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ORIGINAL RESEARCH

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Effects of combined aerobic and resistance training on the inflammatory profile of children with overweight/obesity: A randomized clinical trial

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Summary

Background: We assessed the effects of a 20-week combined (aerobic and resistance) exercise training programme on the inflammatory profile of prepubertal children with overweight or obesity.

Methods: Totally 109 participants (10.1 ± 1.1 years, 41% girls) were randomly allocated to an exercise or control group. Adiponectin, C-reactive protein, epidermal growth factor, insulin-like growth factor-1, interleukin (IL)-1β, IL-6, leptin, tumour necrosis factor-α and vascular endothelial growth factor A (VEGFA) were analysed in plasma. Total white blood cell (WBC) count and immune subpopulations (eosinophils, basophils, neutrophils, lymphocytes and monocytes) were also determined.

Results: No intervention effect was found for any of the analysed biomarkers (all $p \geq 0.05$). We observed a significant sex by intervention interaction for IL-1β ($p = 0.03$). When stratifying the sample by sex, the exercise programme induced a significant effect on IL-1β levels (mean *Z*-score difference, 0.66 [95% confidence interval 0.32–1.01]) in girls, but not in boys. A lower number of girls in the exercise

group showed a meaningful reduction in IL-1β (i.e., ≥0.2 standard deviations) than in the control group (15% vs. 85%, $p = 0.01$).

Conclusions: This exercise programme failed to improve the inflammatory profile in prepubertal children with overweight/obesity. Future studies should explore the effect of longer exercise interventions and in combination with diet.

KEYWORDS
adipokines, cytokines, growth factors, physical exercise, youth

1 | INTRODUCTION

Childhood overweight and obesity increases the risk of developing cardiovascular diseases and type II diabetes later in life.[1] Both conditions involve expansion of subcutaneous and visceral adipose tissue (VAT), which may cause a hypoxic microenvironment—at least in the VAT—associated with adipocyte necrosis.[1,2] In turn, necrosis results in the release of damage-associated molecular patterns (typically known as 'DAMPs') that are recognized by macrophages, triggering the release of pro-inflammatory molecules from these cells, eventually leading to systemic chronic inflammation.[3,4]

Previous cross-sectional studies have reported higher circulating levels of pro-inflammatory cytokines (e.g., interleukin [IL]-1β, IL-6 and tumour necrosis factor [TNF]-α) or acute phase proteins such as C-reactive protein (CRP) in children with obesity compared with their peers with healthy weight.[5–7] Lower levels of adiponectin have been observed in children and adolescents with obesity compared with their controls with normal weight,[7] whereas the data on the association between obesity and insulin-like growth factor (IGF)-1 levels remain inconclusive.[8–10] In addition, no difference in vascular endothelial growth factor (VEGF) levels has been reported between children of different weight categories, yet with higher levels of endothelial growth factor (EGF) in the heavier weight categories.[5] The white blood cell (WBC) count is also considered an inflammatory biomarker linked to obesity, with total WBC and its different subfractions positively associated with body mass index (BMI).[11]

Regular exercise can induce an overall anti-inflammatory effect, partly by reducing VAT mass as well as the expression of toll-like receptors on monocytes and macrophages.[12] A recent study has shown that exercise training is ineffective for reducing TNF-α, IL-6, and CRP in prepubertal children.[13] Yet, the evidence is more limited for the endothelial and hormonal adaptation to regular exercise.[14–16] The VEGF signalling pathway might be upregulated throughout the exercise-derived hypoxia-inducible factor-1,[14] whereas EGF levels seem to be reduced in response to acute exercise.[15] It is noteworthy that changes in IGF-1 levels could be compromised since obesity in children has been demonstrated to attenuate the growth hormone (GH) response to a single bout of exercise.[16]

Based on the evidence available and following the World Health Organization (WHO) guidelines for physical activity in youth,[17] there is a need to investigate the effect of combined aerobic and resistance training on the inflammatory profile of prepubertal children with overweight/obesity.[13] The aim of the present study was to examine the effect of a 20-week combined exercise (aerobic plus resistance) training on circulating cytokines, growth factors and WBC in prepubertal children with overweight/obesity. In addition to the group differences, we explored the inter-individual responses to the intervention to gain further insight into the role of exercise in the inflammatory profile in the context of paediatric obesity.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

The ActiveBrains project is a parallel-grouped randomized clinical trial among children with overweight or obesity. The trial protocol has been previously described.[18] Briefly, this project aimed to examine the effects of a 20-week exercise programme on brain and cognition, as well as on physical and mental health outcomes. We aimed to study prepubertal children of both sexes with overweight or obesity aged 8–11 years, not having any physical disability or neurological disorder that affects their physical/cognitive performance and, in the case of girls, not having started the menstruation at baseline assessments. Left-handedness participants with a score in the Attention-Deficit Hyperactivity Disorder rating scale IV above the 85th percentile or with a psychiatric diagnosis at baseline were excluded. The recruitment occurred mainly at the paediatric units of the San Cecilio and Virgen de las Nieves University Hospitals (Granada, Spain). In addition, we used advertisements in local media and school contacts in the city to recruit the remaining participants. The effects of the ActiveBrains exercise programme on brain health and cardiometabolic risk factors can be found elsewhere.[19,20] Nevertheless, the present study is focused on a subset of cardiometabolic risk factors not yet investigated in this study, that is, biomarkers of systemic inflammation. Of note, although in our ClinicalTrial original registration we referred broadly to risk factors of cardiometabolic diseases as an outcome, which included many blood markers, the present study is focused on reporting and discussing specifically the effects of the intervention on the systemic inflammation biomarkers which have not be reported anywhere else.

Owing to practical and feasibility reasons, the *ActiveBrains* trial was conducted in three waves instead of the two waves that were originally planned. Among 115 children initially screened for

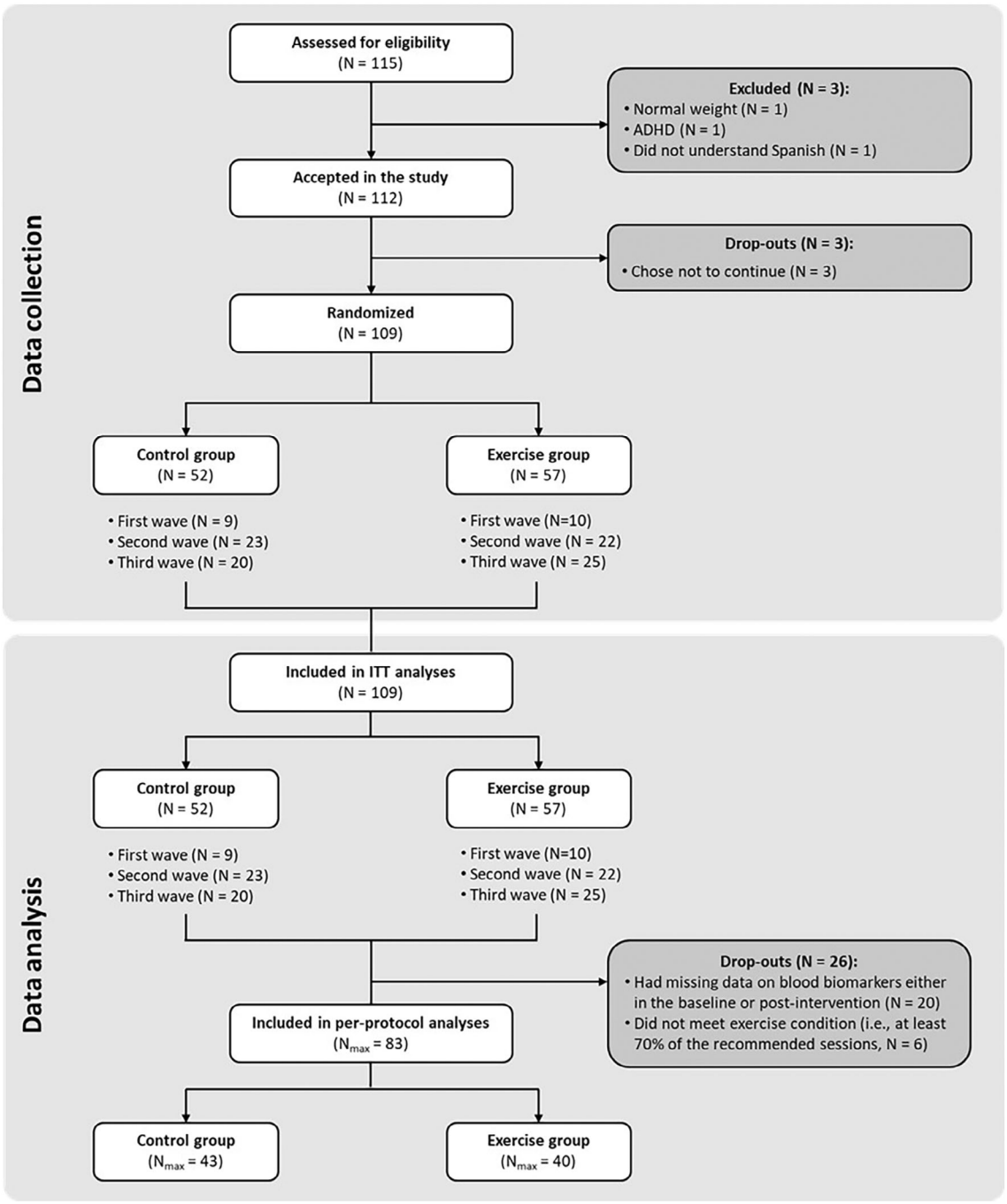

FIGURE 1 Flowchart of the study. ADHD, Attention-deficit hyperactivity disorder; N_{max}, Maximum N for analyses, it changes depending on the variable, see Tables 1 and 2.

participation, 109 were enrolled and randomized to a wait-list control group ($N = 52$) or exercise group ($N = 57$) (Figure 1). Following this strategy, participants of the wait-list group also receive the exercise programme once the whole project has been completed. A computer random number generator in SPSS software for Windows (version 25.0; Armonk, NY, USA) was used to perform participants' simple random allocation into the exercise or the control group. In this context, a "blinded" researcher (FBO) not involved in the assessments nor exercise programme was responsible of the randomization. Equal probability of being allocated to either of the two groups (ratio 1:1) was warranted. The following actions were carried out to reduce the risk of bias: (1) the person that conducted computer random generation was not involved in the assessments; (2) the randomization was performed immediately after the pre-intervention assessments; and (3) the staff in charge of the exercise programme did not take part in the assessments or randomization processes. All baseline and postintervention data were collected from November 21, 2014, to June 30, 2016. A participant information sheet was given to the parents or

legal guardians and a written informed consent was obtained. The **ActiveBrains** project was approved by the Ethics Committee on Human Research of the University of Granada (Reference: 848, February 2014), and was registered at ClinicalTrials.gov (NCT02295072). This trial followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline.

2.2 | The ActiveBrains exercise programme

The exercise programme had a duration of 20 weeks, and its design was based on meeting the international physical activity guidelines at the time of the study design,[21] consistent with recent updates.[17] Both guidelines highlight that physical activity in youth should be mostly aerobic, yet muscle- and bone-strengthening as well as activity of high intensity should be done at least 3 times per week to maximize the health benefits in youth.[17] Participants in the exercise group were asked to attend ≥3 (of 5 offered) sessions per week, all of which were performed during off-school hours and supervised by a Sport and Exercise Specialist. Accordingly, the attendance criterion was set to a minimum of three times/week, and participants meeting 70% of the recommended sessions were included in per-protocol analyses. The sessions were conducted on a group basis and based on the playful component in order to increase adherence to the programme. Each session was structured into four parts: (1) a 5–10 min warm-up consisting of 1–2 physical games of 5 min each; (2) a 60-min aerobic part consisting of around four to five physical multi-games requiring moderate-to-vigorous intensities, with special emphasis on high-intensity activities; (3) a 20-min resistance training part consisting of muscle- and bone-strengthening game-based activities. The resistance part included exercises involving large-muscle-groups for which therabands, fitballs as well as participant's own body weight were used; and (4) a 5–10 min cool-down part consisting of stretching and relaxation exercises. The aerobic part of the session included five playground games/sports in the aerobic training part, including motor skills components (i.e., playful balance, coordination, hand-eye coordination, leg-arm coordination, spatial orientation, and reaction to moving objects or persons). In this regard, the space, number of collaborators/opponents, number of objects, size of the objects and signal-assigned movements were modified during each game. The main objective of the resistance training part was to strengthen the core, arms and legs, which was done in a more analytic way than in the aerobic part. Five to ten exercises focused on the pushing, pulling and throwing patterns were used.

The exercise intensity in both aerobic and resistance parts was monitored by recording heart rate (HR) with a portable telemeter (Polar RS300x, Polar Electro Ltd.; Kempele, Finland). Because children who spend more time above 80% of their maximum HR seem to have more cognitive benefits (*ActiveBrains* main outcome),[22] we also programmed the HR monitors individually at 80% of the maximal HR and at the level of the second ventilatory threshold (VT2, determined during the cardiorespiratory fitness test, see description below), so that we could later obtain the accumulated time over the 80% of the maximum HR and over the VT2. Participants' progress relative to exercise intensity was checked weekly by trained personnel to (i) adapt the intensity of the programme progressively according to the improvements of the participants and (ii) to identify whether any child was training at lower intensities than expected, thus requiring higher motivation during the exercise sessions.

Whereas HR is the most common indicator of the intensity of aerobic training, it is not commonly used as indicator of intensity in resistance training, since it does not directly reflect the stimulus at a muscular level. However, HR is informative of the exercise stimulus at the heart level in any type of exercise, including resistance training, and therefore, when interpreting the intensity of the whole programme at a cardiorespiratory level, we used the HR data of the whole session, i.e., aerobic plus resistance training parts. Training volume was monitored by collecting the number of sessions completed.

Participants in the control group continued their usual routines. An ad hoc question was administered to the control group at post-intervention to check whether they had generally maintained their usual lifestyle or not: "*Did you make any major change in your physical/sport activity participation during the intervention period?*" From these data, we detected that one participant in the control group was enrolled in a swimming club with a heavy training load and competitions. We therefore decided to exclude this participant from the main analyses. Both the control and exercise groups were provided with information about healthy nutrition and recommendations for physical activity at the beginning of the study.

2.3 | Cardiorespiratory fitness

Cardiorespiratory fitness was assessed during a maximal incremental treadmill test at the Andalusian Centre of Sports Medicine (Granada, Spain). Gas exchange variables were analysed breath-by-breath (taking 10-second averages) using a metabolic cart with HR recorded using ECG (General Electric Corp; Boston, MA). Participants walked on the treadmill (h/p/cosmos sports and medical gmbh; Nussdorf-Traunstein, Germany) at a constant speed (4.8 km/h) with a 6% slope with grade increments of 1% every minute until volitional exhaustion. We defined that a maximal effort was achieved if the participants met three of four criteria: reaching >85% of aged-predicted maximal HR, a respiratory exchange ratio of ≥1.0, volitional fatigue (i.e., >8 points in the OMNI scale), and a plateau in the $\dot{V}O_2$ during the last two exercise workloads (i.e., increases <2.0 mL/kg/min).[18] Heart rate was measured with an electrocardiogram. Before the treadmill exercise test, the OMNI scale was explained to children to ensure that they understood the meaning of each category of the scale. However, because of uncertainty about fulfilment of the secondary criteria of maximal effort in children,[23] we collected the peak value of VO_2 attained upon the incremental treadmill exercise test (i.e., VO_{2peak}). We also ran sensitivity analyses in the subsample of children that met the criteria for maximal effort. The VT2 was defined as the workload point where an increase in the ventilatory equivalent for oxygen occurred without a concomitant increase in the ventilatory equivalent for carbon dioxide

production. This was confirmed by inspecting the non-linear increase in ventilation relative to oxygen uptake.

2.4 | Inflammatory profile

Blood samples were obtained between 08.00 and 10.00 am by venipuncture (antecubital vein) after an overnight fast of ≥12 h. The blood samples were drawn into EDTA tubes that were spun immediately (1000 g for 10 min at 4°C). Plasma was isolated, aliquoted and stored at −80°C in the Centre of Biomedical Research in Granada, Spain.

Plasma concentrations of adiponectin, EGF, IGF-1, IL-1β, IL-6, leptin, TNF-α and VEGFA were quantified with the Luminex IS 100/200 system (Luminex Corporation; Austin, TX), using xMAP technology (MILLIPLEX MAP, EMD Millipore Corporation; Burlington, MA), whereas CRP was determined by immunoturbidimetry (Alinity c CRP Vario Reagent, Abbott; Chicago, IL) (intra- and inter-assay coefficients of variation and sensitivity for the different measurements shown in Table S4).

Total WBC count and WBC subpopulations (eosinophils, basophils, neutrophils, lymphocytes and monocytes) were determined with a chemiluminescent immunoassay supplied by Beckman Coulter Inc (Miami, FL), which establishes a sensitivity of 96% and a specificity of 94%.

2.5 | Anthropometry and somatic maturation

The body mass and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, using an electronic scale and a precision stadiometer (SECA 861 and 225, respectively; SECA Corp.; Hamburg, Germany). The BMI was calculated as body mass/height squared (kg/m^2) and the participants were deemed to have overweight or obesity according to the sex- and age-specific cut-off points defined by Cole et al.[24] The peak height velocity (PHV) offset was used as maturational landmark and was predicted through age and anthropometric measures (i.e., height in girls and sitting height in boys) using validated algorithms for boys and girls.[25]

2.6 | Physical activity

Physical activity (PA) was objectively measured by tri-axial accelerometers (GT3X+, ActiGraph, Pensacola, FL, USA) for consecutive days. The participants wore the accelerometers on the non-dominant wrist during 24 h a day and removed it only while bathing or swimming. Accelerometer raw data were processed using the GGIR package for R (GGIR Package, v.1.5.−24, https://cran.r-project.org/web/packages/GGIR/). In brief, raw data were aggregated as the Euclidean Norm Minus One g (ENMO) over 5-s epochs with all negative values rounded to 0. The ENMO metric was used to determine the time spent in moderate-to-vigorous PA by applying previously proposed cut-points validated against metabolic equivalents (measured with indirect calorimetry) moderate-to-vigorous PA.[26]

2.7 | Statistical analyses

Data were analysed using the SPSS version 25.0 for Windows (IBM Corp., Armonk, NY) and statistical significance was defined as $p < 0.05$. The sample size was calculated to achieve at least 80% of statistical power based on the results of a cross-sectional study exploring brain structures (i.e., hippocampus) differences according to cardiorespiratory fitness levels.[18] Data were checked for normality using visual check of histograms, Q-Q plots and box plots.

We reported the findings from the per-protocol analyses because we aimed to study the efficacy of the programme rather than its effectiveness. The analyses of the effects of the intervention were tested using analysis of covariance, with blood parameters as dependent variables, group (exercise vs. control) as fixed factor and the baseline of the study outcome as a covariate. The intervention effects are presented in the raw units of measure and as *Z*-scores of changes. Post-exercise *Z*-scores were calculated relative to the baseline mean and standard deviation (SD) as a standardized measure of the effect size.[27] This effect size can be considered small, medium and large according to the standard benchmarks (0.2, 0.5 and 0.8 SD, respectively).[28] Then, we ran the analyses stratifying the sample by sex. For intervention effects, we kept the standard 5% alpha error for consistency with the reporting of the intervention effects in this trial.[20]

The within-individual change distribution was studied, and the changes exceeding 0.2 Cohen's *d* were considered meaningful (accepted threshold for relevant standardized effect size).[29] Chi-square tests were used to compare the rate of meaningful changes observed in the exercise and the control group. Additionally, the differences between boys and girls on intensity parameters in the exercise group were assessed with analysis of variance.

3 | RESULTS

Of the 109 participants enrolled, six did not meet the exercise intervention requirements (i.e., completion of ≥70% of the recommended training sessions), and 20 had missing data on blood biomarkers either in the baseline or post-intervention (flowchart shown in Figure 1). A total of 83 participants were included in the per-protocol analysis (41% girls; mean (SD) age 10 (1) years; BMI 26.8 (3.6) kg/m^2; and PHV offset −2.2 (0.9)) (Table 1).

Participants in the exercise group showed no significant change in any of the blood parameters assessed ($p \geq 0.05$) (Table 2). These results were consistent when using intention-to-treat instead of per-protocol analyses (Table S2). Figure 2 shows the within- and between-group pre-post differences for boys and girls separately. The tabulated data expressing the outcomes in raw units and standardized are shown in Table S1. We observed a significant sex by intervention interaction effect for IL-1β ($p = 0.03$). The separate analysis by sex

TABLE 1 Descriptive characteristics of the ActiveBrains participants at baseline.

	All		Boys		Girls		Control group		Exercise group	
	N	Mean (SD)	*N*	Mean (SD)	*N*	Mean (SD)	*N*	Mean (SD)	*N*	Mean (SD)
Age (y)	109	10.0 (1.1)	64	10.2 (1.2)	45	9.9 (1.1)	52	10.1 (1.1)	57	9.9 (1.1)
Peak height velocity (y)	109	−2.2 (0.9)	64	−2.6 (0.8)	45	−1.7 (0.9)	52	−2.1 (1.1)	57	−2.4 (0.9)
Height (cm)	109	144.2 (8.4)	64	144.9 (7.9)	45	143.1 (8.9)	52	145.2 (7.9)	57	143.4 (8.9)
Body mass (kg)	109	56.2 (11.2)	64	57.1 (11.2)	45	54.9 (11.3)	52	55.7 (9.4)	57	56.7 (12.8)
Body mass index (kg/m^2)	109	26.8 (3.6)	64	26.9 (3.7)	45	26.6 (3.5)	52	26.3 (2.9)	57	27.3 (4.1)
Moderate-to-vigorous PA (min)	105	54.9 (21.1)	62	62.9 (22.3)	43	43.2 (12.0)	50	54.1 (19.4)	55	55.5 (22.7)
Cytokines and growth factors										
IL-1β (pg/mL)	101	1.6 (0.9)	59	1.5 (0.9)	42	1.7 (0.9)	50	1.6 (0.9)	51	1.6 (0.9)
IL-6 (pg/mL)	97	1.5 [0.7–2.6]	58	1.4 [0.7–2.6]	39	1.6 [0.9–2.5]	46	1.7 [0.7–2.9]	51	1.4 [0.7–2.5]
TNF-α (pg/mL)	97	4.1 (1.4)	60	3.9 (1.6)	43	4.3 (1.4)	41	4.1 (1.4)	56	4.1 (1.4)
CRP (mg/L)	78	2.4 [0.9–4.4]	49	2.5 [1.1–4.5]	29	2.2 [0.8–4.4]	37	2.9 [1.1–4.9]	41	2.0 [0.7–3.4]
Leptin (ng/mL)	104	11.1 (5.6)	61	11.2 (5.6)	43	10.9 (5.5)	51	10.8 (4.6)	53	11.3 (6.3)
Adiponectin (μg/mL)	103	8.1 (5.8)	60	8.2 (5.9)	43	8.1 (5.6)	51	8.4 (6.0)	52	7.9 (5.5)
IGF-1 (ng/mL)	103	95.6 (37.8)	60	86.2 (28.4)	43	108.4 (45.1)	51	97.3 (34.2)	52	94.0 (41.3)
VEGFA (pg/mL)	100	37.4 [24.8–63.3]	58	39.3 [25.6–61.1]	42	34.9 [21.7–84.4]	50	35.9 [25.9–78.7]	50	37.7 [22.4–59.9]
EGF (pg/mL)	94	2.6 [1.4–6.3]	57	2.6 [1.6–6.8]	37	2.4 [1.1–5.3]	48	2.9 [1.5–6.5]	46	2.4 [1.4–6.0]
White blood cells										
Total leucocytes (1000 cells/μL)	101	7.3 (1.7)	62	7.2 (1.7)	39	7.6 (1.6)	50	7.6 (1.6)	51	7.1 (1.7)
Eosinophils (1000 cells/μL)	100	0.3 [0.2–0.5]	61	0.3 [0.1–0.5]	39	0.2 [0.1–0.4]	50	0.3 [0.1–0.5]	50	0.3 [0.2–0.5]
Basophils (1000 cells/μL)	100	0.04 [0.02–0.05]	61	0.0 [0.0–0.1]	39	0.0 [0.0–0.1]	50	0.04 [0.03–0.06]	50	0.04 [0.02–0.04]
Neutrophils (1000 cells/μL)	100	3.8 (1.3)	61	3.7 (1.4)	39	3.9 (1.2)	50	3.9 (1.1)	50	3.8 (1.6)
Lymphocytes (1000 cells/μL)	101	2.6 (0.8)	62	2.5 (0.8)	39	2.7 (0.7)	50	2.7 (0.9)	51	2.5 (0.6)
Monocytes (1000 cells/μL)	100	0.5 [0.4–0.5]	61	0.5 [0.4–0.6]	39	0.5 [0.4–0.5]	50	0.5 [0.4–0.6]	50	0.5 [0.4–0.5]

Note: Data are presented by means ± standard deviation (SD) and median and interquartile range.
CRP, C-reactive protein; EGF, epidermal growth factor; IGF-1, insulin-like growth factor-1; IL, interleukin; PA, physical activity; TNF-α, tumour necrosis factor-alpha; VEGFA, vascular endothelial growth factor A.

indicated that the exercise programme increased IL-1β levels significantly in girls (mean *Z*-score difference, 0.66 [95% confidence interval, 0.32 to 1.01]) but not in boys (0.04, −0.37 to 0.42). Likewise, the exercise programme significantly reduced basophil count in girls (−0.59, −1.14 to −0.11), but not in boys (−0.38, −1.04 to 0.33). The aforementioned IL-1β results were consistent when using intention-to-treat instead of per-protocol analyses, except for basophil count (Table S2 and Figure S1).

Figure 3 shows the individual change distribution in IL-1β levels and basophil count for both control and exercise groups, and by sex. Fewer girls in the exercise group showed a meaningful reduction in IL-1β levels (i.e., ≥0.2 SD) compared with the control group (Panel B, 15% vs. 85%, $p = 0.01$). No differences in the individual response to exercise were found in boys for IL-1β (Panel A, 42% vs. 52% for exercise vs. control group, $p \geq 0.05$). These results were consistent when using intention-to-treat instead of per-protocol analyses (Figure S2, Panel A and B). With regard to basophil count, no differences were found between the exercise and control group when analysing separately girls (Panel D, 58% vs. 35%, $p = 0.18$) or boys (Panel C, 33% vs. 35%, $p = 0.58$). Figure 4 shows the mean differences between boys and girls on intensity parameters in the exercise group. Girls spent less time at exercise intensities above the VT2 than boys during the aerobic part (Panel D, 3.0 vs. 9.1 min respectively, $p = 0.01$) and during the entire sessions (Panel F, 4.9 vs. 14.9 min respectively, $p = 0.01$).

4 | DISCUSSION

The present study investigated the impact of a 20-week exercise programme combining both aerobic and resistance training on the inflammatory profile of prepubertal children with overweight/obesity. The main finding was that this intervention exerted no significant effect on the studied cytokines, growth factors and WBC. We observed,

TABLE 2 Effects of the ActiveBrains exercise programme on cytokines, growth factors and white blood cells (WBCs) (per-protocol analyses).

		Mean (95% CI)				
	N_{all}	*N*	Exercise group	*N*	Control group	Difference between groups	*p*
Cytokines and growth factors							
IL-1β	78	39		39			
Raw score			1.7 (1.5 to 1.9)		1.4 (1.2 to 1.6)	0.3 (0.0 to 0.5)	0.05
Z-score			0.11 (−0.09 to 0.32)		−0.19 (−0.39 to 0.02)	0.29 (0.00 to 0.59)	
IL-6[b]	74	37		37			
Raw score			2.0 (1.6 to 2.5)		1.9 (1.5 to 2.2)	0.2 (−0.3 to 0.7)	0.41
Z-score			0.20 (−0.07 to 0.48)		0.04 (−0.24 to 0.32)	0.16 (−0.23 to 0.56)	
TNF-α[a]	82	42		40			
Raw score			4.2 (3.7 to 4.6)		4.1 (3.6 to 4.5)	0.1 (−0.5 to 0.7)	0.74
Z-score			0.03 (−0.23 to 0.29)		−0.03 (−0.29 to 0.24)	0.06 (−0.31 to 0.43)	
CRP [b]	57	28		29			
Raw score			3.6 (2.5 to 4.9)		2.9 (2.1 to 3.8)	0.8 (−0.6 to 2.4)	0.55
Z-score			−0.01 (−0.29 to 0.28)		−0.13 (−0.41 to 0.15)	0.12 (−0.28 to 0.52)	
Leptin[a]	82	42		40			
Raw score			10.8 (9.4 to 12.1)		11.6 (10.3 to 13.0)	−0.9 (−2.8 to 1.1)	0.39
Z-score			−0.08 (−0.32 to 0.16)		0.07 (−0.17 to 0.32)	−0.15 (−0.49 to 0.19)	
Adiponectin[a]	82	42		40			
Raw score			6.4 (5.2 to 7.7)		5.7 (4.4 to 6.9)	0.7 (−1.1 to 2.6)	0.18
Z-score			−0.38 (−0.57 to −0.19)		−0.57 (−0.76 to −0.37)	0.18 (−0.09 to 0.45)	
IGF-1[a]	82	42		40			
Raw score			97.1 (90.1 to 104.0)		95.1 (87.9 to 102.2)	1.9 (−8.0 to 11.9)	0.69
Z-score			0.00 (−0.18 to 0.18)		−0.05 (−0.24 to 0.14)	0.05 (−0.21 to 0.32)	
VEGFA[a]	76	38		38			
Raw score			47.2 (38.6 to 55.8)		40.4 (31.8 to 49.1)	6.7 (−5.4 to 18.9)	0.26
Z-score			−0.05 (−0.27 to 0.18)		−0.22 (−0.45 to −0.00)	0.18 (−0.14 to 0.49)	
EGF[b]	62	31		31			
Raw score			3.1 (2.1 to 4.3)		4.0 (2.9 to 5.3)	−0.9 (−2.6 to 0.8)	0.29
Z-score			−0.28 (−0.33 to −0.23)		−0.24 (−0.29 to −0.19)	−0.05 (−0.14 to 0.04)	
White blood cells							
Total leukocytes	83	40		43			
Raw score			7.7 (7.2 to 8.2)		7.4 (6.9 to 7.9)	0.3 (−0.4 to 0.9)	0.44
Z-score			0.17 (−0.12 to 0.47)		0.01 (−0.27 to 0.29)	0.16 (−0.25 to 0.57)	
Eosinophils[b]	82	39		43			
Raw score			0.4 (0.3 to 0.4)		0.3 (0.3 to 0.4)	0.1 (−0.1 to 0.1)	0.55
Z-score			0.14 (−0.09 to 0.38)		0.05 (−0.17 to 0.27)	0.09 (−0.22 to 0.42)	
Basophils[b]	82	39		43			
Raw score			0.0 (0.0 to 0.1)		0.1 (0.0 to 0.1)	−0.0 (−0.0 to 0.0)	0.09
Z-score			−0.05 (−0.35 to 0.32)		0.36 (0.07 to 0.65)	−0.42 (−0.85 to 0.07)	
Neutrophils[a]	82	39		43			
Raw score			3.9 (3.5 to 4.3)		3.6 (3.2 to 3.9)	0.4 (−0.2 to 0.9)	0.17
Z-score			0.09 (−0.24 to 0.42)		−0.25 (−0.57 to 0.06)	0.34 (−0.12 to 0.79)	
Lymphocytes[a]	82	39		43			
Raw score			2.8 (2.6 to 2.9)		2.8 (2.6 to 3.0)	−0.1 (−0.4 to 0.2)	0.88
Z-score			0.21 (−0.08 to 0.49)		0.29 (0.03 to 0.57)	−0.09 (−0.49 to 0.31)	

(Continues)

TABLE 2 (Continued)

		Mean (95% CI)					
	N_{all}	*N*	Exercise group	*N*	Control group	Difference between groups	*p*
Monocytes[a]	82	39		43			
Raw score			0.5 (0.5 to 0.6)		0.6 (0.5 to 0.6)	−0.0 (−0.1 to 0.1)	0.52
Z-score			0.37 (0.02 to 0.72)		0.53 (0.19 to 0.86)	−0.16 (−0.64 to 0.33)	

Note: Data analyses were primarily conducted under the per-protocol principle (i.e., follow-up completed data and attending to 70% of the sessions). Data are presented as mean and 95% confidence interval (CI). Each analysis was adjusted for baseline outcomes. Between-group difference represents exercise programme values minus control group values. Baseline *Z*-score of the outcomes were calculated by subtracting the mean value and dividing by the standard deviation (SD) of each outcome. Post-exercise *Z*-scores were calculated relative to the mean and SD of the baseline values, being a *Z*-score of the change in each outcome. i.e., (post-exercise – baseline mean) / baseline SD. *Z*-score values indicate how many SD show follow-up changed with respect to the baseline mean and SD (e.g., a 0.50 *Z*-score means that the mean value at follow-up is 0.50 SD higher than the mean value at baseline, indicating a positive change; with negative values indicating the opposite).

Abbreviations: CRP, C-reactive protein; EGF, epidermal growth factor; IGF-1, insulin-like growth factor-1; IL, interleukin; TNF-α, tumour necrosis factor-alpha; VEGFA, vascular endothelial growth factor A.

[a]Values were log transformed before analysis, but non-transformed values are presented.

however, an interaction by sex and, after stratifying the sample by sex, the intervention significantly increased IL-1β levels while decreasing the basophil count only in girls. Nevertheless, caution should be taken when interpreting this finding due to the small number of girls in the exercise group and future studies should confirm—or contrast—these findings.

4.1 | Intervention effects on the inflammatory profile in the whole sample

Several intervention studies have examined the effects of regular, combined (aerobic and resistance) exercise on inflammatory cytokines in youth with overweight or obesity.[30–35] Our findings are in accordance with previous studies in that exercise training did not induce changes on CRP,[30–35] TNF-α[34,35] or IL-6[34,35] levels in prepubertal children with overweight or obesity. Compared with our intervention, these studies used aerobic[31,33–35] or combined[30,32] exercise programmes, with similar weekly frequencies, lower intensities achieved and lower duration except for the study of Davis et al.[33] which lasted 32 weeks. Additionally, unlike our study, most of the studies including CRP used the high-sensitivity CRP method,[30–33,35] and the results are consistent showing no exercise effect either when using this alternative biomarker. Despite the significant reduction in BMI and fat mass induced by our intervention,[19] adiponectin and leptin levels remained unchanged with exercise training in our study. In line with our findings, some studies have reported no effect of combined exercise training on adiponectin levels in prepubertal children with overweight or obesity.[30,36,37] Compared with our study, these interventions used similar frequency (three sessions/week), but lower intensities (65–70% of participants' maximal HR) and duration (12 weeks). Regarding the intervention effect on leptin levels, this is the first study including prepubertal children of both sexes in the same analysis. These results support the hypothesis that a combined exercise programme shorter than 24 weeks does not seem to reduce the inflammatory levels associated with excessive adipose tissue accumulation in children with overweight/obesity.[38] Future randomized controlled trials should determine circulating levels of novel anti-inflammatory adipokines (e.g., asprosin, spexin and irisin) in order to unravel whether an anti-inflammatory effect occurs with exercise training.

4.2 | Intervention effects on the inflammatory profile stratified by sex

Since the inflammatory profile may vary according to sex as the child matures,[39] several studies have investigated the effect of combined exercise in adolescent boys and girls separately. Our study failed to find a significant intervention effect on CRP, TNF-α, or IL-6 levels in either sex. In contrast with our results, a 12-week high-intensity interval training reduced CRP (standard measurement) and IL-6 levels in prepubertal boys with overweight or obesity.[40] Moreover, Paahoo et al.[40] observed that exercising at 100–110% of maximum aerobic speed exerted greater reductions in CRP and IL-6 than exercising at 40–70% of HR reserve. The latter observation might be explained by the beneficial effect of higher intensities on circulating asprosin, spexin and irisin, which are novel anti-inflammatory adipokines that regulate obesity and related systemic inflammation.[41] Studies comparing the effect of different training intensities on anti-inflammatory adipokines and their role on the inflammatory status are needed. Regarding TNF-α levels, our results are in line with those by Murphy et al.[34] who found no effect of an unsupervised dance-based aerobic exercise programme on TNF-α levels.

It is noteworthy that we found a significant increase of IL-1β (a potent pro-inflammatory cytokine) only in girls. Moreover, less girls in the exercise group showed a meaningful reduction in IL-1β levels compared with the control group and this result was consistent in per-protocol and intention-to-treat analyses. This result disagrees with Scheett et al.[42] who found that a 5-week aerobic exercise programme increased IL-1β levels in prepubertal boys with healthy

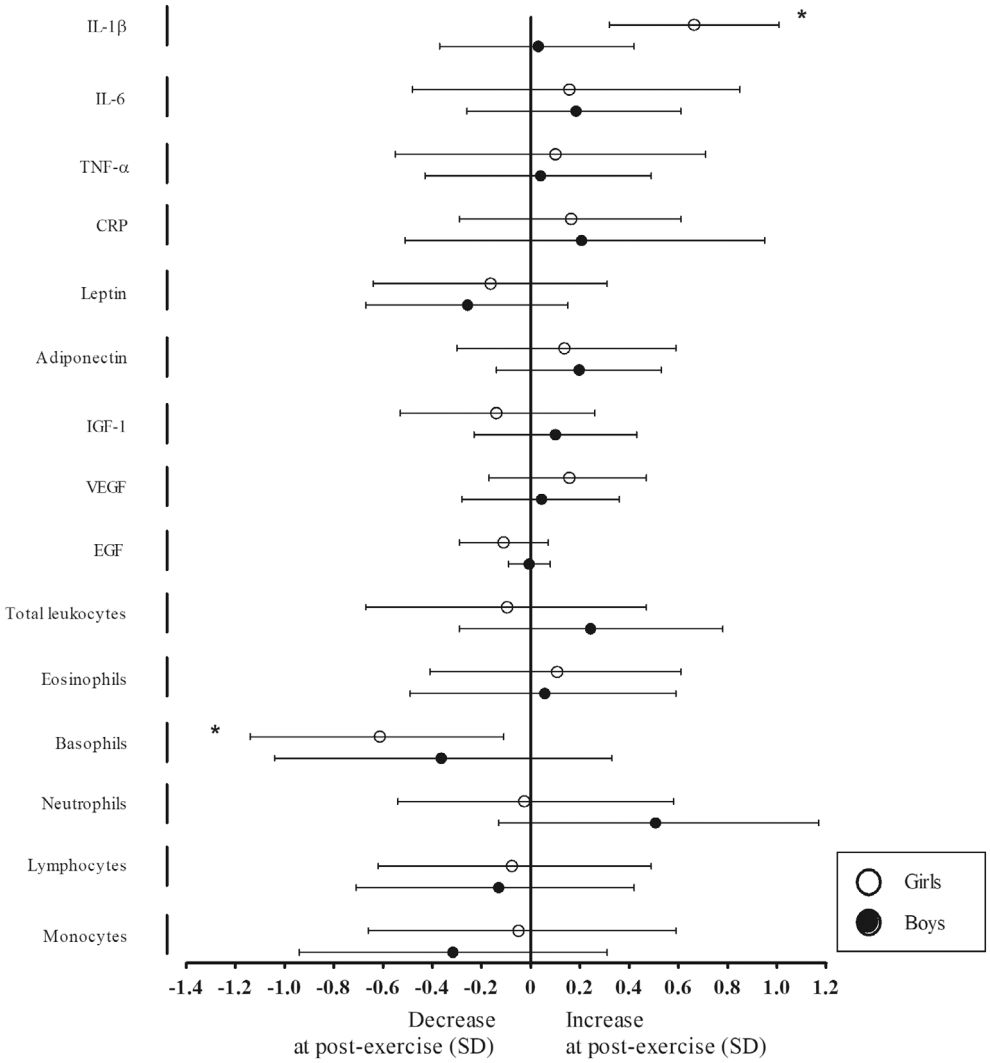

FIGURE 2 Effects of the ActiveBrains exercise intervention on inflammatory cytokines, growth factors and white blood cells (WBCs) by sex (per-protocol analyses). * Indicates a significant interaction term (sex × group) $p < 0.05$. CRP, C-reactive protein; EGF, epidermal growth factor; IGF-1, insulin-like growth factor-1; IL, interleukin; TNF-α, tumour necrosis factor-alpha; VEGFA, vascular endothelial growth factor A.

weight. Interestingly, Khakroo-Abkenar et al.[43] reported a reduction in IL-1β levels after a 12-week moderate-intensity aerobic exercise (Nordic walking) intervention in young men with healthy weight. This result was attributed to the fact that exercise triggers autophagy via AMP-activated protein kinase (commonly known as AMPK), reduces oxidative stress levels, and down-regulates inflammasome activation and subsequent IL-1β secretion.[44] With this in mind, we checked differences between boys and girls on intensity parameters in the exercise group and found that girls spent less time at intensities above the VT2 than boys during the sessions. Hence, we speculated that intensity could have played role in the potential effects on IL-1β levels in children with overweight or obesity. Nevertheless, the significant increase in IL-1β levels should be interpreted cautiously due to the small number of girls in the exercise group.

With regard to adipokines, our intervention did not influence adiponectin or leptin levels in either sex. In this effect, Fazelifar et al.[45] found that a 12-week combined exercise programme had no effect on adiponectin levels in boys with obesity. By contrast, the same authors observed a significant reduction in leptin levels in the same sample.[46] Similarly, previous studies have found that a 12[47] and 16-week[48] aerobic exercise programme significantly reduced leptin levels in boys and girls with obesity. These inconsistent results might be explained by the spexin expression in response to exercise training in this population. Spexin is indeed known to be negatively associated with leptin levels; however, the effect of exercise training on spexin in prepubertal children remained unknown.

Our combined exercise programme failed to modify the levels of growth factors. The data available on IGF-1 adaptations to physical exercise in children are limited. Nevertheless, there is recent meta-analytical evidence for a lack of change in IGF-1 in response to an aerobic exercise training,[16] which is in line with our findings. Theoretically, obesity in children has been demonstrated to decrease GH response to acute bouts of exercise, and this might also affect IGF-1 levels after a chronic stimulus.[49] To our knowledge, ours is the second study examining the effect of an exercise intervention on circulating levels of EGF and VEGFA in youth with overweight/obesity. A previous study reported an increase in VEGF levels after a 12-week combined exercise programme in children with overweight/obesity.[30]

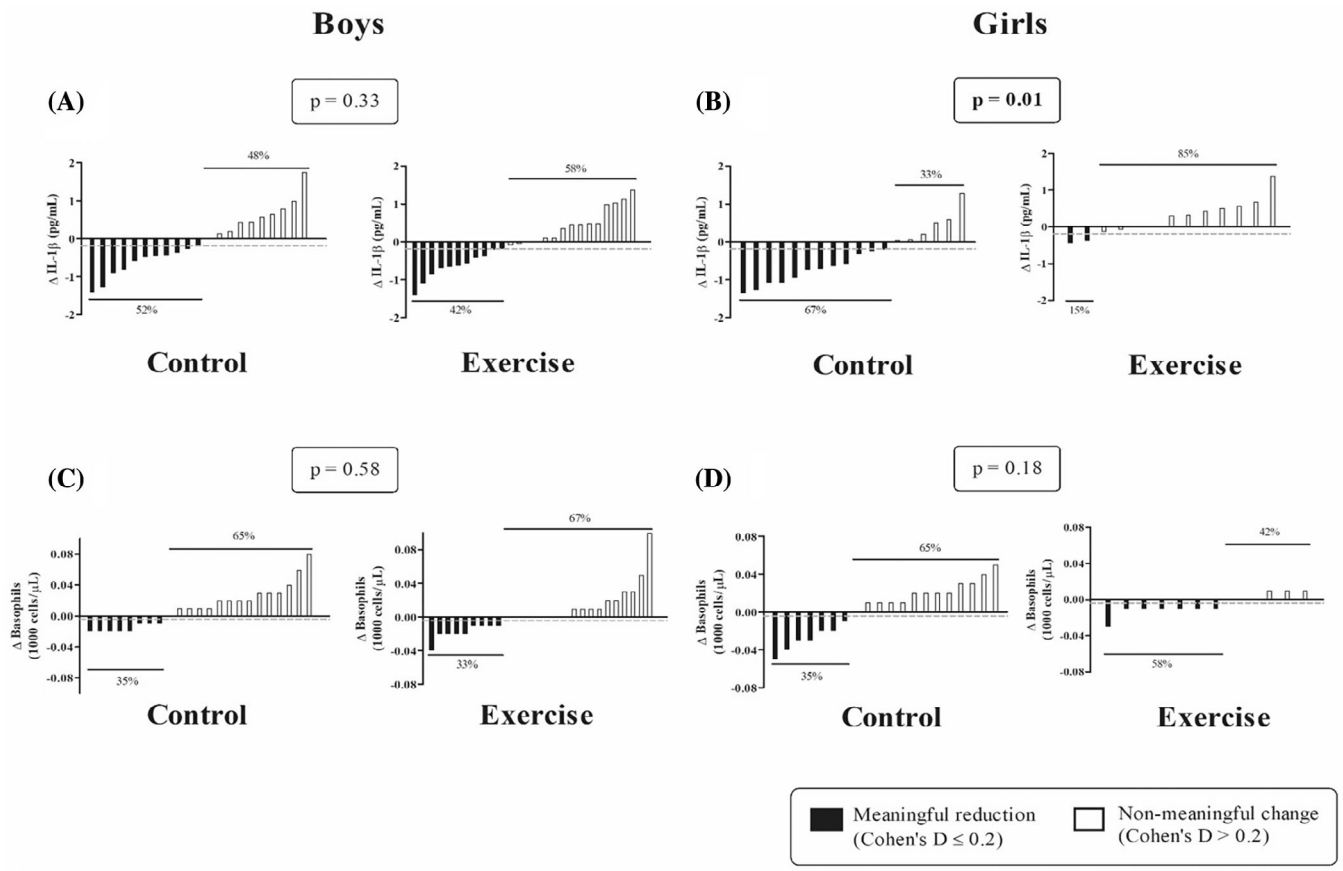

FIGURE 3 Individual change distribution in IL-1β levels (panels A and B) and basophil count (panels C and D) for both control and exercise groups, and by sex. Data analyses were primarily conducted under the per-protocol principle. Dashed lines indicate a meaningful reduction regarding baseline levels. IL, Interleukin; *p*-value from chi-squared test.

Although muscles can secrete VEGF to regulate angiogenesis, modulate blood flow and increase nutrient availability to support tissue growth,[14] we did not observe a significant increase in VEGF levels after the exercise programme. On the other hand, and contrary to our results, Ramirez-Velez et al.[50] found a significant reduction in EGF levels after a 6-month combined exercise programme based on low-to-high-intensity physical education classes in adolescents with overweight or obesity. Given that EGF may play a role in the generation of reactive oxygen species,[15] we speculate that the decrease in EGF levels might represent, a least partly, an exercise adaptation to prevent oxidative stress. However, it is worth noting that, in our study, the children in the exercise arm spent on average 38% of total exercise time (i.e., 25 min per session) at high intensities (above 80% of their maximum HR).[20] In this regard, high-intensity exercise may induce high levels of oxidative stress, which has been reported to modulate EFG signalling.[51]

4.3 | Strengths and limitations

Our study has several limitations. First, the sample size was relatively small, and the inflammatory profile was not a primary outcome of the trial. Second, the intervention only involved physical exercise, and it is known that the combination of exercise and diet is more effective than combined exercise alone to eventually change the inflammatory profile in youth with obesity.[52] The strengths of our study include the randomized design, the determination of a broad set of cytokines, adipokines and growth factors involved in inflammation, and the fact that the intensity of exercise sessions was individually programmed using HR.

5 | CONCLUSION

The findings from our 20-week programme combining aerobic and resistance training do not support an attenuating intervention effect on systemic inflammation biomarkers—that is, circulating levels of cytokines, growth factors and WBC—in prepubertal children with overweight/obesity. In fact, we observed some evidence for an increase in IL-1β (a pro-inflammatory cytokine) in response to exercise in girls. Yet, this finding must be replicated in future studies due to the small number of girls in our exercise group. Future studies should also investigate the effect of longer exercise interventions and in combination with diet.

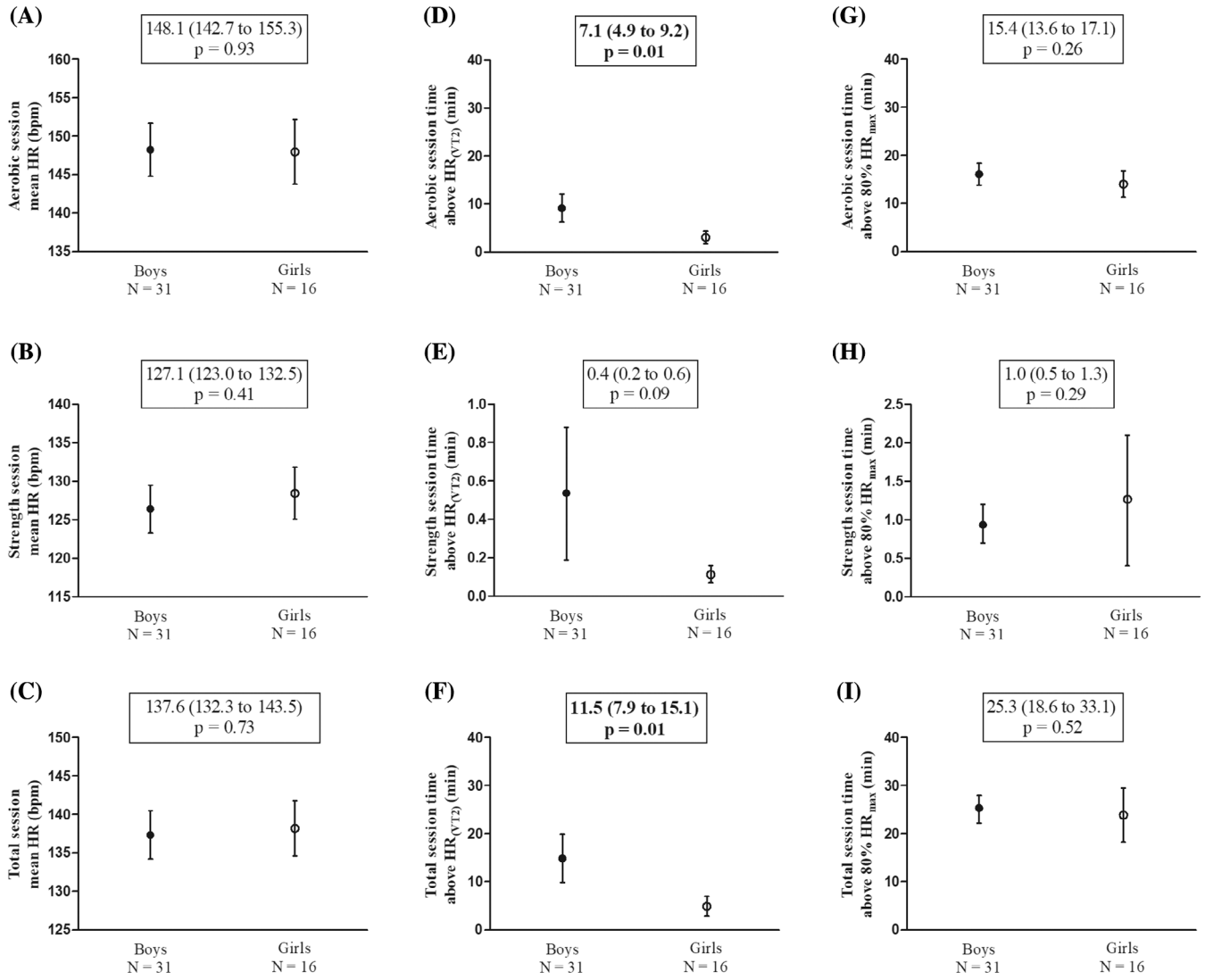

FIGURE 4 Mean differences between boys and girls on intensity parameters in the exercise group. Error bars represent the 95% CI for each parameter. VT2: second ventilatory threshold; bpm: beats per minute; HR: heart rate.

AUTHOR CONTRIBUTIONS

Drs Ortega, Migueles, Cadenas-Sanchez and Labayen participated in the study concept and design. Drs Gil-Cosano, Plaza-Florido, Gracia-Marco, Migueles, Cadenas-Sanchez, Olvera-Rojas, Ubago-Guisado, Labayen, Lucia and Ortega participated in the data acquisition, analysis, or interpretation. Dr Gil-Cosano drafted the manuscript. Plaza-Florido, Gracia-Marco, Migueles, Cadenas-Sanchez, Olvera-Rojas, Ubago-Guisado, Labayen, Lucia and Ortega critically review the final version of the manuscript. Drs Gil-Cosano, Plaza-Florido, Gracia-Marco, Migueles, Cadenas-Sanchez, Lucia and Ortega and participated in the statistical analyses. All authors approved the final version of the manuscript.

The following members contributed to the ActiveBrains project: José Mora-Gonzalez, PhD, Irene Esteban-Cornejo, PhD, Alejandra Mena-Molina, MSc, Ignacio Merino-De Haro, MD, PhD, Juan Pablo Zavala-Crichton, PhD, Patricio Solis-Urra, PhD, Pablo Molina-García, PhD, Lucia V. Torres-Lopez, PhD, Maria Rodriguez-Ayllon, PhD, Miguel Martin-Matillas, PhD, and Jonatan R. Ruiz, PhD, University of Granada, participated in the evaluations or intervention in this project; Gala Maria Enriquez, MSc, María V. Escolano-Margarit, MD, PhD, José Gómez-Vida, MD, PhD, José Maldonado, MD, PhD, and Maria Jose Heras, MSc, "San Cecilio" and "Virgen de las Nieves" Hospitals, assisted with recruitment and screening of participants; Carlos de Teresa, MD, PhD, Rosa Maria Lozano, MSc, and Socorro Navarrete, MD, Centro Andaluz de Medicina del Deporte, provided medical support and realization of physical health evaluations; Maria Elisa Merchan, PhD, Victoria Munoz-Hernandez, PhD, and Wendy Daniela Martinez-Avila, PhD, University of Granada, supported the dietary and nutritional evaluations of the project; Angel Gil, PhD, Belen Pastor-Villaescusa, PhD, Concepcion M. Aguilera, PhD, and Maria Cruz Ruiz, MSc, Centre for Biomedical Research, University of Granada, supported the blood sampling processing and storing; Andrés Catena, PhD, and Juan Verdejo-Roman, PhD, the University of Granada, provided input to the project design and brain analyses; and

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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