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Cardiorespiratory fitness in children with overweight/obesity: Insights into the molecular mechanisms

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Objectives: High cardiorespiratory fitness (CRF) levels reduce the risk of developing cardiovascular disease (CVD) during adulthood. However, little is known about the molecular mechanisms underlying the health benefits of high CRF levels at the early stage of life. This study aimed to analyze the whole-blood transcriptome profile of fit children with overweight/obesity (OW/OB) compared to unfit children with OW/OB.

Design: 27 children with OW/OB (10.14 ± 1.3 years, 59% boys) from the ActiveBrains project were evaluated. VO_2peak was assessed using a gas analyzer, and participants were categorized into fit or unfit according to the CVD risk-related cut-points. Whole-blood transcriptome profile (RNA sequencing) was analyzed. Differential gene expression analysis was performed using the limma R/Bioconductor software package (analyses adjusted by sex and maturational status), and pathways' enrichment analysis was performed with DAVID. In addition, in silico validation data mining was performed using the PHENOPEDIA database.

Results: 256 genes were differentially expressed in fit children with OW/OB compared to unfit children with OW/OB after adjusting by sex and maturational status (FDR < 0.05). Enriched pathway analysis identified gene pathways related to inflammation (eg, dopaminergic and GABAergic synapse pathways). Interestingly, in silico validation data mining detected a set of the differentially expressed genes to be related to CVD, metabolic syndrome, hypertension, inflammation, and asthma.

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Conclusion: The distinct pattern of whole-blood gene expression in fit children with OW/OB reveals genes and gene pathways that might play a role in reducing CVD risk factors later in life.

KEYWORDS

aerobic fitness, childhood, gene expression, RNA-seq, transcriptome

1 | INTRODUCTION

Childhood obesity is associated with increased cardiovascular disease (CVD) risk factors (eg, high fasting glucose, triglycerides, and inflammatory markers) that might promote the development of CVD during adulthood,[1] the main cause of mortality worldwide. Cardiorespiratory fitness (CRF) is a powerful marker of health in youth[2] and is inversely associated with CVD risk factors.[3,4] Notably, youth with low CRF levels have a higher risk to develop CVD during adulthood.[5,6] In fact, there is extensive evidence to support the fat-but-fit paradigm, which shows that CRF is able to counteract the adverse effects of obesity on CVD risk factors.[3,4] Understanding the molecular mechanisms underlying the health benefits of fitness will promote the use of exercise as a form of medicine in a more precise and personalized way.

Previously, a single-gene analysis approach demonstrated higher expression of *PPARG* gene in leukocytes of normal-weight children with high CRF levels compared to normal-weight children with low CRF levels.[7] The *PPARG* gene is involved in lipid, glucose metabolism, and inflammatory response and is a proposed therapeutic target for CVD treatment.[8] Another cross-sectional study using microarray analysis detected higher expression of mitochondrial genes and lower expression of inflammatory genes in leukocytes of healthy young adult endurance athletes compared with healthy young adult non-athletes.[9] These are the first studies to explore the molecular mechanisms in fitness in young population, and clearly, more research is warranted.

Due to ethical considerations in pediatric studies, muscle and/or adipose tissue biopsies are limited and the blood is the primary tissue to study using the cutting-edge omics platforms. Thus, recent technological advances let us to explore, for the first time, the molecular mechanisms related to fitness using high-throughput technology such as RNA-seq in children with OW/OB. Importantly, whole blood includes immune cells that play an important role in the atherothrombotic process and might manifest the organism's systemic inflammatory state associated with obesity and CVD.[10,11] Furthermore, the transcriptome profile in blood cells has been informative to provide biomarkers for molecular diagnostics and management of CVD.[12] Altogether, this indicates that the whole-blood transcriptome profile could provide accessible biomarkers related to CVD and CRF levels in children with OW/OB.

The present study aimed to characterize the whole-blood transcriptome profile (RNA-seq) of fit children with OW/OB compared to unfit children with OW/OB. Our findings will promote a better understanding of the fat-but-fit paradigm and how fitness can counteract some of the adverse effects of obesity on CVD risk factors.

2 | MATERIALS AND METHODS

2.1 | Study sample

The present cross-sectional study used data from the ActiveBrains project (Clinical Trial: NCT02295072). Twenty-seven children with OW/OB (10.14 ± 1.3 years, 59% boys) were included in the current study. The methodology of the project, as well as the inclusion/exclusion criteria, has been reported in detail elsewhere.[13] This study was approved by the Committee for Research Involving Human Subjects at the University of Granada (Reference: 848, February 2014). According to the Declaration of

Helsinki, the study's information was provided to all parents/legal guardians, which gave written informed consent.

2.2 | Maturational status and body composition

Peak height velocity (PHV) was calculated as an indicator of maturational status using age and height in validated algorithms for boys and girls.[14] Body weight and height were collected using an electronic scale and a stadiometer (Seca Instruments, Germany, Ltd). Body mass index (kg/m^2) was calculated, and participants were accordingly classified as OW/OB following the World Obesity Federation body mass index standards, specific for sex and age.[15] Waist circumference (WC) was reported as an indicator of central fat distribution. Body composition parameters were measured by dual-energy X-ray absorptiometry (DXA, discovery densitometer from Hologic) following the recommendations from the International Society of Clinical Densitometry.

2.3 | Cardiorespiratory fitness

Cardiorespiratory fitness (ie, VO_2peak) was quantified using a gas analyzer (General Electric Corporation) while performing a maximal incremental treadmill test (HP-Cosmos ergometer). The incremental test adapted for children with weight disturbances consisted of walking as long as possible at a constant speed (4.8 Km/h). The slope started at 6% with grade increments of 1% every minute until volitional exhaustion. Oxygen consumption, HR (beats/min), and respiratory exchange ratio (RER) were continuously measured and recorded every 10 s, while 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. CRF (ie, VO_2peak) was reported relative to body weight (mL/kg/min). We classified the participants as "fit" and "unfit" according to the health-related cut-points for CRF, that is, 42 and 35 mL/kg/min relative to body weight for boys and girls, respectively, derived from a meta-analysis of studies relating CRF to CVD risk in children and adolescents.[16]

2.4 | Blood sampling and analysis

Blood sampling was performed in the morning (8–9 AM) after an overnight fasting. Venous blood was drawn and collected in EDTA tubes. For transcriptome analyses, 500 μL of whole blood with 1.3 mL RNAlater (Ambion, Inc.; Austin, Texas, USA) was stored at −80ºC until further processing. In regard to inflammatory marker quantification, blood was centrifuged at 1000×g for 10 min, and isolated plasma was stored at −80ºC.

2.4.1 | RNA extraction and sequencing

Briefly, blood samples that contained RNA later were processed to isolate total RNA using RiboPureTM-Blood Kit (Thermo Fisher Scientific; Waltham, Massachusetts, USA), and the abundant globin mRNA content of erythrocytes was blocked using the GlobinLock mechanism.[17] The modified version of the single-cell tagged reverse transcription (STRT) protocol was followed to perform the full transcriptome analysis as described before.[18] High-quality RNA (10 ng) was converted into cDNA and amplified to form an Illumina-compatible library. The processing of the raw sequencing reads, alignment to the hg19 genome, and the quantification of the expression levels were done using the STRTprep pipeline, available at https://github.com/shka/STRTprep/tree/v3dev. The RNA-seq data are available in the Gene Expression Omnibus (GEO) repository, accession number GSE164873.

2.4.2 | Inflammatory markers

Pro-inflammatory cytokines IL-1β, TNF-α, and IL-6 were detected by multiple analyte profiling technologies (MILLIPLEX® MAP Human High Sensitivity T Cell Magnetic Bead Panel, EMD Millipore Corporation, Missouri, USA). For IL-1β and TNF-α, the inter- and intra-assay coefficients of variation were ≤15% and ≤5%, respectively, with sensitivity of 0.14 pg/mL for IL-1β and of 0.16 pg/mL for TNF-α. The inter- and intra-assay coefficients of variation for IL-6 were ≤20% and ≤5%, respectively, with a sensitivity of 0.11 pg/mL.

2.5 | Statistical analysis

The sample characteristic differences between fit and unfit children with OW/OB were tested using the Student t test and chi-squared test for continuous and categorical variables, respectively. ANCOVA was performed to obtain adjusted mean differences between fit and unfit children with OW/OB after including sex and maturational status (ie, PHV) as confounders. The analysis was performed using SPSS version 21.0 (IBM Corporation, NY, USA); statistical significance was defined at the level of $p < 0.05$.

Gene expression data were normalized using a quantile normalization. Subsequently, differential expression

analysis between fit and unfit children with OW/OB was performed with the limma R/Bioconductor software package and was adjusted by sex and PHV (maturation), since these two factors are known to be highly influential at this period of life. Statistically significant differentially regulated genes were defined by a FDR <5% (Benjamini and Hochberg correction on multiple testing). Scripts used to perform this analysis are available for readers: https://osf.io/neuys/. These genes were characterized by functional enrichment analysis using DAVID Bioinformatic resource. Pathways with an EASE score <0.05 were considered significantly enriched. EASE score is a modified Fisher exact P value in DAVID Bioinformatic resource used for functional enrichment analysis (EASE score $p = 0$ shows a perfect enrichment). In addition, in *silico* validation mining was performed with gene lists associated with different diseases publicly available in the PHENOPEDIA database. Briefly, PHENOPEDIA provides information about genetic association studies in relation to different diseases, which is continuously updated from PubMed. Thus, differentially expressed genes in our study were overlapped with lists of genes involved in different diseases, that is, CVD, metabolic syndrome, hypertension, inflammation, and asthma.

3 | RESULTS

Descriptive characteristics are presented in Table 1. In the fit group, 25% of children were boys and 75% girls, while in the unfit group, 87% of children were boys and 13% girls. Fit children presented higher CRF (ie, VO_2peak relative to body weight; unadjusted mean difference of 3 mL/kg/min, and a difference of 8.5 mL/kg/min in adjusted models) and lower values of pro-inflammatory cytokine IL-1β (unadjusted mean difference of −0.50 pg/mL, and a difference of −1.12 pg/mL in adjusted models) compared with unfit children after adjusting for sex and PHV (adjusted p value <0.05). Also, borderline differences were found for pro-inflammatory cytokine IL-6 after adjusting for sex and PHV (adjusted p value

TABLE 1 Characteristics of the participants

Variables	Total sample $n = 27$ (16 boys/11 girls)	Fit $n = 12$ (3 boys/9 girls)	Unfit n=15 (13 boys/2 girls)	Unadjusted p value	Adjusted p value*
Age and maturational status					
Age (years)	10.1 ± 1.3	10.1 ± 1.2	10.2 ± 1.4	0.74	0.17
PHV offset (years)	−2.15 ± 0.94	−1.76 ± 0.80	−2.47 ± 0.96	0.50	N.A.
BMI group					
Overweight/Obesity	6 (22.2%)	3 (25.0%)	3 (20.0%)	0.56	N.A.
	21 (77.8%)	9 (75.0%)	12 (80.0%)		
Body composition and anthropometry					
Weight (kg)	57.31 ± 10.30	58.07 ± 9.23	56.70 ± 11.37	0.74	0.49
Height (cm)	145.65 ± 9.06	147.18 ± 8.16	144.43 ± 9.83	0.44	0.30
Waist circumference (cm)	91.72 ± 7.26	91.50 ± 5.54	91.89 ± 8.58	0.89	0.09
BF (%)	42.73 ± 4.71	42.91 ± 5.37	42.58 ± 4.29	0.86	0.05
DXA FM (kg)	24.22 ± 5.71	24.50 ± 5.10	24.00 ± 6.31	0.83	0.16
DXA total VAT (g)	414.29 ± 85.91	424.48 ± 89.43	406.14 ± 85.22	0.59	0.72
DXA LM (Kg)	30.71 ± 5.43	31.12 ± 5.81	30.38 ± 5.30	0.73	0.51
Inflammatory markers					
IL-1β (pg/mL)	1.77 ± 0.72	1.49 ± 0.56	1.99 ± 0.77	0.08	**0.002**
IL-6 (pg/mL)	2.31 ± 1.98	1.79 ± 0.62	2.25 ± 1.33	0.26	0.09
TNF-α (pg/mL)	3.92 ± 1.13	3.91 ± 1.22	3.93 ± 1.11	0.95	0.37
Cardiorespiratory fitness					
VO_2peak BW (mL/kg/min)	37.68 ± 4.44	39.39 ± 5.27	36.32 ± 3.21	0.07	**<0.001***

Note: Data are presented as non-adjusted means ± SDs, and as number and frequency. Bold numbers indicate $p < 0.05$; *p values derived from ANCOVA models adjusted for sex and maturation (ie, PHV). IL-1β and IL-6 ($n = 25$), TNF-α ($n = 26$).

Abbreviations: BMI, body mass index; BF, body fat; FM, fat mass; VAT, visceral adipose tissue; LM, lean mass; PHV, peak height velocity; BW, body weight; LM, LEAN mass; abs, absolute; N.A., not applicable.

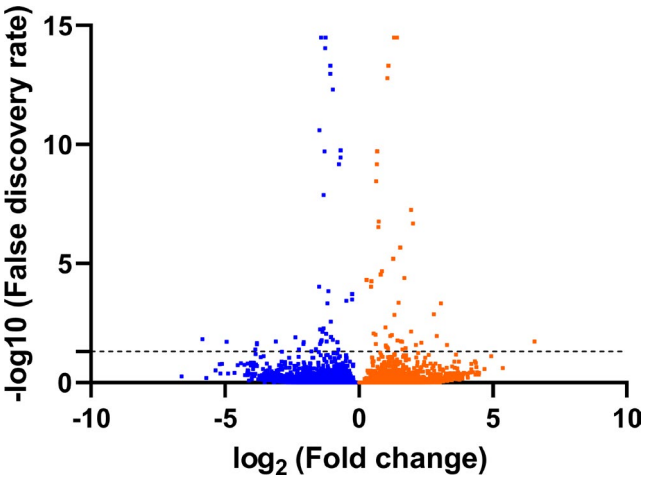

FIGURE 1 Volcano plot indicating the 256 differential expressed genes (up-regulated in yellow and down-regulated in blue) between fit and unfit children with overweight/obesity. The *x*-axis reflects the $\log_2$ fold change (FC) value, while the y-axis corresponds to the false discovery rate (FDR). Statistically significance threshold (ie, FDR <0.05 corresponds to –log10 > 1.30 in the horizontal dashed line)

TABLE 2 Pathway enrichment analysis of differentially expressed genes in fit children compared to unfit children with overweight/obesity using DAVID Bioinformatic resource (EASE score <0.05)

Selected pathways (KEGG)	**Genes identified in each pathway**
Dopaminergic synapse	*GNAO1GNAL↑ GNG10↑ GNG8↓ CREB3L3↑ PPP2R5E↑*
GABAergic synapse	*GNAO1↑ GNG10↑ GNG8↓ GABA RAP↑ GABBR1↓*
Molecular function	**Genes involved**
Histone deacetylase binding	*DNMT3B↓ KLF4↓ CCND1↑ KAT2 B↑ MEF2B↑ ZMYND15↑*
zinc ion transmembrane transporter activity	*SLC30A4↓ SLC30A8↑ SLC39A4↓*

Abbreviation: KEGG, Kyoto Encyclopedia of Genes and Genomes.

= 0.09; unadjusted mean difference of −0.46 pg/mL, and a difference of −1.06 pg/mL in adjusted models).

Two hundred and fifty-six genes were differentially expressed (145 up-regulated and 111 down-regulated, $\log_2$FC ranged from −5.83 to 6.55, FDR <0.05) in fit children compared with unfit children after adjusting by sex and PHV (Figure 1; Table S1). The differentially expressed genes were enriched in two pathways related to inflammation: dopaminergic synapse and GABAergic synapse (EASE score <0.05) (Table 2). Genes identified in dopaminergic and GABAergic synapse pathways were linked to obesity (Table S2). In silico validation data mining within the PHENOPEDIA database detected that 9 of the differentially expressed genes between fit and unfit children were involved in CVD, 11 genes in metabolic syndrome, 30 genes in hypertension, 25 genes inflammation, and 13 genes in asthma (Table 3). Further, 33 top genes were selected based on the highest $\log_2$FC (threshold ≥1.5) (Figure 2). Three of these 33 genes were enriched in the detected dopaminergic synapse and GABAergic synapse pathways: *GNG10*, *CREB3L3*, *and PPP2R5E* (Table 2), while 3 of these 33 genes were detected in the *in silico* validation data mining using the PHENOPEDIA database: *IL2RA*, *GRB2*, *and MAL* (Table 3).

4 | DISCUSSION

Our study highlights different transcriptome profiles between fit and unfit children with OW/OB, where a number of molecular pathways related to immune system and inflammation are involved, such as dopaminergic and GABAergic synapse pathways.

Exercise and physical activity are the main environmental factors able to modify CRF, and therefore, fit and unfit groups might be in part indicative of more and less active children, respectively. Importantly, fit and unfit groups presented an unadjusted mean difference of 3 mL/kg/min in VO2peak and a difference of 8.5 mL/kg/min in adjusted models. A threshold of 1.75 mL/kg/min in VO2peak has been considered clinically relevant.[19] Thus, the transcriptome analyses between fit and unfit groups could be of interest to gain a better understanding of the molecular mechanisms related to CRF and health in children with OW/OB. In our study, differentially expressed genes between fit and unfit children enriched dopaminergic and GABAergic synapse pathways; most of the genes were up-regulated in these pathways (*GNAO1*, *GNAL*, *GNG10*, *CREB3L3*, *PPP2R5E*, and *GABARAP*). In this context, exercise could increase levels of neurotransmitters, such as dopamine and amino acid γ-aminobutyric acid (GABA) in plasma and in different brain regions in humans.[20-22] Besides, neurological disorders have elucidated that dopamine might play an important role in controlling movement.[23] Interestingly, impairments in dopamine synthesis, release, and receptor function (mainly in the nervous system cells) could be underlying the lack of physical activity in humans with obesity.[23]

Importantly, dopaminergic and GABAergic receptors are expressed in different types of immune cells with different roles in the immune system.[24,25] Thus, dopaminergic pathways have been related to obesity-associated inflammation, although the specific molecular mechanisms in different types of immune cells need to be clarified.[25] Furthermore, GABA reduced the

TABLE 3 In silico validation of differentially expressed genes in fit vs unfit children with overweight/obesity using PHENOPEDIA database

PHENOPEDIA input term	Genes overlapped
Cardiovascular disease	*TNNI3*↓ *TNFRSF11A*↓ *APOC3*↑ *KAT2B*↑ *MEIS2*↑ *SPTA1*↑ *AHSG*↑ *SLC30A8*↑ *NPY*↑
Metabolic syndrome	*TNNI3*↓ *PTGES*↓ *IGF2R*↓ *TNFRSF11A*↓ *APOC3*↑ *CCND1*↑ *AHSG*↑ *SLC30A8*↑ *NPY*↑ *LRP8*↑ ***GRB2***↑
Hypertension	***IL2RA*** ↓ *MYBPH*↓ *TNNI3*↓ *DYNC1H1*↓ *NAT8*↓ *PTGES*↓ *IGF2R*↓ *TNFRSF11A*↓ *TOX2*↓ *ARVCF*↓ *ATP1B1*↓ *APOC3*↑ *CCND1*↑ *CXCL13*↑ *DDAH1*↑ *IL9*↑ *MLXIP*↑ *LTBP4*↑ *MFAP2*↑ *SPTA1*↑ *TNKS*↑ *NF1*↑ *SLC30A8*↑ *MYLK*↑ *NDST1*↑ *TXN*↑ *NPY*↑ *RFX7*↑ *LRP8*↑ ***GRB2***↑
Inflammation	***IL2RA*** ↓***MAL***↓ *SMAD7*↓ *DNMT3B*↓ *MYBPH*↓ *C3orf18*↓ *PTGES*↓ *IGF2R*↓ *GABBR1*↓ *TNFRSF11A*↓ *CDH6*↓ *KIF3B*↓ *APOC3*↑ *CCND1*↑ *CXCL13*↑ *DDAH1*↑ *GNAO1*↑ *IL9*↑ *RGMA*↑ *KAT2B*↑ *AHSG*↑ *SLC30A8*↑ *MYLK*↑ *TXN*↑ *NPY*↑
Asthma	*MYLK*↑ ***IL2RA*** ↓ *NPY*↑ *PTGES*↓ *TNFRSF11A*↓ *SLC22A15*↓ ***GRB2***↑ *IGSF11*↑ *TMEM79*↓ *CCND1*↑ *CXCL13*↑ *IL9*↑ *NRXN1*↑

Note: Bold genes are contained in the list of 33 genes with the highest $\log_2$ fold changes (FC) (threshold $\log_2$FC 1.5; false discovery rate, FDR <0.05).

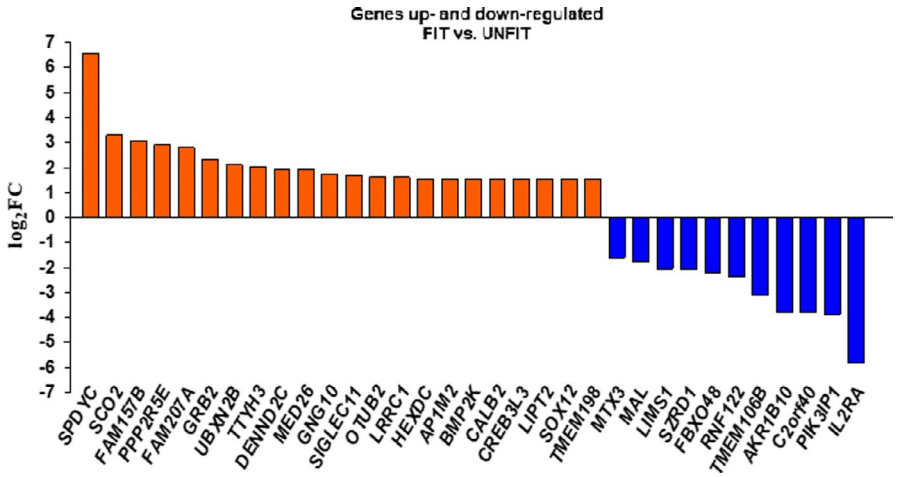

FIGURE 2 33 top genes significantly up- and down-regulated (fold change [FC] ≥1.5) in fit children compared to unfit children with overweight/obesity according to the limma analysis (up-regulated in orange and down-regulated in blue). False discovery rate, FDR <0.05

secretion of 47 cytokines (IL-1β included) in peripheral blood mononuclear cells (PBMCs) and CD4+ T cells of type 1 diabetes patients.[26] Also, Reyes-García et al. reported that GABA decreased IL-6 production in peripheral macrophages of rodents,[27] while Bhat et al. showed that increased GABAergic activity reduced autoimmune inflammation.[28] Therefore, dopaminergic and GABAergic pathways in immune cells have been related to inflammation such as an emergent research area.[24,25] Indeed, we did observe in our study that fit children presented a more favorable inflammatory profile than unfit children, that is, lower values of circulating pro-inflammatory cytokines such as IL-1β and IL-6, which are involved in CVD. Importantly, obesity might impair the dopaminergic and GABAergic systems.[23,29,30] Thus, good CRF levels (modifiable by physical activity and exercise) could attenuate the negative impact of obesity on the dopaminergic and GABAergic systems (ie, the *fat-but-fit* paradigm). These findings, however, should be interpreted with caution, as, for example, IL-6 can exert both pro- and anti-inflammatory effects.[31] High levels of circulating IL-6 (measured at resting conditions) could induce pro-inflammatory effects and are related to pediatric obesity, insulin resistance, and lipid metabolism.[32] Otherwise, IL-6 has been considered a pleiotropic myokine with anti-inflammatory properties when it is released by skeletal muscle in response to acute exercise.[31] In our study, IL-6 was considered a pro-inflammatory cytokine because it was quantified at resting conditions in children with overweight/obesity. The interpretation of circulating IL-6 levels could be different in response to acute exercise.

It is well known that low CRF levels are associated with more CVD events,[33] a higher risk of asthma incidents,[34]

and unfavorable cardiometabolic and inflammatory profiles.[35] In order to test the validity of our findings, that is, of the differentially expressed genes in fit vs. unfit children with OW/OB, we performed an *in silico* validation data mining using PHENOPEDIA database. Our findings showed that differentially expressed genes according to fitness groups were involved in CVD, metabolic syndrome, hypertension, inflammation, and asthma, matching therefore well with previous epidemiological evidence.[33-35] These results suggest that these differentially expressed genes could contribute partially to a better cardiovascular profile in those children with higher CRF levels. Further studies should analyze these genes' mechanistic role in developing CVD in the pediatric population with weight disturbances.

On single-gene level, of specific interest are *SCO2* and *IL2RA* genes that showed the highest and lowest regulation in fit vs. unfit OB/OW children. The IL-2 receptor (IL-2R) comprises three subunits (IL-2Rα, IL-2Rβ, and IL-2γc). The IL-2Rα subunit encoded by *IL2RA* gene regulates T lymphocyte activation, playing an important role in the atherothrombotic process, although the precise mechanisms are unclear.[36,37] Interestingly, high concentrations of plasma soluble IL-2Rα have been positively associated with CVD risk factors and mortality in older adults.[38] Therefore, that the *IL2RA* gene was found downregulated in the fit group in our study supports the notion that this could be one of the mechanisms why higher CRF linked to better cardiovascular health.

Interestingly, the bioenergetic capacity of PBMC (ie, higher maximal respiration of PBMC) was associated with lower circulating IL-6 in adults with OW/OB.[39] In this regard, SCO2 protein is fundamental for the assembly of cytochrome c oxidase, which is essential for cellular respiration and the aerobic ATP production in the mitochondria.[40] Markedly, an increase in age has been negatively associated with *SCO2* gene expression, while exercise training increased the *SCO2* gene expression levels in cardiac cells of old and young rodents.[41] Interestingly, mutations in *SCO2* gene have been associated with infantile cardioencephalomyopathy.[42] Furthermore, promoter hypermethylation and reduced *SCO2* gene expression were reported in cardiac cells of patients with congenital heart diseases.[43] We hypothesize that a lower *IL2RA* and higher *SCO2* gene expression levels in blood cells of fit children could promote a better cardiovascular profile in those children compared with unfit children.

Our study presents three main limitations. First, the cross-sectional study design does not allow us to assume causal relationships. Second, our sample size was relatively small and most of children in the fit group were girls (9 of 12) while most of children in the unfit group were boys (13 of 15), which could have influenced the analysis. Nevertheless, the limma analysis was controlled by sex, to attenuate the potential confounding role in this analysis. Third, whole-blood samples were used to perform whole transcriptome analysis. In this regard, it is known that different leukocyte populations have specific roles in the immune system and CVD. Nonetheless, the whole-blood RNA-seq reflects the general system's response to the stimulus and it has served as useful approach to identify "aberrant" gene expression patterns associated with different diseases.

Despite these limitations, some strengths in our study need to be acknowledged. To our knowledge, this is the first study to analyze the whole-blood transcriptome profiles using high-throughput technologies such as RNA-seq in fit children compared to unfit children with OW/OB. Besides, transcriptome analysis was performed using blood samples obtained in first hour in the morning at fasting conditions in a unified manner. Furthermore, GlobinLock molecular mechanism was applied as a novel robust method to block abundant globin mRNA in erythrocytes,[17] which hinder the whole-blood transcriptome analysis.

In conclusion, differentially expressed genes between fit and unfit children with OW/OB are involved in dopaminergic and GABAergic synapse pathways. Further, in *silico* validation data mining using PHENOPEDIA database detected several differentially expressed genes related to CVD, metabolic syndrome, hypertension, inflammation, and asthma. The top candidate genes involved in link between CRF and CVD include *IL2RA*, *SCO2*, *GRB2*, *MAL*, *GNG10*, *CREB3L3*, and *PPP2R5E*. Our results promote a better understanding of how fitness might contribute to a favorable CVD risk factor profile in youth and potentially reduce CVD later in adulthood.

5 | PERSPECTIVE

CRF is a powerful marker of health in children,[2] which is modifiable by physical activity and exercise. For the first time, a distinct pattern of whole-blood transcriptome profile (RNA-seq) was identified in fit children with overweight/obesity (OW/OB) compared to unfit children with OW/OB. The identified whole-blood transcriptome profile in fit children with OW/OB might be related to inflammation and promote a better understanding of how fitness might contribute to reduce CVD later in adulthood. Therefore, understanding the molecular mechanisms underlying the health benefits of CRF promotes the use of exercise as a form of medicine in a more precise and personalized way in children with OW/OB.

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CONFLICT OF INTEREST

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 in Gene Expression Omnibus (GEO) repository, accession number GSE164873.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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