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Effects of exercise on whole-blood transcriptome profile in children with overweight/obesity

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Abstract

Background: The current knowledge about the molecular mechanisms underlying the health benefits of exercise is still limited, especially in childhood. We set out to investigate the effects of a 20-week exercise intervention on whole-blood transcriptome profile (RNA-seq) in children with overweight/obesity.

Signe Altmäe and Abel Plaza-Florido contributed equally to this study.

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Methods: Twenty-four children (10.21 ± 1.33 years, 46% girls) with overweight/obesity, were randomized to either a 20-week exercise program (intervention group; $n = 10$), or to a no-exercise control group ($n = 14$). Whole-blood transcriptome profile was analyzed using RNA-seq by STRT technique with GlobinLock technology.

Results: Following the 20-week exercise intervention program, 161 genes were differentially expressed between the exercise and the control groups among boys, and 121 genes among girls (p-value <0.05), while after multiple correction, no significant difference between exercise and control groups persisted in gene expression profiles (FDR >0.05). Genes enriched in GO processes and molecular pathways showed different immune response in boys (antigen processing and presentation, infections, and T cell receptor complex) and in girls (Fc epsilon RI signaling pathway) (FDR <0.05).

Conclusion: These results suggest that 20-week exercise intervention program alters the molecular pathways involved in immune processes in children with overweight/obesity.

1 | INTRODUCTION

Childhood overweight/obesity (OW/OB) is related to higher morbidity and mortality later in life (Ortega et al., 2016). Exercise has the potential to attenuate this adverse consequences by improving a wide range of health-related markers (e.g., fitness, body composition, cardiometabolic risk markers, among others) (García-Hermoso et al., 2018). Currently, there is a limited understanding of the molecular processes underlying the health benefits of regular exercise in pediatric population. Studying the molecular changes that occur following exercise will advance the understanding of how physical activity improves and preserves health in children with OW/OB.

Probably, the ethical and technical difficulty in obtaining biopsies from skeletal muscle and adipose tissue in children has limited the investigation on the effects of exercise at molecular level, compared to research in adult population. An alternative target tissue for studying the effects of exercise on molecular processes is the whole-blood, which is less invasive compared to biopsies and therefore more suitable for pediatric research. In fact, a recent study predicted tissue-specific gene expression from the whole-blood transcriptome across 32 tissues and found that individual's whole-blood transcriptome predicted skeletal muscle expression levels for 81% of the genes and 75% of the genes in adipose tissue (Basu et al., 2021). Hence, a significant part of the muscular and adipose tissue transcripts are reflected in the whole-blood transcriptome (Basu et al., 2021), and additionally whole blood could indicate the low-grade systemic inflammation that is observed in obesity (Maurizi et al., 2018), and could be influenced by exercise (Gleeson et al., 2011).

A few studies have investigated the effects of exercise interventions on gene expression profiles in blood cells of young and middle-aged adults (Denham et al., 2016; Denham et al., 2015; Dias et al., 2015). A 8-week resistance exercise intervention changed the expression of growth factor genes (*GHRH* and *FGF1*) in leukocytes of healthy young men, and a 4-week exercise intervention based on sprints altered the expression of genes involved in cardiovascular health in leukocytes of this same group (Denham et al., 2015). Likewise, an endurance exercise intervention of longer duration (e.g., 18-week) regulated the expression of genes involved in immune function and development in peripheral blood mononuclear cells (PBMCs) of healthy young men (Dias et al., 2015). In the middle-aged group, a 24-week exercise intervention up-regulated the expression of genes involved in inflammation and immune responses (e.g., *IL-2*, *IL-4*, *IL-8*) in men (Thompson et al., 2010). By contrast, a 12-week exercise intervention showed that several genes detected in oxidative phosphorylation gene pathway were up-regulated by exercise in whole-blood of middle-aged women (Rampersaud et al., 2013). To the best of our knowledge, the effects of an exercise intervention (i.e., the effects of

chronic exercise) on whole-blood transcriptome profile in children and adolescents are so far unstudied.

In pediatric population, the effects of short-term/acute exercise on PBMCs transcriptome profile in healthy boys and girls has been shown (Radom-Aizik et al., 2009), genes involved in pediatric inflammatory processes and diseases likes' asthma, apoptosis and, cytotoxic killing factors were altered by acute exercise, suggesting that acute exercise could initiate a "danger" response in PBMCs that might be of benefit to the organism (Radom-Aizik et al., 2009). These are the first studies performed in children that help to unravel the effects of acute exercise on molecular processes, and clearly more studies in different pediatric target groups and exercise programs are warranted.

We set out to investigate the effects of a 20-week exercise intervention on whole-blood transcriptome profile in pre-pubertal children with OW/OB to advance the understanding of the beneficial effects of exercise on molecular processes in childhood obesity. As there is a growing evidence of sex differences in transcriptome landscapes (Lopes-Ramos et al., 2020; Shapiro et al., 2021), we analyzed boys and girls separately.

2 | METHODS

2.1 | Study design and participants

One hundred and nine children with OW/OB were included in the ActiveBrains randomized controlled trial (ClinicalTrials.gov [identifier: NCT02295072]) (Ortega et al., 2022). The ActiveBrains randomized controlled trial aimed to test the effects of a 20-week exercise intervention on brain health outcomes in children with OW/OB (Ortega et al., 2022). Due to budgetary restrictions, a sub-sample of 31 participants was selected for transcriptome analyses in this preliminary study. Two participants were excluded due to low adherence to the exercise program (<66% adherence), 1 participant was excluded for low RNA quality (RIN score <7), and 4 participants for failure in RNA sequencing. Finally, 24 children (10.21 ± 1.33 years, 46% girls) qualified for our preliminary study (also considering the >66% participation/adherence to the training intervention, samples obtained in all time-points, and the RNA quality for transcriptome analysis). Ten children from the exercise group (5 boys and 5 girls) and 14 children from the control group (8 boys and 6 girls). Children included in the current study were pre-pubertal (8–11 years) with OW/OB according to the sex- and age-specific international body mass index (BMI) standards (World Obesity Federation).

The ActiveBrains project was approved by the Committee for Research Involving Human Subjects at the University of Granada (Reference: 848, February 2014). All parents received information about the study and gave their consent following the Declaration of Helsinki guidelines. The assessments were performed from October 2014 to February 2016. Detailed design and methods of this randomized controlled trial have been described elsewhere (Ortega et al., 2022). Briefly, the randomization was done after the pre-intervention data collection. Likewise, the person who performed the computer random generation and exercise trainers were not involved in the pre-intervention data collection.

2.2 | Exercise training intervention

The 20-week exercise training intervention has been described in detail (Ortega et al., 2022). In short, the exercise training intervention based on multi-games is in line with the international physical activity guidelines (Bull et al., 2020). The minimum frequency of exercise was three sessions per week, and the desired frequency was five sessions. Each session lasted for 90 min. The general structure of the sessions was: (1) warm-up (5–10 min) consisting of 1–2 physical games; (2) aerobic part (60-min) at moderate-to-vigorous intensities based on 4–5 multi-games (emphasis above 80% of heart rate (HR) max) and (3) resistance training (20-min) comprising of strength exercises involving large-muscle-groups in sets of 10–12 repetitions using bodyweight, theraband and/or fitballs; and (4) cool-down (5–10 min) based on relaxation and stretching exercises.

The intensity of the exercise intervention was recorded in each session using heart rate monitors (Polar RS300X, Polar Electro Oy Inc, Kempele, Finland). The maximum heart rate achieved by each child in a maximal incremental treadmill test was used to individually program the heart rate monitors. The heart rate data were assessed for the aerobic and resistance training part independently due to the different physiological demand of the cardiovascular system in these parts of the sessions. The children assigned to the control group were advised to continue with their everyday life and information of healthier lifestyle regarding physical activity and nutrition were provided.

2.3 | Body composition measurements and maturational status

Body composition measurements were performed before and after the exercise intervention period. Body weight and height were measured with an electronic scale and a stadiometer (Seca instruments, Germany, Ltd) and BMI

was calculated as kg/m^2. Waist circumference was evaluated as an indicator of central fat using the International Society for the Advancement of Kinanthropometry (ISAK) procedures. Fat mass index (FMI), body fat percentage (BFP) and lean mass index (LMI) were measured by dual energy X-ray absorptiometry (DXA, Discovery densitometer from Hologic), and were calculated as the ratio between the fat mass and fat-free mass (kg) with the squared height (m^2) and the percentage (%) of adipose tissue relative to body weight, respectively. Also, visceral adipose tissue (VAT) was measured. The maturational status of the participants was reflected by the peak height velocity (PHV). This variable was derived from height and seated height using Moore equations: in boys: $-8.1 + (0.0070346 \times (\text{age} \times \text{sitting height})$; and in girls: $-7.7 + (0.0042232 \times (\text{age} \times \text{height})$. Maturity offset was calculated by subtracting the PHV age from the chronological age.

2.4 | Cardiorespiratory fitness

Cardiorespiratory fitness (i.e., VO_2peak) was assessed before and after the 20-week exercise intervention period in the laboratory using a gas analyzer (General Electric Corporation) while performing a maximal incremental treadmill test (HP-Cosmos ergometer) adapted for low-fit children (Plaza-Florido et al., 2021). Briefly, the incremental test consisted of walking on a treadmill at a constant speed (4.8 km/h) starting at a 6% slope with grade increments of 1% every minute until volitional exhaustion (Plaza-Florido et al., 2021). Oxygen consumption (ml/min), HR (beats/min) and respiratory exchange ratio (RER) were continuously recorded every 10 s, whilst the rating of perceived exertion (RPE) scale was reported at the end of each 1 min stage using children's OMNI scale ranging from 0 to 10. Absolute peak oxygen consumption (VO_2 peak absolute; ml/min) and oxygen consumption relative to body weight (VO_2 peak; ml/kg/min) were provided as indicators of cardiorespiratory fitness.

2.5 | Blood sampling and analysis

Peripheral blood samples were was collected at two timepoints, before and after the exercise intervention period. Participants arrived at the laboratory between 8:00 and 9:00 AM after an overnight fasting of at least 12 h. Venous blood (3 mL) was collected into EDTA tube, which was subsequently centrifuged at 1000 × g for 10 min, and the plasma was isolated and stored at −80°C until biochemical analyses. For RNA-seq analysis, 500 μL of whole-blood was collected into the tube that contained 1.3 mL RNA later solution (Ambion Inc; Austin, Texas) and was stored at −80°C until further processing.

2.5.1 | Pro-inflammatory markers

Interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) cytokines were quantified by multiple analyte profiling technology (MILLIPLEX® MAP Human High Sensitivity T Cell Magnetic Bead Panel, EMD Millipore Corporation, Missouri, USA) using a kit plex (HCYIL6-MAG Anti-Human IL-6 Beads set, HCYIL1B-MAG Anti-Human IL-1β Bead, and HCYTNFA-MAG Anti-Human TNFα Beads set). Vascular endothelial growth factor A (VEGFA) was measured using multiple analyte profiling technology (MILLIPLEX® MAP Growth Factor Magnetic Bead Panel 1, EMD Millipore Corporation, Missouri, USA). The intra- and inter-assay coefficients of variation for IL-6 were ≤5% and ≤20%, and sensitivity was 0.11 pg/mL. For IL-1β and TNF-α the intra- and inter-assay coefficients of variation were ≤5% and ≤15%, respectively, with a respective sensitivity of 0.14 and 0.16 pg/mL.

2.5.2 | RNA extraction and sequencing

The blood samples for transcriptome analyses were obtained in spring 2015 and were stored in RNA solution. RNA extractions were performed in June 2016, and libraries and RNAseq analyses were conducted in September 2016. Total RNA was isolated from the whole-blood samples using RiboPureTM-Blood Kit (Thermo Fisher Scientific; Waltham, Massachusetts), followed by treatment with GlobinLock system (GL) in order to block the high globin mRNA content of erythrocytes, which is abundant in the blood and could hamper the whole-blood RNA analyses. The RNA integrity number (RIN) higher than 7 was considered as high quality for transcriptome analyses. The RIN values in our sample included for analyses ranged from 7.4 to 9.2 except for one participant that presented a RIN number <7 and was excluded.

Total transcriptome analysis was performed following the single-cell tagged reverse transcription (STRT2) RNA-seq protocol as described in detail before (Krjutškov et al., 2016). Briefly, 10 ng of high-quality input RNA was converted into cDNA and amplified to form an Illumina-compatible library. The STRTprep pipeline v.3.0.0, available at https://github.com/shka/STRTprep/tree/v3dev, was used for processing the raw sequencing reads, aligning to the hg19 genome and

quantitating the expression levels. RNA-seq data is publicly available at www.ncbi.nlm.nih.gov/geo under accession number GSE193771.

2.6 | Data analyses

2.6.1 | Descriptive and physiological effects of exercise training

Descriptive data are presented as the mean and standard deviation (mean ± SD), or frequency and percentage for categorical variables. The effects of exercise intervention were performed on body composition variables, cardiorespiratory fitness, and inflammatory markers in boys and girls separately. The analyses were performed using SPSS version 21.0 (IBM Corporation, NY), and the statistical significance was defined at the level of $p < 0.05$. Analysis of covariance (ANCOVA) was performed using post-exercise data as dependent variables, group (i.e., exercise vs. control) as a fixed factor, and baseline data as covariates. The z-scores for each variable at post-exercise (i.e., (post-exercise individual value − baseline value)/baseline SD) were calculated as an effect size indicator of the changes caused by the exercise, being considered a value around 0.2 a small effect size, 0.5 medium effect size and, 0.8 a large effect size. Including the baseline value of the study outcome (i.e., pre-intervention value) as a covariate and the post-intervention value as the outcome is equivalent to studying the change in the outcome; therefore, this model indicates the time × group interaction intended to know the effects of the intervention (Ortega et al., 2022).

2.6.2 | Gene expression profiles

Quantile normalization was performed for RNA-seq data. Subsequently, the differential gene expression analysis between the exercise and the control groups (i.e., interaction: group [exercise; control] × time [pre-intervention; post-intervention]) was performed using the Limma R/Bioconductor software package (Ritchie et al., 2015), in boys and girls separately. For more information, our scripts files of the Limma analyses are available in the Open Science Framework (https://osf.io/5a9nh/).

The differentially expressed genes (i.e., interaction analyses) were further characterized by enrichment analysis using the LIMMA function topKEGG in R software environment ($p < 0.05$). All analyses were controlled for Benjamini-Hochberg multiple testing correction (false discovery rate [FDR] < 0.05). Pathway direction was calculated as the median $\log_2$FC fold change of significant transcripts in each pathway (Contrepois et al., 2020). Further, the gene networks were computed with QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity), using as input the list of differentially expressed genes between the exercise and control groups (boys and girls separately). Networks were generated based on the connectivity among genes and top-IPA networks, with the cut-point of 35 molecules (default settings) and the highest IPA score per network.

2.7 | In silico data mining and validation

We performed in silico data mining using PHENOPEDIA database and validation of the transcriptome data in two steps. First, PHENOPEDIA database was used to perform in silico data mining with the aim of finding common genes/pathways with obesity and its associated diseases. PHENOPEDIA provides lists of genes involved in different diseases (based on genetic associations studies), which are continuously updated from PubMed. Genes regulated by exercise intervention (i.e., exercise vs. control) in our study were compared with lists of genes involved in obesity and other diseases and risk factors associated with obesity (i.e., CVD, inflammation, and metabolic syndrome). Next, the in silico validation was performed using a up-to-date database https://extrameta.org/, which is derived from an extensive meta-analysis database using 43 publicly available transcriptome data from human skeletal muscle and blood in response to acute and exercise interventions (Amar et al., 2021). Our validation was focused on genes that were identified in: (1) KEGG gene pathways (FDR <0.05) (i.e., pathways analyses using the LIMMA function topKEGG in R); (2) Top-IPA networks; and (3) were detected in at least one of the PHENOPEDIA disease terms.

3 | RESULTS

3.1 | Sample characteristics and physiological effects of training

Descriptive characteristics of participants at baseline are summarized in Table 1. The study sample had an average chronological age of 10.76 ± 1.34 and biological maturation age of −2.17 ± 0.97 years for boys, while for girls the chronological age was 9.57 ± 1.35, and biological maturation age − 1.88 ± 0.87 years.

The main effects of the exercise intervention on body composition and cardiorespiratory fitness are reported in

TABLE 1 Descriptive characteristics of the study sample.

Variables	Total sample ($n = 24$, 46% girls)	Boys ($n = 13$, exercise 5/control 8)	Girls ($n = 11$, exercise 5/control 6)
Age and maturational status			
Age (years)	10.21 ± 1.33	10.76 ± 1.34	9.57 ± 1.05
PHV offset (years)	−2.04 ± 0.92	−2.17 ± 0.97	−1.88 ± 0.87
Body composition			
Weight (kg)	58.37 ± 9.99	59.73 ± 11.82	56.76 ± 7.53
Height (cm)	146.53 ± 9.03	146.04 ± 10.34	143.56 ± 6.43
Waist circumference (cm)	92.46 ± 6.82	92.10 ± 7.95	92.88 ± 5.55
BMI (kg/m^2)	27.01 ± 2.51	26.61 ± 2.48	27.49 ± 2.57
FMI (kg/m^2)	11.52 ± 2.11	10.82 ± 1.88	12.36 ± 2.15
BF (%)	43.15 ± 4.69	41.14 ± 4.12	45.52 ± 4.33
Visceral adipose tissue (g)	426.03 ± 83.67	422.91 ± 92.81	429.71 ± 75.75
LMI (kg/m^2)	14.35 ± 1.03	14.67 ± 1.07	13.98 ± 0.90
Cardiorespiratory fitness			
VO2peak absolute (ml/min)	2194.83 ± 485.54	2336.62 ± 578.54	2027.27 ± 289.06
VO2peak relative (ml/kg/min)	37.56 ± 4.63	39.07 ± 5.48	35.77 ± 2.62
Inflammatory markers			
IL-1β (pg·mL-1)[a]	1.80 ± 0.75	1.68 ± 0.63	1.93 ± 0.89
IL-6 (pg·mL-1)[a]	2.11 ± 1.10	2.03 ± 1.23	2.22 ± 0.95
TNF-α (pg·mL-1)[a]	4.02 ± 1.17	3.87 ± 1.24	4.21 ± 1.11
VEGFA (pg·mL-1)[a]	28.08 ± 13.97	32.04 ± 14.65	23.32 ± 12.11

Note: Data presented as mean ± SD, and as number and frequency. BMI: Body mass-index, BF: Body fat, FMI: Fat mass index, IL: interleukin, PHV: Peak height velocity, TNF-α: tumor necrosis factor alpha, VEGFA: vascular endothelial growth factor A.
[a]Sample size baseline: IL-1β (boys 12; girls 10); IL-6 (girls 9); TNF-α (girls 10); VEGFA (boys 12; girls 10).

the complete sample from the ActiveBrains project (Ortega et al., 2022). In this subsample ($n = 24$) with transcriptome data from all time-points, in boys, the exercise intervention reduced waist circumference and improved significantly cardiorespiratory fitness (medium and large effect sizes: −0.41 SDs to 1.29, all $p \leq 0.041$) (Supplemental Table S1). Among girls, body fat percentage and inflammatory marker VEGFA decreased after exercise intervention (large effect sizes: −0.87 SDs, $p = 0.080$ and −1.44 SDs, $p = 0.056$) (Supplemental Table S2). Time at >80%HR max during exercise sessions was significantly higher in boys compared to girls (Supplemental Table S3), supported by the significant improvement of VO_2 values in boys but not in girls.

3.2 | Gene expression profiles

Following the training program, 161 genes were differentially expressed (71 up-regulated and 90 down-regulated; Figure 1) in boys allocated in the exercise group compared to boys in the control group (log_2FC ranged from −1.93 to 2.36; $p < 0.05$) (Supplemental Table S4). Among girls, 121 genes were differentially expressed (51 up-regulated and 70 down-regulated; Figure 2) in the exercise group compared to the control group (log_2FC ranged from −2.35 to 1.65; $p < 0.05$) (Supplemental Table S4). Among the two gene lists, only eight genes overlapped between the sexes: *RAB7A*, *TXNL4B*, *CKS1B*, *TPP1*, *CCN1*, *CALR*, *EMD*, and *LASP1*. After applying multiple hypothesis testing, none of the genes (161 genes in boys and 121 genes in girls) were significantly differentially expressed between exercise and control groups (all FDR >0.05) (Supplemental Table S4).

The KEGG pathway analysis of the genes regulated by exercise in boys was significantly enriched in five pathways, such these include antigen processing and presentation, and infections (yersinia, salmonella, human immunodeficiency virus, pathogenic Escherichia coli) that were up-regulated after exercise and GO (Gene

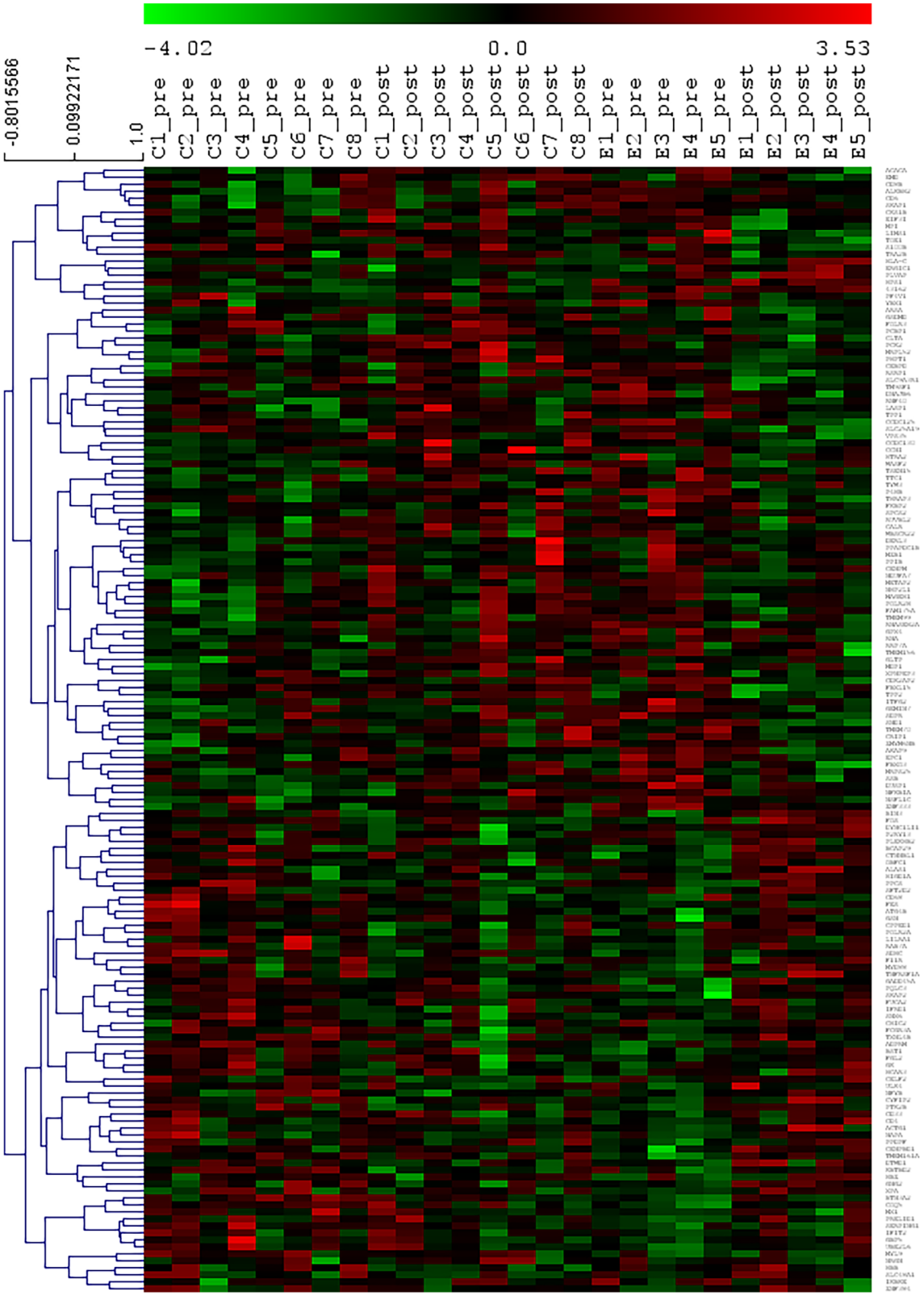

FIGURE 1 Heatmap of differentially expressed genes between exercise and control groups at different time-points (pre- and after intervention) in boys (161 genes, unadjusted *p*-value <0.05). Red indicates up-regulated genes and green down-regulated genes (z-values of quantile-normalized expression levels). Columns represent each subject in the specific group (i.e., exercise or control) and the time-point (i.e., pre- or post-intervention).

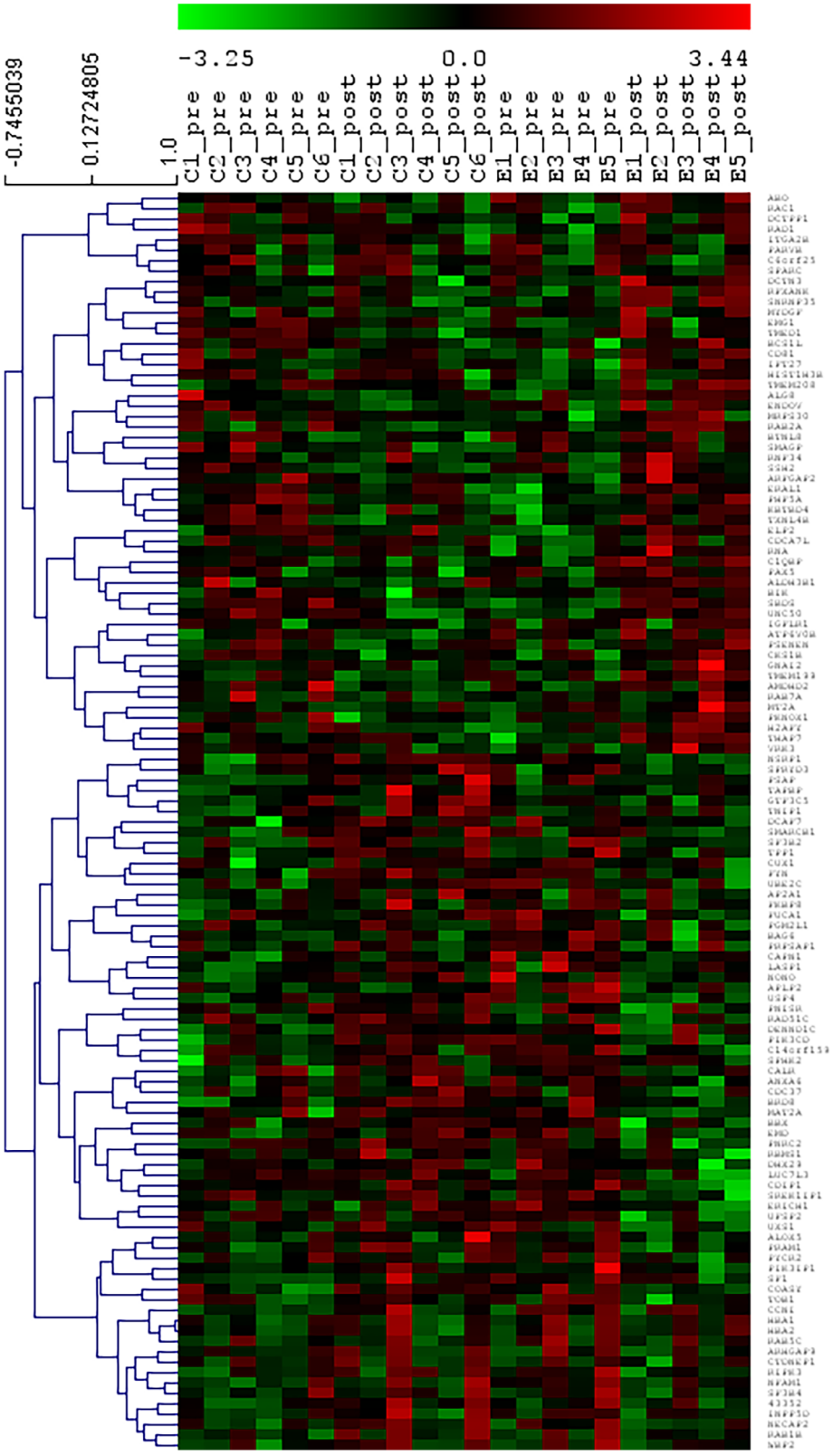

FIGURE 2 Heatmap of differentially expressed genes between exercise and control groups at different time-points (pre- and after intervention) in girls (121 genes, unadjusted *p*-value <0.05). Red indicates up-regulated genes and green down-regulated genes (z-values of quantile-normalized expression levels). Columns represent each subject in the specific group (i.e., exercise or control) and the time-point (i.e., pre- or post-intervention).

ontology) terms T cell receptor complex and endoplasmic reticulum chaperone complex that were down-regulated by exercise (FDR <0.05) (Table 2). In addition, immune gene pathways such as NOD-like receptor, B cell receptor signaling, and adipocytokine signaling pathway were down-regulated by exercise in boys ($p < 0.05$), while other known immune pathways such as Toll-like receptor and nuclear factor-κB (NF-κB) did not show a clear direction or were up-regulated respectively (Supplemental Table S5 and Supplemental Figure S3).

TABLE 2 Enrichment analyses of differentially expressed genes by a 20-week exercise intervention (i.e., exercise vs. control) in boys and girls (statistically significant pathways FDR < 0.05).

KEGG pathways and GO terms	Genes identified in each pathway
Boys, 5 KEGG gene pathways and 2 GO terms	
Antigen processing and presentation (KEGG)	***CD4*** (↑), ***HLA-C*** (↑), *NFYB* (↑), ***CD8B*** (↓), ***CALR*** (↓)
Yersinia infection (KEGG)	*ACTG1* (↑), ***CD4*** (↑), ***CD8B*** (↓), *FOS* (↑), ***MYD88*** (↑), ***NFKBIA*** (↓), ***PTK2B*** (↑), *SKAP2* (↑), *WASF2* (↓)
Salmonella infection (KEGG)	*ACTG1* (↑), *CYFIP2* (↑), *DYNC1LI1* (↑), *FOS* (↑), *GSDMD* (↓), ***MYD88*** (↑), *MYL9* (↑), ***NFKBIA*** (↓), *RAB7A* (↑), ***TNFRSF1A*** (↑)
Human immunodeficiency virus 1 infection (KEGG)	***CALR*** (↓), ***CD4*** (↑), *FOS* (↑), *GNG2* (↑), ***HLA-C*** (↑), ***MYD88*** (↑), ***NFKBIA*** (↓), ***PTK2B*** (↑), ***TNFRSF1A*** (↑)
Pathogenic Escherichia coli infection (KEGG)	*ACTG1* (↑), *CYFIP2* (↑), *FOS* (↑), ***MYD88*** (↑), ***NFKBIA*** (↓), *SLC9A3R1* (↓), ***TNFRSF1A*** (↑), *WASF2* (↓)
T cell receptor complex (GO)	***CD4*** (↑), *SKAP1* (↓), ***CD8B*** (↓), ***CD6*** (↓)
endoplasmic reticulum chaperone complex (GO)	*PPIB* (↓), *P4HB* (↓), *MZB1* (↓)
Girls, 1 KEGG gene pathway and 6 GO terms	
Fc epsilon RI signaling pathway (KEGG)	***RAC1*** (↑), ***FYN*** (↓), ***INPP5D*** (↓), *ALOX5* (↓), ***PIK3CD*** (↓)
Endoplasmic reticulum-Golgi intermediate compartment membrane (GO)	*TMED1* (↑), *RAB2A* (↑), ***CALR*** (↓), *TAPBP* (↓), *RAB1B* (↓)
Platelet alpha granule membrane (GO)	***ITGA2B*** (↑), ***SPARC*** (↓), *APLP2* (↓)
Endoplasmic reticulum-Golgi intermediate compartment (GO)	*TMED1* (↑), *MYDGF* (↑), *RAB2A* (↑), ***CALR*** (↓), *TAPBP* (↓), *RAB1B* (↓)
Melanosome (GO)	***RAC1*** (↑), *RAB2A* (↑), *RAB7A* (↑), *RAB5C* (↓), ***ANXA6*** (↓), *TPP1* (↓)
Pigment granule (GO)	***RAC1*** (↑), *RAB2A* (↑), *RAB7A* (↑), *RAB5C* (↓), ***ANXA6*** (↓), *TPP1* (↓)
U12-type spliceosomal complex (GO)	*SNRNP35* (↑), *SF3B4* (↓), *SF3B2* (↓)

Note: (↑): gene up-regulated (exercise vs. control), (↓): gene down-regulated (exercise vs. control). Bold indicate genes overlapped between genes identified in gene pathways regulated by exercise and Top-IPA gene networks. GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

However, the abovementioned pathways were not significant after multiple correction testing (FDR >0.05) (Supplemental Table S5 and Supplemental Figure S3).

In girls, Fc epsilon RI signaling pathway and GO terms including endoplasmic reticulum-Golgi intermediate compartment membrane, platelet alpha granule membrane, endoplasmic reticulum-Golgi intermediate compartment, melanosome, pigment granule, and U12-type spliceosomal complex were down-regulated after exercise intervention (FDR < 0.05) (Table 2). In addition, gene pathways involved in inflammation such as Fc gamma R-mediated phagocytosis, VEGF signaling, and platelet activation were down-regulated by the exercise intervention in girls ($p < 0.05$), nevertheless they were not significant after multiple correction testing (FDR > 0.05) (Supplemental Table S5 and Supplemental Figure S4). Several pathways involved in immune processes, including antigen processing and presentation, B cell receptor signaling, phagosome, lysosome, and leukocyte transendothelial migration were common pathways regulated by exercise in both obese/overweight boys and girls ($p < 0.05$), however did not pass the multiple correction testing (FDR >0.05) (Supplemental Table S5).

Top-IPA gene network in boys (IPA score 41) highlighted the molecular functions such as cell death and survival, cellular development, and inflammatory response influenced by exercise intervention in boys (Figure 3), where genes *CD4*, *HLA-C*, *TNFRSF1A*, *MYD88*, *PTK2B*, *CD8B*, *CD6*, *CALR*, *NFKBIA* are of special interest as they also belong to the KEGG pathways significantly influenced by exercise (Table 2; Supplemental Figure S1). In girls, the top-IPA gene network (IPA score 51) highlighted the involvement of cell-mediated immune response, cellular function and maintenance, and hematological system development and function stimulated by exercise intervention (Figure 4), where genes *RAC1*, *ITGA2B*, *FYN*, *INPP5D*, *PIK3CD*, *CALR*, *TAPBP*, *SPARC*, *ANXA6* are of interest as they were also detected in the KEGG pathway analyses influenced by exercise (Table 2; Supplemental Figure S2).

3.2.1 | In silico data mining and validation

In silico data mining of the differentially expressed genes in boys after exercise intervention using the PHENOPEDIA database detected 21 of the genes to be involved in obesity, 9 genes in CVD, 31 genes in inflammation, and 10 genes in metabolic syndrome. Interestingly, genes *CD4*, *CD6*, *HLA-C*, *TNFRSF1A*, *MYD88*, *PTK2B*, *CALR*, *NFKBIA* were identified in our gene pathways (Table 2), Top-IPA network (Figure 3), and in PHENOPEDIA disease terms. Furthermore, five out of

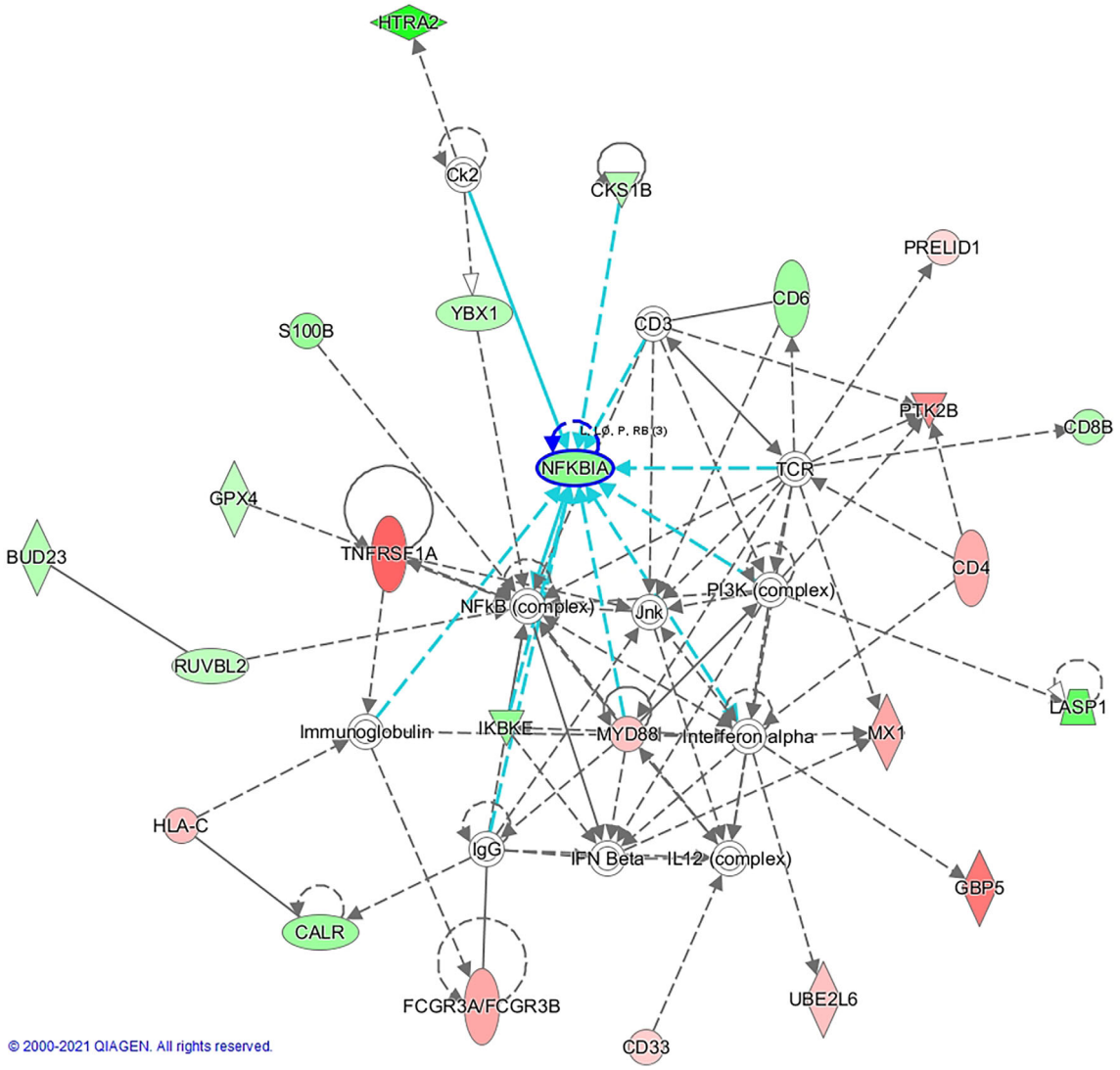

FIGURE 3 Top-IPA gene network in boys, enriched with IPA disease terms and functions such as cell death and survival, cellular development, and inflammatory response. The red color indicates up-regulated genes and the green color down-regulated genes in the exercise group compared to control group. Molecule shapes and relationship lines are available in: IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity. Transcription regulator: ⬭, transmembrane receptor: ⬯, enzyme: ⟠, kinase: ▽, transporter: ⏢, other: ○. Dashed lines and solid lines reflect indirect and direct relationships between molecules. Circles lines shows that the molecule interacts with itself (e.g., autophosphorilation). Blue lines indicate the hub gene (i.e., *NFKBIA*) with more interactions inside the gene network. For more details about molecule shapes and relationship lines, please see: https://qiagen.secure.force.com/KnowledgeBase/articles/Basic_Technical_Q_A/Legend.

these eight genes, *CD6*, *HLA-C*, *TNFRSF1A*, *MYD88*, *and NFKBIA* validated in the large meta-analysis database of exercise effects identified as significantly (although reported high I^2) regulated genes by exercise (https://extrameta.org/).

In girls, 11 of the genes regulated by exercise were related to obesity, four genes involved in CVD, 21 genes involved in inflammation, and 10 in metabolic syndrome. Eight genes, *RAC1*, *ITGA2B*, *FYN*, *INPP5D*, *PIK3CD*, *CALR*, *SPARC*, *ANXA6* were identified in our gene pathways (Table 2), Top-IPA network (Figure 4), and in PHENOPEDIA disease terms. Six out of these top eight genes, *RAC1*, *ANXA6*, *FYN*, *INPP5D*, *PIK3CD* and *SPARC* validated in the comprehensive meta-analysis database of exercise effects as significantly regulated genes by exercise (https://extrameta.org/).

4 | DISCUSSION

This preliminary study shows that a 20-week exercise intervention altered whole-blood transcriptome profile

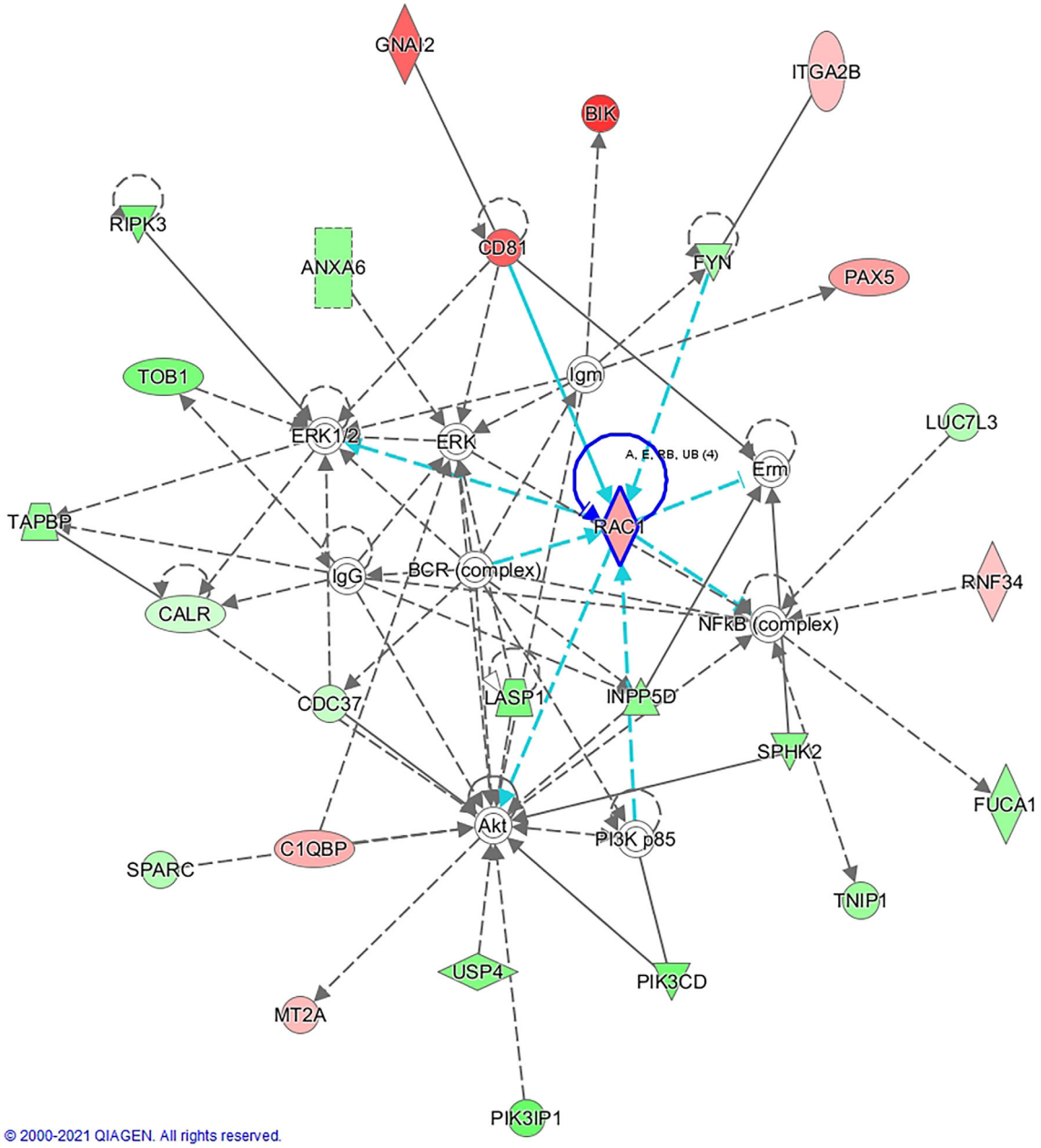

FIGURE 4 Top-IPA gene network in girls enriched with IPA disease terms and functions such as cell-mediated immune response, cellular function and maintenance, and hematological system development and function. The red color indicates up-regulated genes and the green colo down regulated genes in the exercise group compared to control group. Molecule shapes and relationship lines are available in: IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity. Transcription regulator: ⬭, transmembrane receptor: ⬯, enzyme: ⟠, kinase: ▽, peptidase: ⧟ phosphatase: △, transporter: ▱ ion channel: ⬚, other: ○ Dashed lines and solid lines reflects indirect and direct relationships between molecules. Circles lines shows that the molecule interacts with itself (e.g., dimerization). Blue lines indicate the hub gene (i.e., *RAC1*) with more interactions inside the gene network. Please see: https://qiagen.secure.force.com/KnowledgeBase/articles/Basic_Technical_Q_A/Legend for more details about molecule shapes and relationship.

(RNA-sequencing) in pre-pubertal children with OW/OB. Differential gene expression analyses detected 161 and 121 genes regulated by exercise in boys and girls respectively. Nevertheless, the effects were pronounced on molecular pathway level rather than on a single gene level. A number of gene pathways involved in immune responses were enriched with genes altered by the exercise intervention, with distinct differences between boys and girls.

Although few genes were in common that were influenced by exercise in both boys and girls, a number of gene pathways involved in immune processes were

detected, including antigen processing and presentation, phagosome, lysosome, leukocyte transendothelial migration and B cell receptor signaling pathway. Knowledge about exercise effects on the molecular level is limited, especially for pediatric populations, but the stimulating effect of physical exercise on immune responses is one of the few molecular processes commonly detected in previous studies (Gleeson et al., 2011; Nieman & Pence, 2020). Regardless of the few shared molecular processes between the sexes in our current study, the whole-blood transcriptome profiles in pre-pubertal OB/OW children were rather different in boys and girls. We believe that, in addition to the biological sex differences, the distinct molecular responses of the two sexes could be accounted, at least in part, to the longer duration and higher intensity (i.e., over the 80% of the maximal heart rate) observed in boys compared to girls, which may lead to different effect of training on body composition and cardiorespiratory fitness.

Some evidence suggests that women experience less adaptation than males in response to long-term training (i.e., males demonstrate greater absolute and relative $\dot{V}O_2$peak increases than females) (Ansdell et al., 2020), as a result of less mitochondrial biogenesis and angiogenesis within the skeletal muscle, differences on left ventricular mass that can determine cardiac output, among other factors (Ansdell et al., 2020; Howden et al., 2015). However, this aspects in skeletal and cardiac muscle cannot be explored in the current study. Concerning the reduction of body fat percentage observed in girls but not in boys, it is possible a ceiling effect, that is, boys presented a better adiposity profile (i.e., lower body fat percentage) than girls at baseline. Also, some research showed that women have a greater reliance on fat oxidation than men during submaximal exercise (Cano et al., 2022; Chenevière et al., 2011), while a one year long-term endurance training intervention showed a greater decrease in body fat percentage in females compared to males (Howden et al., 2015).

4.1 | Exercise intervention and blood transcriptome in boys

We observed that exercise intervention influenced antigen processing and presentation and T cell receptor complex (down-regulated) gene pathways in boys. Antigen-presenting cells (i.e., monocytes, macrophages, dendritic cells) lead to the recognition of foreign proteins (antigen) by T lymphocytes (particularly T-cell receptors on T-helper cells) (Gaudino & Kumar, 2019). T-helper cells release cytokines to activate other immune cells (e.g., can stimulate antibody production by B lymphocytes) (Gaudino & Kumar, 2019). Interestingly, the abovementioned gene pathways were up-regulated after a single bout of exercise in transcriptome analysis of PBMCs of healthy boys (Radom-Aizik et al., 2009) and middle-aged adults (Contrepois et al., 2020). These findings support the stimulation of the immune system after acute exercise and the possible down-regulation of immune pathways related to low-grade systemic inflammation during resting conditions in obesity (Gjevestad et al., 2015).

In addition, exercise regulated other gene pathways related to different infections (e.g., salmonella, yersinia) that involve diverse immune processes, although the biological implication of the up-regulation of these gene pathways after the exercise intervention in children is challenging to elucidate. Further, several genes regulated by exercise that were detected in infection pathways and in silico validation (e.g., *NFKBIA*, *MYD88*) were detected in well-characterized immune gene pathways such as Toll-like receptor, NOD-like receptor, nuclear factor-κB (NF-κB). Toll-like and NOD-like receptors are important for identifying pathogen-associated molecular patterns by antigen-presenting cells (Fukata et al., 2009), while the transcription factor NF-κB is a master regulator of inflammation inducing pro-inflammatory cytokine production (e.g., IL-6, TNF-α, IL-1β) (Liu et al., 2017). In fact, Toll-like receptors can induce the production of the pro-inflammatory cytokine IL-1β through the activation of NF-κB (Fukata et al., 2009).

Interestingly, *NFKBIA* was identified as a hub gene in the "Top" IPA network in boys (also detected within in silico validation). This gene encodes the protein NF-κB Inhibitor Alpha (IκBα) (Liu et al., 2017). In this regard, up-regulation of *NFKBIA* could be expected by a long-term exercise intervention leading to the inhibition of NF-κB, contributing partially to decreased levels of pro-inflammatory cytokines in circulation. However, the current study reported changes in the opposite direction (e.g., exercise intervention reduced *NFKBIA* gene expression). Intriguingly, a previous study reported a positive association of body mass index and insulin resistance with IκBα protein and gene expression levels (i.e., *NFKBIA*) in PBMCs of adults with type II diabetes compared to healthy controls (He et al., 2010). IκBα may be regulated by NF-kB activity (higher in type II patients compared to healthy controls), when NF-kB activity is increased will increase the expression of IκBα quickly to reduce the activity of NF-kB (He et al., 2010). Another study showed that *NFKBIA* gene expression was significantly higher in whole-blood of non-survivors melioidosis patients than survivors (Yimthin et al., 2021). Thus, the reduction of *NFKBIA* gene expression levels could partially indicate a lower pro-inflammatory profile after exercise intervention in boys.

However, in our limited sample size we did not detect any differences in pro-inflammatory cytokines IL-6, TNF-α, and IL-1β. On the other hand, CRF increased and waist circumference reduced after exercise intervention, improving CVD risk profile in boys, independently of changes on pro-inflammatory cytokines. Similarly, a 12-week exercise intervention improved CRF levels, while exercise did not affect cardiometabolic blood markers and total body fat in children with obesity (Dias et al., 2018).

4.2 | Exercise intervention and molecular responses in girls

The whole-blood transcriptome analysis in girls demonstrated that the exercise intervention down-regulated the Fc epsilon RI signaling pathway (considered a pro-inflammatory pathway (Mkaddem et al., 2019). Fc receptors modulate humoral and innate immunity and are important for preventing chronic inflammation and autoimmune diseases (Mkaddem et al., 2019). Interestingly, acute exercise down-regulated the expression of miR-96 in neutrophils of healthy adults (Radom-Aizik et al., 2010). To note, miR-96 targets genes in the Fc epsilon RI signaling pathway (Radom-Aizik et al., 2010).

Otherwise, platelet activation is involved in the atherothrombotic process and long-term exercise interventions can reduce platelet activation in adults with hypertension (De Meirelles et al., 2009). Also, platelet activation releases growth factors into the circulation, such as VEGFA, which has been considered a pro-inflammatory marker in rheumatoid and pediatric obesity-associated inflammation (Gil-Cosano et al., 2020; Rodriguez et al., 2021). VEGFA is an angiogenic factor that may regulate adipose tissue expansion by forming new blood vessels in adipose tissue (Cullberg et al., 2013). Some studies reported positive associations between circulating VEGFA levels and adiposity/body weight (Cullberg et al., 2013; Miyazawa-Hoshimoto et al., 2003). In this regard, VEGFA levels decreased in circulation after weight loss induced by multicomponent interventions (i.e., exercise, behavioral and nutritional counseling) (Cullberg et al., 2013).

Interestingly, in our study, an exercise intervention down-regulated the platelet alpha granule membrane gene pathway, while circulating VEGFA levels and body fat percentage decreased after exercise intervention in girls with OW/OB. In addition, other gene pathways (e.g., Fc gamma R-mediated phagocytosis, VEGF signaling, and platelet activation) related to inflammation and platelet function may be down-regulated by the exercise intervention in girls with OW/OB.

RAC1 (up-regulated by exercise in the current study) was a hub gene in the "Top" IPA network and identified in in silico validation in girls. RAC1 is a member of the Rac family of guanosine triphosphate phosphohydrolases (GTPases) that might contribute to inflammation and cardiovascular disease (Marei & Malliri, 2017). Also, the inhibition of RAC1 in macrophages from rodents could reduce the risk of atherosclerosis (Bandaru et al., 2020).

In our study, the exercise intervention increased *RAC1* gene expression levels in the whole-blood of girls with OW/OB (in the opposite direction of the expected results based on previous literature (Bandaru et al., 2020)). However, we cannot assume that increased *RAC1* gene expression after exercise intervention is directly related to increased RAC1 protein expression levels in whole blood (post-transcriptional mechanisms will regulate protein levels). In addition, post-translation modifications play a key role in RAC1 protein activation by regulating the protein localization in cell (Marei & Malliri, 2017). Besides, the exercise intervention did not affect plasma indicators of systemic inflammation (IL-6, TNF-α, IL-1β). At the same time, VEGFA (interpreted as a pro-inflammatory marker) decreased after exercise intervention in girls with OW/OB. Thus, we cannot interpret the increase of *RAC1* gene expression in whole blood after exercise intervention as an indicator of a higher pro-inflammatory status after exercise in girls.

4.3 | Limitations

Although providing novel information, several limitations need to be acknowledged. Whole-blood gene expression profile was analyzed, while blood is a complex tissue comprised of different cell populations (e.g., T cells, B cells, NK killers, neutrophils, among others) with different gene expression profiles. Likewise, the relatively low sample size limited our statistical power to detect statistically significant individual gene expression differences between groups. Also, blood samples collection was performed in a time window of 2–4 weeks after the last exercise session of the program, and we cannot rule out a possible detraining effect. Future studies should try to standardize the timing of sample collection in relation to the exercise training sessions. A normal-weight group of children was not included as a reference in the current study.

Importantly, the current study did not report data about the immune function of blood cells and the protein expression corresponding to gene transcripts regulated by the exercise intervention. In addition, at the protein level, we did not detect any effect of exercise intervention in the expression of well-known pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β in the plasma of children with overweight/obesity. All these limitations make the physiological and clinical interpretation of the results

presented in our study difficult. Therefore, future studies should try to overcome the abovementioned limitations.

Despite these limitations, our study is the first randomized controlled trial to report the effects of a 20-week exercise intervention on whole-blood transcriptome profile in boys and girls with OW/OB. Likewise, RNA-sequencing method was used to perform whole-transcriptome untargeted analysis. Additionally, the high globin mRNA of erythrocytes was reduced by a locking assay enabling better detection of less abundant transcripts in the blood.

5 | CONCLUSION

We hereby present novel findings on whole-blood transcriptome in pediatric population, increasing the understanding into the beneficial effects of regular physical activity on molecular processes. We were able to identify several immune processes to be influenced by the exercise intervention in OB/OW children, with some overlap in gene pathways in boys and girls. The general trend of transcriptome differences between boys and girls could partly be explained, in addition to sex differences, by the longer time at higher exercise intensity during the endurance part of the intervention sessions in boys compared to girls. We believe that our preliminary findings will be helpful input to advance the understanding of the molecular basis of the health benefits of exercise in children with OW/OB.

AUTHOR CONTRIBUTIONS

Conceptualization: Signe Altmäe, Abel Plaza-Florido, Francisco B. Ortega; **Formal analysis**: Francisco J. Esteban; **Funding acquisition**: Francisco B. Ortega; **Investigation (experiments)**: Abel Plaza-Florido, Francisco B. Ortega, Signe Altmäe, Kaarel Krjutškov, Shintaro Katayama, Elisabet Einarsdottir, and Juha Kere; **Methodology**: Signe Altmäe, Abel Plaza-Florido, Francisco J. Esteban, Francisco B. Ortega; **Project administration**: Signe Altmäe, Francisco B. Ortega; **Resources**: Signe Altmäe, Francisco B. Ortega; **Supervision**: Signe Altmäe, Francisco B. Ortega; **Roles/Writing - original draft**: Signe Altmäe, Abel Plaza-Florido; **Writing - review & editing**: Signe Altmäe, Abel Plaza-Florido, Francisco J. Esteban, Augusto Anguita-Ruiz, Kaarel Krjutškov, Shintaro Katayama, Elisabet Einarsdottir, Juha Kere, Shlomit Radom-Aizik, and Francisco B. Ortega.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GEO at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi/acc=GSE193771, reference number GSE193771.

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