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Targeted proteomics involved in cardiovascular health and heart rate variability in children with overweight/obesity

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Abstract

Background: Children with overweight/obesity often exhibit alterations in their plasma protein profiles and reduced heart rate variability (HRV). Plasma proteomics is at the forefront of identifying biomarkers for various clinical conditions. We aimed to examine the association between plasma-targeted proteomics involved in cardiovascular health and resting vagal-related HRV parameters in children with overweight/obesity.

Methods: Forty-four children with overweight/obesity (10.2 ± 1.1 years old; 52% boys) participated in the study. Olink's technology was used to quantify 92 proteins involved in cardiovascular health. HRV was measured using a heart rate monitor (Polar RS800CX). Four resting vagal-related HRV parameters were derived in time- and frequency-domain.

Results: Eight proteins (KIM1, IgG Fc receptor II-b, IDUA, BOC, IL1RL2, TNFRSF11A, VSIG2, and TF) were associated with at least one out of the four vagal-related HRV parameters (β values ranging from −0.188 to 0.288; all $p < .05$), while KIM1, IDUA, and BOC associated with ≥ three vagal-related HRV parameters. Multiple hypothesis testing corrections did not reach statistical significance (false discovery rate [FDR >0.05]).

Abel Plaza-Florido and Marcos Olvera-Rojas contributed equally to this work and co-first authors.

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Conclusion: Plasma-targeted proteomics suggested novel biomarkers for resting vagal-related HRV parameters in children with overweight/obesity. Future studies using larger cohorts and longitudinal designs should confirm our findings and their potential clinical implications.

1 | INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality in the world (Vaduganathan et al., 2022). Childhood overweight/obesity is associated with an adverse CVD risk profile during adulthood (Umer et al., 2017). In this context, identifying novel non-invasive biomarkers associated with CVD risk factors during childhood overweight/obesity is a matter of interest to prevent the development of CVD later in life. In the last decades, the development of sophisticated "omics" technologies (e.g., proteomics) lets researchers and clinicians associate tens to thousands of proteins with different clinical outcomes using a relatively small amount of blood (Mokou et al., 2017).

Resting heart rate variability (HRV) is the variation in time between heart beats, or R–R intervals, which is considered a non-invasive health-related biomarker that predicts adverse CVD events and mortality (Hillebrand et al., 2013; Thayer & Lane, 2007). HRV is an index of neurocardiac activity determined by the complex interplay between several physiological systems, such as the autonomic nervous system and immune and endocrine systems (Draghici & Taylor, 2016), being higher values in vagal-related HRV parameters at rest associated with a healthier cardiovascular profile (Task Force, 1996). Conversely, lower values on vagal-related HRV parameters at rest are related to increased CVD risk factors in children and adolescents with overweight/obesity (Parish et al., 2016; Plaza-Florido, Alcantara, et al., 2021; Plaza-Florido, Migueles, Mora-Gonzalez, Molina-Garcia, Rodriguez-Ayllon, Cadenas-Sanchez, Esteban-Cornejo, Navarrete, et al., 2019; Plaza-Florido, Migueles, Mora-Gonzalez, Molina-Garcia, Rodriguez-Ayllon, Cadenas-Sanchez, Esteban-Cornejo, Solis-Urra, et al., 2019). However, the molecular mechanisms underlying the potential protective effects of higher values on vagal-related HRV parameters at rest remains poorly understood in children with overweight/obesity. Thus, human biology is interested in a better understanding of the relationship between vagal-related HRV parameters and proteomics. More specifically, studies addressing these issues in pediatric populations with overweight/obesity are relevant to advancing current knowledge in the pediatric human biology field.

This exploratory cross-sectional study aimed to examine the association between plasma-targeted proteomics (92 proteins) known to be involved in cardiovascular health and resting vagal-related HRV parameters in a sample of 44 children with overweight/obesity. We hypothesized that a "better" profile in cardiovascular health-related proteins would be associated with resting vagal-related HRV parameters in children with overweight/obesity.

2 | METHODS

2.1 | Participants and study design

This cross-sectional study was performed under the umbrella of the ActiveBrains project (Cadenas-Sánchez et al., 2016; Ortega et al., 2022), and used data from the baseline. From a total of 109 children with overweight/obesity, we retrospectively included a subsample of 44 (10.2 ± 1.1 years old; 52% boys) with valid plasma-targeted proteomics and HRV data. Body weight and height were measured with an electronic scale and a stadiometer (Seca Instruments, Germany, Ltd.), respectively. Subsequently, body mass index (BMI) was calculated as kg/m^2 to classify children as overweight or obesity using sex- and age-specific cutoff points (Cole & Lobstein, 2012). The ActiveBrains project study protocol was approved by the Committee for Research Involving Human Subjects at the University of Granada (Reference: 848, February 2014), and it was conducted according to the Declaration of Helsinki. All parents had received information about the study and gave written informed consent. The eligibility criteria were described in detail elsewhere (Cadenas-Sánchez et al., 2016; Ortega et al., 2022). Inclusion/exclusion criteria were: (i) children with overweight/obesity according to sex- and age-specific international body mass index standards (World Obesity Federation); (ii) girls not having started the menstruation; (iii) not to present neurological disorders or take medications that affect nervous system function.

2.2 | Targeted proteomics involved in cardiovascular health

Proteomics analyses were extensively described previously (Plaza-Florido et al., 2023). Briefly, the blood

samples were collected, from the antecubital vein, in the morning after 12 h of fasting and were immediately centrifuged at 1000×*g* for 10 min. The isolated plasma was stored at −80°C. The 92 proteins involved in cardiovascular health were quantified using one microliter of plasma at the Olink laboratory in Uppsala using the Proximity Extension Assay (PEA) technology (Proseek Multiplex Cardiovascular panel 96 × 96 reagents kit [Olink® Bioscience, Uppsala, Sweden]). The PEA technology has been described in detail (https://www.olink.com/) (Plaza-Florido et al., 2023). The protein expression values are presented in arbitrary units (NPX values; https://olink.com/faq/what-is-npx/) in the $\log_2$ scale. Intra- and inter-assay coefficients of variations, detection limits, and biological information for each protein are reported on the manufacturer's website (https://www.olink.com/).

2.3 | Heart rate variability

The HRV assessment and analysis was described elsewhere (Plaza-Florido, Migueles, Mora-Gonzalez, Molina-Garcia, Rodriguez-Ayllon, Cadenas-Sanchez, Esteban-Cornejo, Navarrete, et al., 2019; Plaza-Florido, Migueles, Mora-Gonzalez, Molina-Garcia, Rodriguez-Ayllon, Cadenas-Sanchez, Esteban-Cornejo, Solis-Urra, et al., 2019). Briefly, the Polar RS800CX (Polar Electro Oy Inc., Kempele, Finland) was used to assess the R–R signal at a sampling frequency of 1000 Hz. Ten minutes were recorded at morning while the participant was lying in the supine position and at rest. Participants were encouraged to breathe normally, do not move or speak during the HRV assessment and stay calm. The Kubios software (HRV analysis, University of Eastern Finland) and the automatized "low filter" were used to process the raw R–R signal (Alcantara et al., 2020). Out of the 10 min recorded, the "best" 5 min were checked for quality (i.e., normal distribution of the R–R intervals, no large R–R interval outliers and R–R intervals equidistance) and then selected to calculate HRV parameters using the Kubios software (Plaza-Florido et al., 2020). Detailed information about data selection, processing, and derivation of HRV parameters using the Kubios software can be found elsewhere (Alcantara et al., 2020; Plaza-Florido et al., 2020).

We computed resting vagal-related HRV parameters in time- and frequency-domain based on the Guidelines of Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology (Task Force, 1996). In the time-domain, we computed the squared root of the mean of the sum of the squares of successive normal R–R interval differences (RMSSD), the standard deviation of all normal R–R intervals (SDNN), and the percentage number of pairs of adjacent normal R–R intervals differing by more than 50 ms in the recording (pNN50). In the frequency-domain, we performed spectral analyses using the non-parametric fast Fourier transformation algorithm and we computed the power in the high (HF) frequency band (HF: 0.15–0.4 Hz; in absolute units [ms^2]). Finally, from the R–R signal we also computed the resting mean heart rate (i.e., average heart rate values from the selected period).

2.4 | Covariates

Participants' sex, peak height velocity (PHV), parental education level, and resting mean heart rate were used as potential confounders in the statistical analyses, according to our previous studies (Plaza-Florido, Migueles, Mora-Gonzalez, Molina-Garcia, Rodriguez-Ayllon, Cadenas-Sanchez, Esteban-Cornejo, Navarrete, et al., 2019; Plaza-Florido, Migueles, Mora-Gonzalez, Molina-Garcia, Rodriguez-Ayllon, Cadenas-Sanchez, Esteban-Cornejo, Solis-Urra, et al., 2019; Plaza-Florido, Migueles, Sacha, & Ortega, 2019). The PHV was reported as an indicator of maturation, which was calculated using age and anthropometric variables following validated algorithms (Moore et al., 2015). In boys was calculated as: $-8.1 + (0.0070346 \times (\text{age} \times \text{sitting height}))$, while in girls: $-7.7 + (0.0042232 \times (\text{age} \times \text{height}))$. Years from PHV were calculated by subtracting the age of PHV from chronological age, so positive values are presented in more mature children (after the PHV) and negative values in less mature children (before the PHV). Parental educational level was self-reported using questionnaires, the responses from both parents were pooled, as neither parent had a university degree; one parent had a university degree; and both parents had a university degree (Plaza-Florido, Migueles, Mora-Gonzalez, Molina-Garcia, Rodriguez-Ayllon, Cadenas-Sanchez, Esteban-Cornejo, Solis-Urra, et al., 2019).

2.5 | Statistical analyses

Descriptive characteristics were reported as means and standard deviations for continuous variables with a normal distribution. Frequencies and percentages were reported for categorical variables. We presented the medians and interquartile ranges for resting vagal-related HRV parameters because they presented a non-normal distribution. The visual inspection of histograms and the Kolmogorov–Smirnov test were performed to check the normal distribution of variables. When needed, we

calculated normal scores using the Blom formula (Blom, 1958) for non-normally distributed variables. Student's *t*-test was performed to examine the differences in the descriptive characteristics between boys and girls. Multiple linear regressions models were performed to explore the association between plasma-targeted proteomics involved in cardiovascular health (92 proteins) and resting vagal-related HRV parameters in time- and frequency-domains (RMSSD, SDNN, pNN50, and HF). As mentioned above, sex, PHV, parental education, and resting mean heart rate were included as covariates in the regression models. The threshold for statistically significant associations was $p < .05$. In addition, we performed multiple hypothesis testing corrections (false discovery rate [FDR] correction, Benjamini and Hochberg) due to the high number of variables included in this study (92 proteins and 4 resting vagal-related HRV parameters). The statistical analyses were performed using R (version 4.2.0; R Foundation for Statistical Computing).

3 | RESULTS

Table 1 shows the descriptive characteristics of the 44 children with overweight/obesity (10.2 ± 1.1 years old; 52% boys) included. Girls presented a higher maturation status than boys ($p < .05$, Table 1). Eight proteins involved in cardiovascular health (cytokine production, lipid metabolism, blood coagulation, among others) (Table 2) were associated with vagal-related HRV parameters in children with overweight/obesity (all $p < .05$; Figure 1) after adjusting for potential confounders. Two proteins (KIM1 [kidney injury molecule-1] and IgG Fc receptor II-b [low affinity immunoglobulin gamma Fc region receptor II-b]) were inversely associated with vagal-related HRV parameters (standardized β ranged from −0.188 to −0.244 all $p < .05$; Figure 1A–C). Six proteins (IDUA [Alpha-L-iduronidase], BOC [Brother of CDO], IL1RL2 [Interleukin-1 receptor], TNFRSF11A [Tumor necrosis factor receptor superfamily member 11A], VSIG2 [V-set and immunoglobulin

TABLE 1 Descriptive characteristics of the sample.

	All		Boys		Girls		
	n		*n*		*n*		*p value*
Physical characteristics	44		23		21		
Age (years)		10 ± 1		10 ± 1		10 ± 1	.197
Weight (kg)		56 ± 11		57 ± 10		56 ± 12	.771
Height (cm)		145 ± 9		146 ± 8		144 ± 9	.459
Years from peak height velocity offset		−2.05 ± 0.97		−2.47 ± 0.78		−1.58 ± 0.94	.001
Body mass index (kg/m^2)		26 ± 4		26 ± 4		26 ± 3	.975
Body mass index category (%)							.870
Overweight	11	25	6	26	5	24	
Obesity type I	24	55	13	57	11	52	
Obesity type II	9	20	4	17	5	24	
Parental education: university level (%)							.886
None of the parents	22	50	11	48	11	52	
One of the two parents	12	27	7	30	5	24	
Both parents	10	23	5	22	5	24	
Heart rate and heart rate variability							
Mean HR (bpm)		82 ± 11		80 ± 10		85 ± 10	.078
RMSSD (ms)		58 [63]		52 [79]		63 [53]	.314
SDNN (ms)		59 [55]		58 [58]		60 [43]	.313
pNN50 (%)		31 [42]		30 [45]		32 [36]	.563
HF (ms^2)		1223 [3845]		1411 [4159]		1221 [3649]	.621

Note: Values presented as mean ± SD or percentages or frequency and percentage. Median [IQR: Interquartile range] are presented for vagal-related heart rate variability parameters.

Abbreviations: BMI, body mass index; HF, high frequency band (0.15–0.4 Hz); HR, heart rate; RMSSD, the square root of the mean of the sum of the squares of differences between adjacent R–R intervals; pNN50, percentage (%) of the total pairs of R–R intervals that differ by more than 50 ms; SDNN, standard deviation of all normal R–R intervals.

TABLE 2 Molecular function, biological process, tissue profile, and disease area of the differentially expressed proteins related to cardiovascular health in children with overweight/obesity.

Protein name	Main function	Molecular function	Biological process	Tissue profile	Disease area
BOC	Component of a cell-surface receptor complex that mediates cell–cell interactions between muscle precursor cells	Promotes differentiation of myogenic cells	Axon guidance, cell–cell adhesion	Skeletal muscle, heart, thymus, kidney and small intestine	Locomotor/ Neurological
IDUA	Hydrolysis of un-sulfated alpha-L-iduronosidic linkages in dermatan sulfate	Signaling receptor binding	Glycosaminoglycan catabolic process Disaccharide metabolic process	Cytoplasmic expression in most tissues, often with a lysosomal pattern	Immunity
IgG Fc receptor II-b	Phagocytosis of immune complexes and the regulation of antibody production by B-cells	Regulation of immune responses	Negative regulation of cytokine production, mature B cell differentiation, dendritic cell activation	Immune cells, endothelial and vascular smooth muscle cells	Inflammation/ immunity
IL1RL2	It is present in epithelial barriers and could take part in local inflammatory response	Interleukin-1 receptor activity	Regulation of interleukin-6 production Cellular defense response	Mostly expressed in skin surface	Inflammation/ immunity
KIM1	Phosphatidylserine receptor that plays an important functional role in regulatory B-cells homeostasis including generation, expansion and suppressor functions. Also, can be a marker of renal injury	Virus receptor activity, B-cells homeostasis, regulates the expression of anti-inflammatory cytokines	Regulation of mast cell activation Phagocytosis, engulfment	Kidney, blood, testicle	Immunity/ neurological
TF	Cell surface glycoprotein involved in the blood coagulation cascades	Initiates blood coagulation	Positive regulation of enothelial cell proliferation, activation of proteins involved in acute inflammatory response, blood coagulation	Immune cells, smooth muscle cells	Immunity
TNFRSF11A	Involved in the regulation of interactions between T-cells and dendritic cells	Transmembrane receptor signaling activity	Adaptive immune response Circadian temperature homeostasis	Cytoplasmic expression in several tissues	Inflammation/ immunity
VSIG2	Unclear, the physiological function of VSIG2 needs to be explored	Unclear, possible modulation of antitumor activity	Lipid metabolic process	High membrane and cytoplasmic expression in glandular cells of gastrointestinal tract	Inflammation, heart failure

Note: The information was gathered from the GeneCards (https://www.genecards.org), TheHumanProteinAtlas (https://www.proteinatlas.org) and UniProtKB website (https://www.uniprot.org), and National Library of Medicine (https://www.ncbi.nlm.nih.gov/gene/2213).
Abbreviations: BOC, brother of CDO; IDUA, alpha-L-iduronidase; IgG Fc receptor II-b, low affinity immunoglobulin gamma Fc region receptor II-b; IL1RL2, interleukin-1 receptor-like 2; KIM1, kidney injury molecule; TF, tissue factor; TNFRSF11A, tumor necrosis factor receptor superfamily member 11A; VSIG2, V-set and immunoglobulin domain-containing protein.

domain-containing protein 2], and TF [Tissue factor]) were positively associated with vagal-related HRV parameters (standardized β ranged from 0.184 to 0.288, all $p < .05$; Figure 1A–D). The KIM1, IDUA, and BOC proteins showed consistent associations, being related with at least three out of the four vagal-related HRV

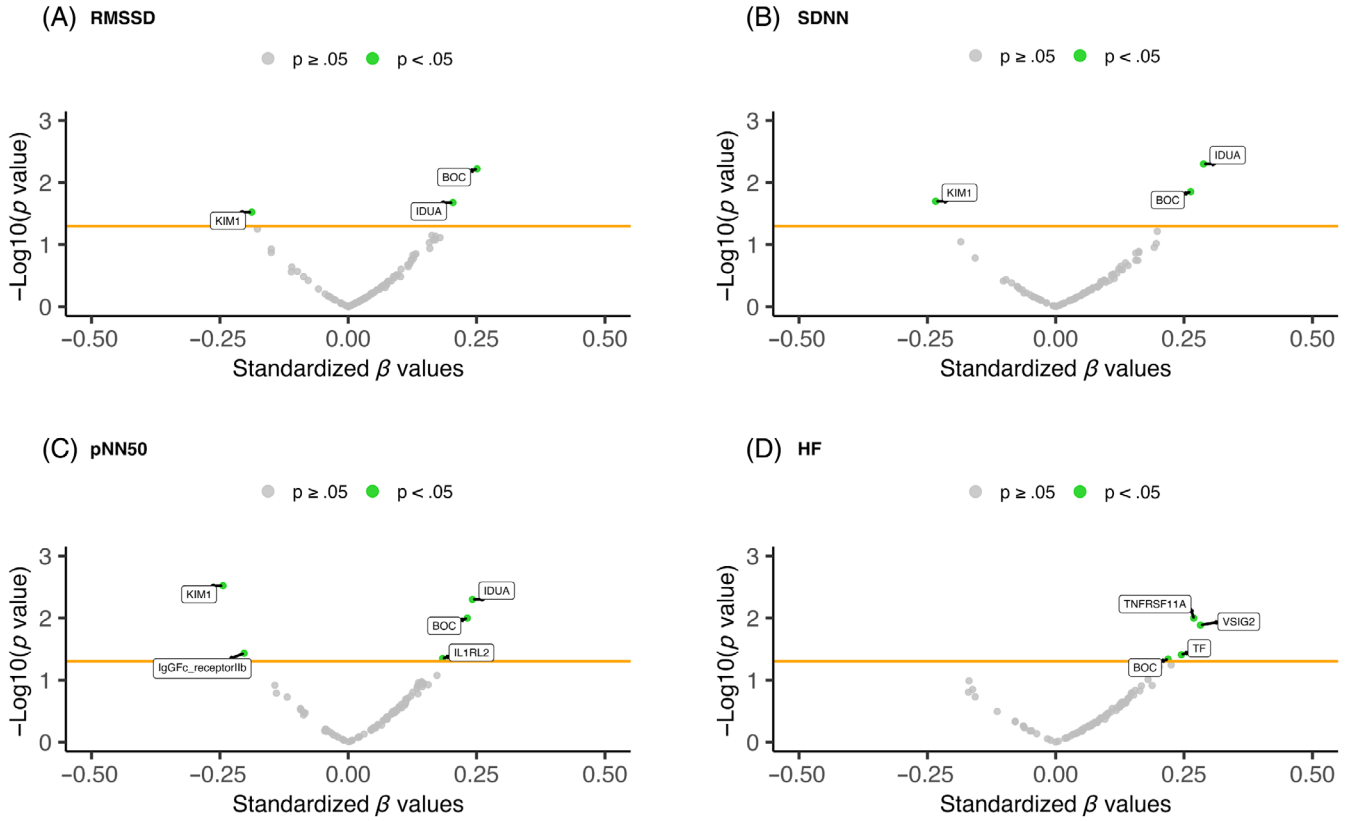

FIGURE 1 Volcano plots show the standardized β values from multiple linear regression models between cardiovascular proteins from the Olink assay and vagal-related heart rate variability parameters in children with overweight/obesity ($n = 44$). The figure displays proteins that were significantly associated ($p < .05$) in green and nonsignificantly ($p \geq .05$) in gray. The *x*-axis indicates the effect size measured in standardized β values. The *y*-axis shows −log transformation of the *p*-values, with a solid orange line used as a cut-point for statistical significance ($p < .05$). Linear regression models are adjusted by sex, peak height velocity, parental education, and mean heart rate. BOC, brother of CDO; HF, high frequency band (0.15–0.4 Hz). IDUA, alpha-L-iduronidase; IgG Fc receptor II-b, low affinity immunoglobulin gamma Fc region receptor II-b; IL1RL2, interleukin-1 receptor-like 2; KIM1, kidney injury molecule; pNN50, percentage (%) of the total pairs of R–R intervals that differ by more than 50 ms; RMSSD, the square root of the mean of the sum of the squares of differences between adjacent R–R intervals; SDNN, standard deviation of all normal R–R intervals; TF, tissue factor; TNFRSF11A, tumor necrosis factor receptor superfamily member 11A; VSIG2, V-set and immunoglobulin domain-containing protein 2.

parameters (Figure 1A–C). Multiple hypothesis testing corrections did not reach statistical significance (FDR >0.05).

4 | DISCUSSION

This cross-sectional study explored the association between plasma-targeted proteomics related to cardiovascular health and resting vagal-related HRV parameters in children with overweight/obesity. Eight proteins were associated with resting vagal-related HRV parameters (KIM1, IgG Fc receptor II-b, IDUA, BOC, IL1RL2, TNFRSF11A, VSIG2, and TF). Specifically, KIM1, IDUA, and BOC showed the most consistent associations. It is important to consider the exploratory nature of this study because of the high number of variables analyzed using a relatively small sample size (44 children); thus, multiple hypothesis testing corrections did not reach statistical significance. These preliminary findings offer valuable insights that could guide researchers and clinicians in designing future longitudinal studies with greater statistical power. Particularly in the context of children with overweight/obesity, our study provides a basis for more comprehensive investigations.

The general biological information and function of the eight proteins of interest is presented in Table 2, thus in this section we will be more focused in the discussion of the three proteins (KIM1, IDUA, and BOC) that showed consistent associations with resting vagal-related HRV parameters. To our knowledge, no previous studies have investigated the relationship between the proteomics data reported in the current study and resting vagal-related HRV parameters, which make difficult

between-studies comparisons. The most relevant proteins detected in this study are discussed in the context of existing knowledge linking these proteins to human CVD in adults or to animal experiments. The KIM1 is a protein related to adverse renal function in patients with heart failure, and elevated levels of plasma KIM1 could increase the number of heart failure (re)hospitalization (Emmens et al., 2016). Interestingly, one study (Egli et al., 2018) showed that higher levels of KIM1 in plasma were related to higher blood pressure, inflammatory markers, and BMI in healthy adults. In our study, higher levels of KIM1 in plasma were related to lower values on resting vagal-related HRV parameters in children with overweight/obesity, which could indicate a worse CVD risk profile.

The IDUA is a glycoprotein found in the lysosomes of cells that regulates the expression of glycosaminoglycans, such as heparan and dermatan sulfate (Ma et al., 2008). IDUA deficiency contributes to the accumulation of these glycosaminoglycans, thus resulting in the fragmentation of the elastin fibers in the heart valves and the aorta (Ma et al., 2008). Likewise, mucopolysaccharidosis type I is an autosomal recessive disorder mainly caused by the lack of IDUA activity (also presented in the pediatric population (Cantú-Reyna et al., 2023)) that has a negative impact on multiple organs and cardiac activity (Osborn et al., 2017). Interestingly, in our study, higher levels of IDUA in plasma were positively associated with resting vagal-related HRV parameters in children with overweight/obesity. Altogether, we can infer that lower levels of KIM1 and higher IDUA in plasma could induce a cardioprotective role in children with overweight/obesity. Additionally, KIM1 and IDUA levels in plasma could be considered potential blood biomarkers related to an adverse CVD risk profile in pediatric populations with overweight/obesity.

The BOC protein mediates cell–cell interactions between muscle precursor cells and promotes the differentiation of myogenic cells, although the role of this protein in cardiovascular health is poorly understood. Recently, one study (Yuan et al., 2023), performed prospective cohort and Mendelian randomization analyses to reveal novel protein biomarkers associated with venous thromboembolism in Swedish adults. In their cohort analyses, they observed that higher levels of BOC in plasma were related to a lower risk of venous thromboembolism (Yuan et al., 2023). This association is in the same line as the direction of the positive association between BOC levels in plasma and resting vagal-related HRV parameters we observed in children with overweight/obesity. Thus, we could hypothesize that higher levels of BOC protein in plasma of children with weight disturbances could exert some beneficial effects on cardiovascular health. However, an intriguing observation was reported in the abovementioned study (Yuan et al., 2023), Mendelian randomization analyses showed that genetically predicted higher levels of BOC in plasma were associated with an increased risk of venous thromboembolism using data from the FinnGen R7 study. It is possible that the different direction of the associations in the cohort and Mendelian randomization analyses might be caused by different proteomic profiling assays (Pietzner et al., 2021; Yuan et al., 2023). In this regard, it has been reported discordance of genetically predicted effect directions between the aptamer-based SomaScan® v4 assay and the antibody-based Olink assays (Pietzner et al., 2021). It is important to standardize the molecular assays in different cohorts for comparability purposes whenever possible.

Regarding the proteins that showed less consistent associations with resting vagal-related HRV parameters, it is interesting to mention the inverse association between IgG Fc receptor II-b in plasma and the pNN50 (Figure 1C). The IgG Fc receptors are expressed in several immune, endothelial, and vascular smooth muscle cells, modulating immune responses and vascular disease pathogenesis (Tanigaki et al., 2015). Interestingly, C-reactive protein is a pro-inflammatory marker related to myocardial infarction, obesity, and is inversely associated with vagal-related HRV parameters (Madsen et al., 2007; Tanigaki et al., 2015). In mice, C-reactive protein can blunt skeletal muscle glucose uptake via IgG Fc receptor II-b inducing insulin resistance (Tanigaki et al., 2013). This could be one of the mechanisms through IgG Fc receptor II-b can play a role in the development of CVD, specifically these related to impaired glucose metabolism. More powered, longitudinal, and observational studies performing Mendelian randomization analyses are warranted to interpret our findings' clinical relevance and causal relationship.

5 | STRENGTHS AND LIMITATIONS

This study presented several limitations that should be mentioned. First, it is not possible to establish causality on the associations examined, considering the cross-sectional study design and exploratory methodology. Second, statistical significance did not persist after multiple hypothesis testing corrections because of the small sample size ($N = 44$) and the high number of variables included (92 proteins and 4 vagal-related HRV parameters). Future studies using larger sample sizes and longitudinal designs should overcome this limitation trying to corroborate our findings. Third, we did not utilize a gold

standard technique to assess HRV; however, assessing HRV using a heart rate monitor as the RS800CX is a valid and reliable procedure for its quantification (Plaza-Florido, Sacha, & Alcantara, 2021). Fourth, it is known that breathing frequency can alter values on HRV parameters (Plaza-Florido, Sacha, & Alcantara, 2021). However, we decided not to control the breathing to avoid disturbing the resting status of the children.

6 | CONCLUSION

We detected novel proteins in plasma (KIM1, IgG Fc receptor II-b, IDUA, BOC, IL1RL2, TNFRSF11A, VSIG2, and TF) associated with resting vagal-related HRV parameters in children with overweight. These proteins were involved in cardiovascular health, immunity, cell–cell adhesion, and insulin resistance, among others. Importantly, KIM1, IDUA, and BOC proteins showed consistent associations across resting vagal-related HRV parameters and could be considered potential therapeutic targets for preventing CVD development later in life.

AUTHOR CONTRIBUTIONS

Abel Plaza-Florido, Marcos Olvera-Rojas, and Francisco B. Ortega participated in the manuscript design/conception. Abel Plaza-Florido and Francisco B. Ortega data acquisition. Marcos Olvera-Rojas data analysis. Marcos Olvera-Rojas tables and figure generation. Abel Plaza-Florido first drafting of the manuscript. Abel Plaza-Florido, Marcos Olvera-Rojas, Juan M. A. Alcantara, Shlomit Radom-Aizik, and Francisco B. Ortega critically revised the manuscript for important intellectual content and writing. All authors have read and approved the final version of the manuscript and agree with the order of presentation of the authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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