biomarkers were checked for normality using both graphical (normal probability plots) and statistical (Kolmogorov−Smirnov test) procedures. Due to their skewed distribution, BDNF, kynurenine, FGF21, CTSB, and BHB were transformed to $\log_{10}$ values before analyses.

2.3.2. Panel of neurology-related proteins

As previously described,[40,41] the 92 neurology-related proteins were analyzed at the Olink laboratory in Uppsala, Sweden using the Proximity Extension Assay technique with the Proseek Multiplex Neurology I 96 × 96 reagents kit (Olink Bioscience, Uppsala, Sweden). The proteins included in the neurology panel are listed in Supplementary Table 1A. Data are presented as arbitrary units—in particular, as NPX (Normalized Protein eXpression). In brief, NPX (the Olink's arbitrary unit) is in $\log_2$ scale, where a high NPX value equals a high protein concentration (i.e., a 1 NPX value difference means a doubling of protein concentration). Intra- and inter-assay CV, detection limits, and specific information for each protein are described on the manufacturer's website (https://www.olink.com/). The neurology panel includes a mix of proteins related to neurobiological processes and neurological diseases (e.g., neural development, axon guidance, or synaptic function) as well as some more exploratory proteins with broader roles in processes such as cellular regulation, immunology, development, and metabolism (https://www.olink.com/products/neurology/). Of the 92 proteins included in the Olink proteomic neurology panel (https://www.olink.com/products/neurology/), a total of 91 (99%) were detected in our sample, with only microtubule-associated protein tau failing to pass the detection control criteria (proteins detected in >75% of the sample).

We used ELISA to validate the effect of exercise on some proteins, as originally assessed by the Proximity Extension Assay technique. In particular, we validated kynureninase (KYNU), macrophage scavenger receptor type-I (MSR1), and plexin-B3 (PLXNB3) using the kits ELH-KYNU-1 (RayBiotech, Norcross, GA, USA; CV = 12.4), SK00489-01 (Aviscera Bioscience, Santa Clara, CA, USA; CV = 8.25) and EK1827 (Boster Bio, Pleasanton, CA, USA; CV = 7.71), respectively. The decision to validate these specific neurology-related proteins (i.e., KYNU, MSR1, and PLXNB3) were based on (a) the effect sizes observed in the main analyses and/or (b) their relationship with BDNF (Supplementary Table 2A).

2.4. Outcome measurements

Details related to the hippocampal volumetric analysis, hippocampal functional connectivity analysis, cognitive performance, and mental health measurements have been described previously.[39,42–44]

2.5. Statistical analyses

2.5.1. Main analyses: Effects of chronic exercise on circulating neurology-related proteins

Statistical procedures were performed using the SPSS software (Version 22.0, IBM Corp., Armonk, NY, USA) and *R*

Version 1.3.1073. A significance difference level of $p < 0.05$ was set. Characteristics of the study sample are presented as mean and SD or frequency and percentage as appropriate.

Main effects on the blood-circulating biomarkers were tested according to the per-protocol principle (participants with valid data who attended at least 70% of the recommended 3 sessions/week). Results from the per-protocol analysis were reported as the main findings since we were interested in knowing the efficacy rather than the effectiveness of our exercise intervention (i.e., we wanted to know the effects of exercise on the study biomarkers when exercise was done (operationally defined as ≥70% attendance)). Main analyses were performed with analysis of covariance using post-exercise program data as dependent variables, group (i.e., exercise *vs*. control) as a fixed factor, and baseline data as covariates. As in previous randomized controlled trials focused on cognitive outcomes,[45] the *z*-scores for each blood-circulating biomarker at post-exercise program were found by dividing the difference of the raw score of each participant from the baseline mean by the baseline SD (i.e., post-exercise individual value − baseline mean / baseline SD). Since this variable illustrates how many SDs the outcome has changed from baseline, we interpreted it as an effect size indicator; a value around 0.2 was considered a small effect size, 0.5 was a medium effect size, and 0.8 was a large effect size.[46] Discovery analysis of the effects of the exercise program on 91 neurology-related plasma proteins was adjusted for multiple comparisons using a false discovery rate based on the Benjamini−Hochberg method.[47]

2.5.2. Secondary analysis: Mediation analysis

We tested whether the effects of the exercise intervention on brain health indicators (i.e., BDNF, hippocampal structure and function, cognitive performance, and mental health) were mediated by changes in blood-circulating biomarkers if exercise showed an effect on them. Mediation analysis was performed using *R* Version 1.3.1073 using the lavaan package, with a resample procedure of 5000 bootstrap samples.

The unstandardized (B) and standardized (β) regression coefficients are presented for four equations: equation 1 regressed the mediator (i.e., change in blood-circulating biomarkers) on the independent variable (group). Equation 2 regressed the dependent variables (i.e., brain health indicators) on the independent variable. Equation(s) 3 regressed the dependent variables on both the mediator (equation 3) and the independent variable (equation 3') (Supplementary Fig. 1A). The indirect effects along with their confidence intervals (CIs) were also presented, and the significance was considered if the indirect effect significantly differed from zero (i.e., if zero is not contained within the CI). Finally, the percentage of total effect was computed to determine how much of the total effect was explained by the mediation: (indirect effect/total effect) × 100.

2.5.3. Sensitivity analyses: Intention-to-treat analyses

Using analysis of covariance, we tested whether participants who completed the baseline and post-exercise evaluations but did not attend at least 70% of the recommended 3 sessions/week differed in terms of main study variables from participants who met per-protocol criteria.

3. Results

A flowchart of the study is presented in Fig. 1. In total, 112 children with OW/OB meeting the eligibility criteria participated in this study. Of them, 109 were randomly allocated to an exercise group, which participated in the exercise program, or to a wait-list control group, which followed their normal life routines. A total of 22 children were excluded from analyses due to missing blood data at 1 or more time points ($n = 21$) or due to poor quality control at the proteomic analysis ($n = 1$), leaving 87 participants for the intention-to-treat analyses. Finally, 81 children with OW/OB met the per-protocol criteria (i.e., attended at least 70% of the recommended 3 sessions/week) and were included in the main analyses. Baseline sample characteristics are displayed in Table 1.

3.1. Main analyses: Effects of exercise on blood-circulating biomarkers

The effects of the exercise intervention on the candidate blood-circulating biomarkers (i.e., BDNF, BHB, CTSB, kynurenine, FGF21, sVCAM-1) are presented in Table 2. Overall, no significant effects were found on any of the selected blood-circulating biomarkers (all $p > 0.05$).

The effects of the exercise program on the 91 neurology-related proteins are presented in Supplementary Table 3A. Significant (unadjusted p-values <0.05) effects of the intervention on these neurology-related proteins are presented in detail in Table 3. Up- and down-regulated proteins and their levels of significance are graphically represented by a volcano plot in Fig. 2. Overall, the exercise group showed significantly reduced levels of 6 circulating neurology-related proteins in plasma compared to the control group, which can be interpreted as a small-to-medium effect size (SDs of change ranged from −0.19 to −0.46; p values ranged from 0.001 to 0.040). In particular, the proteins whose concentration reduced significantly after the intervention program were: (a) carboxypeptidase A2 (CPA2), (b) KYNU, (c) leukocyte-associated immunoglobulin-like receptor 2 (LAIR2), (d) MSR1, (e) PLXNB3, and (f) the lysosome membrane protein 2 (SCARB2). All significant effects disappeared after correcting for multiple testing, with only the MSR1 showing a borderline corrected (false discovery rate) p value of 0.07.

The validation analysis performed by ELISA assays is presented in Table 4 (per-protocol). Briefly, 3 neurology-related proteins were validated with ELISA in our study (i.e., MSR1, PLXNB3, KYNU). Of them, the effect of exercise intervention was only confirmed on MSR1 (−0.33 SD, $p = 0.013$).

3.2. Secondary analysis: Mediation analysis

A visualization of the mediation panel modeling approach is represented in Supplementary Fig. 1A. Mediation analysis was performed with the per-protocol sample. The effects of

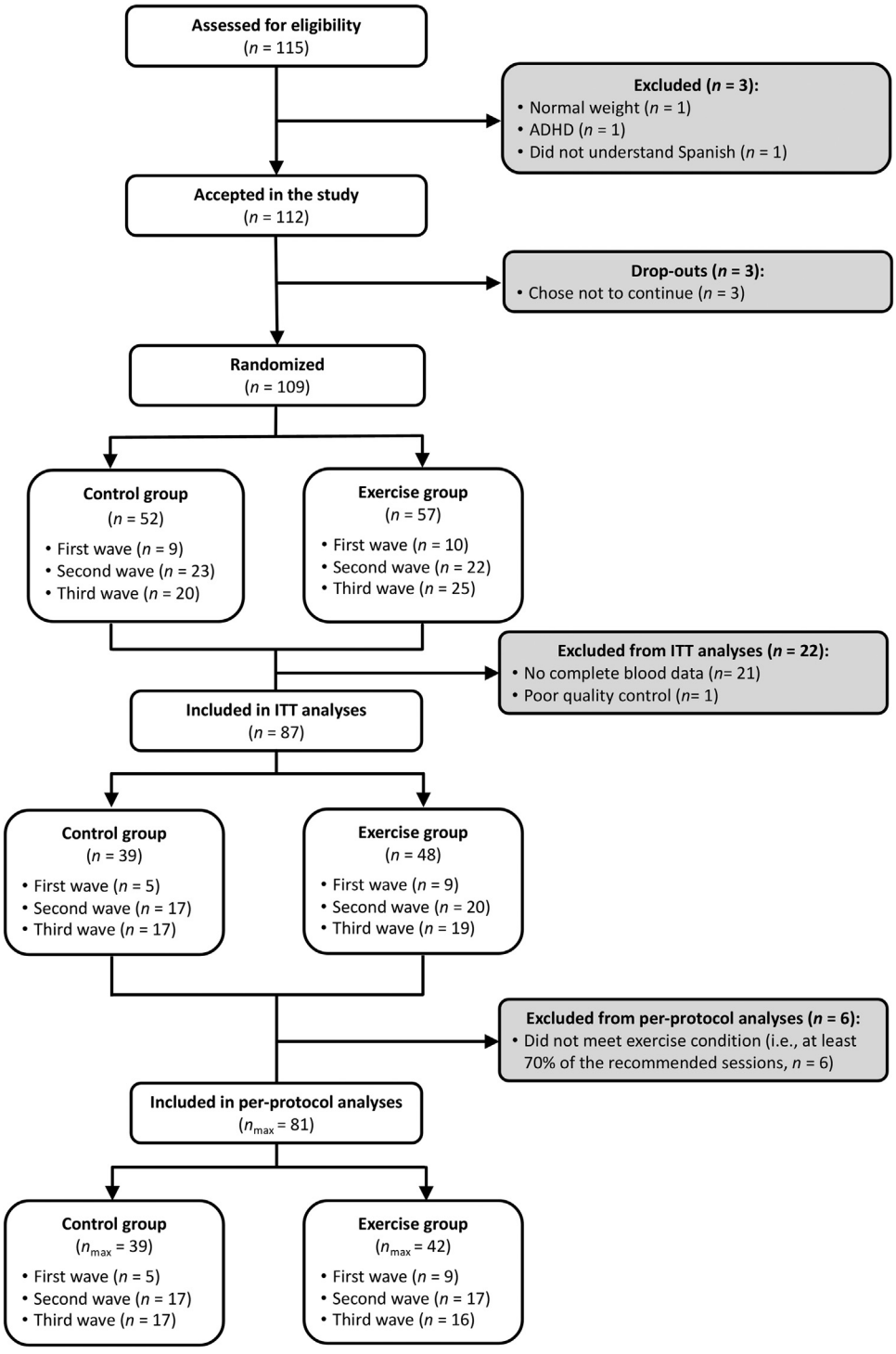

Fig. 1. Flow diagram of the study. ADHD = attention-deficit/hyperactivity disorder; ITT = intention-to-treat; n_{max} = maximum n for analyses (changes depending on the variable; see Supplementary tables X for specific sample sizes per variable).

the exercise intervention on brain health outcomes, mediated by changes in neurology-related proteins (i.e., CPA2, KYNU, LAIR2, MSR1, PLXNB3, SCARB2), are presented in Supplementary Materials B. Briefly, none of the neurology-related proteins mediated the chronic effects of exercise on brain health outcomes after correcting for the false discovery rate.

3.3. *Sensitivity analyses: Intention-to-treat analyses*

As Supplementary Table 4A shows, the effects of the exercise intervention on neurology-related proteins derived from the intention-to-treat analyses were in line with those from the per-protocol analyses described above. The effect sizes were consistent, yet slightly attenuated, which was to be expected since participants who attended few exercise sessions were

Table 1
Descriptive baseline characteristics of the *ActiveBrains* participants meeting per-protocol criteria ($n = 81$).

	All		Exercise group		Control group	
	n	Value	*n*	Value	*n*	Value
Sex						
Girl	81	40.7	42	35.7	39	46.2
Age (year)	81	10.12 ± 1.11	42	10.02 ± 1.13	39	10.23 ± 1.10
Weight (kg)	81	55.85 ± 11.18	42	56.61 ± 12.58	39	55.03 ± 9.54
Height (cm)	81	144.52 ± 8.39	42	143.48 ± 8.84	39	145.65 ± 7.84
Body mass index (kg/m^2)	81	26.52 ± 3.56	42	27.18 ± 3.98	39	25.80 ± 2.93
Peak height velocity offset (year)	81	−2.21 ± 0.97	42	−2.37 ± 0.92	39	−2.04 ± 1.01
Wave of participation	81		42		39	
First	14	17.3	9	21.4	5	12.8
Second	34	42.0	17	40.5	17	43.6
Third	33	20.7	16	38.1	17	43.6

Note: Values are expressed as mean ± SD or %.

less committed to the program overall. These results were also consistent with the candidate blood-circulating biomarkers (Supplementary Table 5A) and ELISA validation (Supplementary Table 6A).

4. Discussion

The main aim of this study was to investigate the effects of a 20-week exercise intervention in children with OW/OB on a broad set of blood-circulating biomarkers potentially related to brain health. Overall, we did not identify any effect of chronic exercise on candidate the blood-circulating biomarkers selected, i.e., BDNF, BHB, CTSB, kynurenine, FGF21, and sVCAM-1. In the discovery analysis, we observed consistent evidence (i.e., cross-validated with 2 different techniques in per-protocol and intention-to-treat analyses) that an exercise program reduces the plasma concentration of MRS1 in children with OW/OB. However, this exercise-induced change in MRS1 did not appear to mediate the chronic effect of exercise on BDNF, hippocampus structure and function, cognition, and mental health in OW/OB children.

This study could not confirm that exercise affects the blood-circulating biomarkers previously identified as potential molecular mechanisms through which exercise might improve

Table 2
Per-protocol effects of the ActiveBrains intervention on raw and *z*-score post-intervention candidate blood-circulating biomarkers.

	n_{all}	n_i	Intervention group	n_c	Control group	Differences between groups	*p*
BDNF (ng/mL)	80	41		39			
Raw score			0.34 (0.21 to 0.47)		0.33 (0.19 to 0.46)	0.01 (−0.17 to 0.20)	0.898
z-Score			−0.20 (−0.48 to 0.08)		−0.22 (−0.51 to 0.06)	0.02 (−0.37 to 0.42)	
BHB (ng/μL)							
Raw score	80	41	1.49 (1.44 to 1.52)	39	1.48 (1.44 to 1.52)	0.00 (−0.05 to 0.06)	0.896
z-Score			−0.31 (−0.67 to 0.05)		−0.34 (−0.72 to 0.03)	0.03 (−0.48 to 0.56)	
CTSB (μg/L)							
Raw score	80	41	1.74 (1.70 to 1.78)	39	1.74 (1.70 to 1.77)	0.004 (−0.05 to 0.06)	0.887
z-Score			−0.03 (−0.29 to 0.23)		−0.05 (−0.32 to 0.21)	0.03 (−0.35 to 0.41)	
Kynurenine (ng/mL)							
Raw score	81	42	3.01 (2.98 to 3.05)	39	3.00 (2.96 to 3.03)	0.01 (−0.04 to 0.06)	0.585
z-Score			0.29 (0.12 to 0.47)		0.22 (0.04 to 0.40)	0.07 (−0.18 to 0.32)	
FGF21 (pg/mL)							
Raw score	79	40	1.79 (1.73 to 1.85)	39	1.88 (1.81 to 1.94)	−0.09 (−0.18 to 0.00)	0.061
z-Score			−0.13 (−0.35 to 0.09)		0.17 (−0.05 to 0.39)	−0.30 (−0.61 to 0.01)	
sVCAM-1 (μg/L)							
Raw score	80	41	1253.04 (1179.14 to 1326.93)	39	1304.15 (1228.38 to 1379.92)	−51.11 (−156.96 to 54.73)	0.339
z-Score			0.03 (−0.24 to 0.30)		0.22 (−0.06 to 0.50)	−0.19 (−0.58 to 0.20)	

Notes: *z*-score values indicate how many standard deviations the post-intervention values have changed with respect to the baseline mean and standard deviation. For example, a 0.50 *z*-score means that the mean value at post-intervention is 0.50 standard deviations higher than the mean value at baseline, indicating a positive change; negative values indicate the opposite. Values are expressed as mean (95%CI). Analyses were adjusted for baseline values. Due to their skewed distribution, BDNF, kynurenine, FGF21, CTSB, and BHB were transformed to $\log_{10}$ values before analysis.
Abbreviations: 95%CI = 95% confidence interval; BDNF = brain-derived neurotrophic factor; BHB = B-hydroxybutyrate; CTSB = cathepsin B; FGF21 = fibroblast growth factor 21; n_{all} = all sample; n_c = sample of the control group; n_i = sample of the intervention group; sVCAM-1 = soluble vascular cell adhesion molecule-1.

Table 3
Significant per-protocol effects of the ActiveBrains intervention on raw and z-score post-intervention Olink neurology-related proteins.

	Intervention group ($n = 42$)	Control group ($n = 39$)	Differences between groups	p	p^{FDR}
CPA2					
Raw score	9.91 (9.79 to 10.04)	10.11 (9.98 to 10.25)	−0.2 (−0.39 to −0.01)	0.040	0.601
z-Score	−0.12 (−0.33 to 0.08)	0.19 (−0.02 to 0.41)	−0.32 (−0.62 to −0.01)		
KYNU†					
Raw score	8.85 (8.71 to 8.98)	9.12 (8.98 to 9.26)	−0.28 (−0.47 to −0.08)	0.005	0.166
z-Score	−0.41 (−0.61 to −0.21)	0.01 (−0.2 to 0.22)	−0.42 (−0.71 to −0.13)		
LAIR2					
Raw score	4.72 (4.6 to 4.85)	4.92 (4.8 to 5.05)	−0.2 (−0.38 to −0.02)	0.028	0.517
z-Score	−0.13 (−0.24 to −0.01)	0.06 (−0.06 to 0.18)	−0.19 (−0.35 to −0.02)		
MSR1†					
Raw score	6.78 (6.72 to 6.84)	6.93 (6.87 to 6.99)	−0.15 (−0.23 to −0.06)	0.001	0.077
z-Score	−0.28 (−0.4 to −0.15)	0.04 (−0.09 to 0.17)	−0.32 (−0.5 to −0.14)		
PLXNB3†					
Raw score	4.18 (4.09 to 4.27)	4.33 (4.24 to 4.43)	−0.15 (−0.28 to −0.02)	0.028	0.517
z-Score	−0.36 (−0.48 to −0.23)	−0.16 (−0.28 to −0.03)	−0.2 (−0.37 to −0.02)		
SCARB2					
Raw score	4.12 (4.06 to 4.18)	4.24 (4.18 to 4.3)	−0.13 (−0.21 to −0.04)	0.004	0.163
z-Score	−0.08 (−0.29 to 0.13)	0.38 (0.16 to 0.6)	−0.46 (−0.77 to −0.16)		

Notes: *Z*-score values indicate how many standard deviations the post-intervention values have changed with respect to the baseline mean and standard deviation. For example, a 0.50 *z*-score means that the mean value at post-intervention is 0.50 standard deviations higher than the mean value at baseline, indicating a positive change; negative values indicate the opposite. Raw scores values are expressed in Normalized Protein eXpression (NPX) units. This means that a high NPX value equals a high protein concentration. Because NPX is in a $\log_2$ scale, a 1 NPX difference means a doubling of protein concentration. If needed, NPX values can be converted into linear scale: 2NPX = linear NPX. Values are expressed as mean (95%CI). Analyses were adjusted for baseline values. $n = 81$ participants. † Proteins assessed by the Proximity Extension Assay technique (i.e., Olink neurological panel) and the enzyme-linked immunosorbent assays. p^{FDR} = adjusted for multiple comparisons using false discovery rate.
Abbreviations: 95%CI = 95% confidence interval; CPA2 = carboxypeptidase A2; KYNU = kynureninase; LAIR2 = leukocyte-associated immunoglobulin-like receptor 2; MSR1 = macrophage scavenger receptor type-I; NPX = Normalized Protein eXpression; PLXNB3 = plexin-B3; SCARB2 = lysosome membrane protein 2.

brain health in children. There are several possible explanations. First, most previous studies investigated these molecular pathways in rodents.[23] Second, the recent controversial studies developed in humans were performed either in healthy adults[22,48–50] or in unhealthy populations (e.g., adults with type-II diabetes or children with autism),[31,51–53] which precludes comparison with our population. Third, the use of different assessment methodologies (e.g., biological medium, centrifugation strategy, temperature, and choice of bioassay) may introduce bias and complicate comparisons between studies. Fourth, the dose of exercise in terms of type, duration, or intensity could have differing effects on blood-circulating biomarkers. Consequently, future studies should standardize the measurement methodology of these biomarkers to improve comparability between studies and explore the effect of other types of exercise interventions of different intensities (e.g., high-intensity interval training) and durations. Furthermore, most of the human studies have gathered evidence with respect to the effects of acute exercise on levels of circulating biomarkers, but we investigated the effects of chronic exercise. While the acute effect of exercise on circulating FGF21 levels is well known, for example, the effect of chronic exercise has not been widely studied.[54] Lastly, it is possible that we did not observe any effect because most of the selected biomarkers are short-life molecules and, consequently, return to basal levels within minutes or hours of experiencing the stimuli.

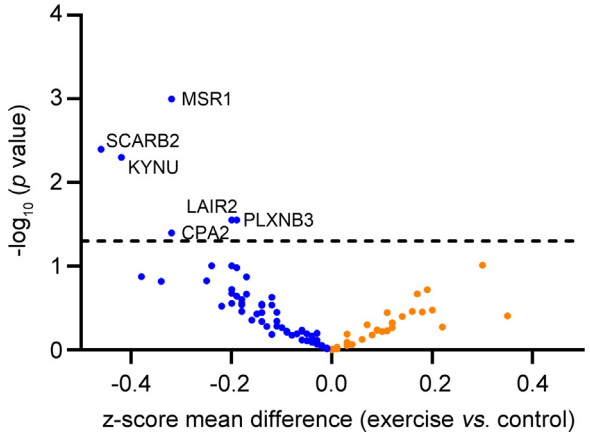

Fig. 2. Volcano plot showing the 6 differentially expressed proteins between exercise and control groups in overweight/obese children controlling for baseline protein levels. Up-regulated proteins are in orange, while down-regulated proteins are in blue. The x-axis reflects the NPX values mean differences (i.e., A 1 NPX value difference means a doubling of protein concentration), while the y-axis indicates statistical significance $p < 0.05$, which is $-\log_{10} > 1.30$ in the horizontal dashed line. CPA2 = carboxypeptidase A2; KYNU = kynureninase; LAIR2 = leukocyte-associated immunoglobulin-like receptor 2; MSR1 = macrophage scavenger receptor type-I; NPX = Normalized Protein eXpression; PLXNB3 = plexin-B3; SCARB2 = lysosome membrane protein 2.

The most robust and consistent finding of the present study is that a 20-week exercise program reduced the protein levels of MSR1 in the plasma of children with OW/OB. MSR1 is a membrane glycoprotein expressed in macrophages, and its relationship to exercise has not been previously explored. We know that it is involved in pathological processes such as

Table 4
ELISA assay technique validation.

Per-protocol	n_{all}	n_i	Intervention group	n_c	Control group	Differences between groups	p
MSR1 (pg/mL)							
Raw score	77	39	238.20 (210.90 to 265.50)	38	288.09 (260.43 to 315.75)	−49.89 (−88.76 to −11.01)	**0.013**
z-Score			−0.14 (−0.32 to 0.04)		0.19 (0.00 to 0.37)	−0.33 (−0.58 to −0.07)	
PLXNB3 (pg/mL)							
Raw score	75	39	2132.66 (1732.94 to 2532.39)	36	2253.75 (1837.57 to 2669.93)	−121.09 (−700.42 to 458.24)	0.678
z-Score			−0.02 (−0.09 to 0.05)		0.00 (−0.07 to 0.07)	−0.02 (−0.12 to 0.08)	
KYNU (ng/mL)							
Raw score	81	42	74.24 (27.13 to 121.34)	39	91.84 (42.95 to 140.74)	−17.60 (−85.65 to 50.44)	0.608
z-Score			0.07 (−0.24 to 0.38)		0.18 (−0.13 to 0.50)	−0.11 (−0.56 to 0.33)	

Notes: *Z*-score values indicate how many standard deviations the post-intervention values have changed with respect to the baseline mean and standard deviation. For example, a 0.50 *z*-score means that the mean value at post-intervention is 0.50 standard deviations higher than the mean value at baseline, indicating a positive change; negative values indicate the opposite. Values are expressed as mean (95%CI). Analyses were adjusted for baseline values.
Abbreviations: 95%CI = 95% confidence interval; KYNU = kynureninase; MSR1 = macrophage scavenger receptor type-I; n_{all} = all sample; n_c = sample of the control group; n_i = sample of the intervention group; PLXNB3 = plexin-B3.

atherosclerosis, non-alcoholic fatty liver disease, and neurological diseases (e.g., Alzheimer's disease and multiple sclerosis).[55–58] Concerning atherosclerosis, oxidized lipoproteins are one of the main factors that contribute to the initiation of the disease.[59,60] Scavenger receptors such as MSR1 lead macrophages to accumulate oxidized lipoproteins and other modified lipoproteins, thus contributing to the formation of atherosclerotic plaque.[55] In this regard, the late stage of atherosclerosis is characterized by increased monocyte and macrophage activity as well as systemic inflammation.[55] Interestingly, a previous study showed that after 6-month of treatment, patients who received atorvastatin showed a significant decrease in MSR1 expression, demonstrating that MSR1 might be involved in the development and/or progression of the acute coronary syndrome.[55] Thus, MSR1 up-regulation in circulating monocytes of patients with acute coronary syndrome might be associated with the late-stage atherosclerosis and inflammation. Concerning non-alcoholic fatty liver disease, Govaere et al.[58] recently found that higher levels of MSR1 were associated with greater accumulation in Kupffer cells (the endogenous hepatic macrophages) resulting in liver inflammation and, in turn, promoting the progression of non-alcoholic fatty liver disease in human and animal models. Taken together, we showed that a 20-week exercise intervention is effective to reduce the circulating plasma levels of MSR1 in children with OW/OB, which might improve some cardiovascular risk factors (e.g., inflammatory profile or lipid levels) associated with the development of subclinical atherosclerosis in youth[61,62] and other neurological diseases (e.g., Alzheimer's disease and multiple sclerosis) later in life.

In the context of neurological disorders, Frenkel et al.[57] reported that amyloid-β (Aβ) accumulation in the brain was positively associated with MSR1 deficiency in mice, while Aβ clearance was increased after pharmacological up-regulation of MSR1 in vitro, which could attenuate Alzheimer's disease progression. On the other hand, in multiple sclerosis, the MSR1 gene was up-regulated in chronic active lesions (in the post-mortem human brain) compared to control tissues and could contribute to demyelination.[63] Additionally, central nervous system demyelination was reduced in $Msr1^{-/-}$ mice (i.e., mice deficient in MSR1) compared to wild-type.[56] In relation to exercise, one study reported that the MSR1 gene was down-regulated in the blood of older adults with prostate cancer who did not practice sports compared to those patients who were "active".[64] In summary, the heterogeneity among studies (e.g., humans or rodents, different tissues analyzed, reported MSR1 protein or MSR1 gene) and the lack of studies on exercise and MSR1 make it difficult to interpret the direction of change in MSR1 in our study. We found that changes in MSR1 did not mediate the effects of chronic exercise on brain health indicators. However, using 2 different techniques (i.e., Olink and ELISA, both of which reported similar effect sizes), we found that chronic exercise did decrease levels of MSR1 protein in the plasma of children with OW/OB. Thus, future studies should examine the effects of exercise (chronic or acute) on MSR1 protein expression in different tissues and combine various human (e.g., different age ranges and clinical conditions) and animal models to reveal the molecular mechanisms underlying the effects of exercise on brain health indicators. In this regard, MSR1 protein may be considered a novel candidate for further studies.

Blood-circulating biomarkers, upon which our exercise program had small effects, did not mediate the effects of exercise on brain health indicators during childhood. Noteworthy is that our exercise intervention showed almost no effects on most of the brain health outcomes included in this study. Therefore, the unmediated role of the explored biomarkers may be partially explained by the small effect of our exercise program on the brain health of children with OW/OB. Additionally, other blood-circulating biomarkers (e.g., repressor element 1 silencing transcription factor, or irisin) not included in this study could be responsible for mediating the effects of exercise on brain health during childhood.[20,65,66] In particular, irisin (a muscle-derived factor secreted from muscle after the shedding of the extracellular portion of the type I membrane protein FNDC5) appears to be increased in the hippocampus of mice following exercise.[20,65] However, measuring irisin with ELISA methods remains challenging and controversial in humans.[67,68] In fact, we decided not to measure irisin in our study due to the lack of specificity in

the currently available ELISA methods, a claim made by numerous authors.[69] Moreover, additional studies are needed to delineate between neurobiological, psychosocial, and behavioral mechanisms responsible for the effects of exercise on brain health in childhood.[70]

A strength of the present study is the design itself, which guarantee that the only difference between the 2 groups (exercise *vs*. control) is their exposure to the treatment of interest. Moreover, much of the previous evidence on the role of exercise in blood-circulating biomarkers is based on acute effect studies. Our study provides evidence of the chronic effects of exercise on these biomarkers, which is novel and has implications for all people who practice exercise on a regular basis. Another strength of this study is the inclusion of a broad set of novel blood-circulating biomarkers, including a candidate-based approach as well as a discovery approach based on a large number of proteins involved in neurological processes. Our study also has a few limitations that need to be considered. Although precautions were taken to reduce the risk of bias in the evaluations (e.g., randomization after baseline assessment, physical trainers not involved in the evaluations), some of the project staff involved in the post-exercise evaluations (e.g., cognitive and mental health assessment) were not blinded to group allocation, which could add some bias to the post-intervention measurements. However, this does not influence the main findings of this study, since all blood-circulating biomarkers were analyzed by personnel who were blinded to group allocation; this also applies to the magnetic resonance imaging outcomes. Finally, our study was conducted in children with OW/OB, and it is unknown the extent to which our findings apply to other populations.

5. Conclusion

We did not find a chronic effect of exercise on the candidate biomarkers of brain health in OW/OB children that were considered in this study. However, our discovery analysis identified a potential reduction effect of exercise on levels of blood-circulating MRS1, which was validated using ELISA methods. Lastly, exercise-induced changes in MRS1 or any other biomarker studied did not appear to mediate changes in brain health indicators. Future studies will confirm or deny the effect of exercise on MRS1 and its implications for health outcomes.

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Authors' contributions

IEC, FBO, and MRA participated in the study design/conception, data acquisition, data analysis, interpretation, manuscript preparation and revision, and the final approval of the manuscript; APF and PSU participated in the data acquisition, data analysis, interpretation, manuscript preparation and revision, and the final approval of the manuscript; AMG participated in the data acquisition, interpretation, manuscript preparation and revision, and the final approval of the manuscript; SA participated in the data analysis, interpretation, manuscript preparation and revision, and the final approval of the manuscript; CMA and AC participated in the interpretation, manuscript preparation and revision, and the final approval of the manuscript. All authors agreed to be accountable for all aspects of the work, contributed to the manuscript writing. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

The authors declare that they have no competing interests.

Supplementary materials

Supplementary materials associated with this article can be found in the online version at doi:10.1016/j.jshs.2022.12.007.

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