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Neurological-related proteomic profiling in plasma of children with metabolic healthy and unhealthy overweight/obesity

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Summary

Objective: Children with overweight/obesity (OW/OB) exhibit poor cardiometabolic health, yet mechanisms influencing brain health remain unclear. We examined the differences in neurological-related circulating proteins in plasma among children with metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUO) and the association with metabolic syndrome markers.

Methods: In this cross-sectional study, we included 84 Caucasian children (39% girls), aged 10.1 ± 1.1 years, from the ActiveBrains project (NCT02295072). A ninety-two-protein targeted approach using Olink's® technology was used.

Results: We identified distinct concentrations of CD38, LAIR2, MANF and NRP2 proteins in MHO compared with MUO. Moreover, individual metabolic syndrome (MS) markers were linked to nine proteins (CD38, CPM, EDA2R, IL12, JAMB, KYNU, LAYN, MSR1 and SMOC2) in children with OW/OB. These proteins play crucial roles in diverse biological processes (e.g., angiogenesis, cholesterol transport, nicotinamide adenine dinucleotide (NAD+) catalysis and maintenance of blood–brain barrier) related to brain health.

Conclusion: Our proteomics study suggests that cardiometabolic health (represented by MHO/MUO or individual MS markers) is associated with the concentration in plasma of several proteins involved in brain health. Larger-scale studies are needed to contrast/confirm these findings, with CD38 standing out as a particularly noteworthy and robust discovery.

KEYWORDS
brain health, cardiometabolic health, children, metabolic healthy obesity, proteomics

1 | INTRODUCTION

The prevalence of childhood overweight/obesity (OW/OB), which is known to have a negative impact on cardiometabolic health (i.e., elevated metabolic syndrome markers), has significantly increased in the last decades.[1] Importantly, an impaired cardiometabolic health may have a detrimental effect on the structure and function of the brain.[1–4] Noteworthy, childhood OW/OB is associated with elevated levels of circulating pro-inflammatory cytokines, which also might impair brain health.[5–7] However, there is a subgroup of children with OW/OB characterized by a "favourable" cardiometabolic profile, known as metabolically healthy OW/OB (MHO).[8] Even so, they continue to face an increased risk of cardiovascular diseases and higher morbidity later in life when compared with individuals with normal weight.[9,10]

Individuals with OW/OB who do not display symptoms of the metabolic syndrome (i.e., high levels of fasting glucose, triglycerides, systolic and diastolic blood pressure, and low high-density lipoprotein [HDL]-cholesterol; excluding waist circumference) are considered MHO.[3] Conversely, metabolically unhealthy OW/OB (MUO) in children is characterized by the presence of at least one to four classification criteria for the metabolic syndrome.[2] In paediatric populations, MHO has mostly included children with both OW or OB to maximize sample size and power.[11]

Several studies aimed to characterize the molecular mechanisms of MHO compared with the MUO phenotype in paediatric populations.[11,12] These studies reported a distinct whole-blood transcriptome profile in children with MHO compared with MUO, which was related to a better inflammatory profile in the former.[11,12] Importantly, chronic low-grade inflammation associated with OW/OB could have a negative influence on brain health in children.[13] In this context, Cadenas-Sanchez et al. showed that children with MHO exhibited larger grey matter volumes than those with the MUO phenotype, which was associated with enhanced academic performance.[4] However, why a subset of the paediatric population shows an MHO phenotype linked to better brain health compared with MUO remains poorly understood.

In this sense, proteomics analyses have the potential to offer a comprehensive understanding of biological pathways related to brain health by assessing the concentrations of a large number of circulating proteins.[7,14] Understanding the intricate interplay between circulating proteins and brain health becomes particularly pertinent in discerning disparities within populations, such as MHO and MUO counterparts.[8] Applying targeted proteomics involved in brain/neurological health allows for a nuanced analysis, enabling the identification of specific protein signatures that may differentiate these subgroups. By unravelling the molecular intricacies through targeted proteomics, we can advance our comprehension of the underlying mechanisms linking cardiometabolic and brain health, paving the way for more effective strategies to promote cognitive well-being in diverse populations.

Hence, the goal of this study is to shed light on the potential molecular mechanisms linking the cardiometabolic health status and brain health in children with OW/OB. We performed a targeted proteomics approach to characterize the differences between MHO and MUO phenotypes in plasma levels of 92 neurological-related proteins in children with OW/OB. Additionally, a secondary analysis explored the association between individual metabolic syndrome markers and the neurological-related circulating proteins.

2 | METHODS

2.1 | Participants and study design

A total of 84 children with OW/OB, aged 10.1 ± 1.1 years, (39% girls), were included in this cross-sectional study under the umbrella of the ActiveBrains project (www.profith.ugr.es/activebrains, Clinical Trial: NCT02295072).[15] The detailed study design, methods and inclusion/exclusion criteria have been previously described.[16,17] The research protocol received approval from the Committee for Research Involving Human Subjects at the University of Granada (Reference: 848, February 2014). Prior to participation, all parents were fully informed about the study's objective, and written informed consent was obtained following the principles outlined in the Declaration of Helsinki.

2.2 | Definition of MHO and MUO in children

The definition for MHO and MUO used in this study was explained elsewhere.[2] The cardiometabolic risk factors used to define the MHO and MUO groups were those included in the definition of metabolic syndrome (i.e., blood pressure, fasting glucose, triglycerides and HDL-cholesterol). We classified participants as MHO and MUO following the methodology outlined by Jolliffe and Janssen.[18] This study conducted a comprehensive review of various criteria and developed age- and sex-specific cut-off points for each marker of metabolic syndrome in adolescents aged 12 to 18 years. Since our sample consisted of children aged 9 to 11 years, we utilized the cut-off points for boys and girls aged 12 years as the closest approximation. These cut-off points align with those proposed for adults by the International Diabetes Federation and Adults Treatment Panel, with adaptations made based on age- and sex-specific growth curves in youth. The metabolic syndrome was defined as the presence of specific criteria: fasting glucose (≥100.9 mg/dL in boys and girls), high-serum triglycerides (≥127.4 mg/dL in boys and ≥ 141.6 mg/dL in girls), low HDL-

cholesterol (≤43.7 mg/dL in boys and ≤ 48.3 mg/dL in girls) and elevated systolic or diastolic blood pressure (systolic ≥121 mmHg in boys and girls; diastolic ≥76 mmHg in boys and ≥ 80 mmHg in girls). Participants who did not meet all the metabolic syndrome (MS) criteria (excluding high waist circumference) were classified as MHO, while those who met one or more of the criteria were classified as MUO. Our analysis included both OW and OB participants ($n = 84$; 23 OW and 61 OB).

2.3 | Neurological targeted proteomics

Blood samples were collected from participants between 8 and 9 a.m. following a 12-hour overnight fast. Venipuncture was performed to withdraw the blood. Samples were collected in Vacutainer Tubes® and centrifuged according to the manufacturer 's instruction to obtain serum (Vacutainer® SST™ II Advance tubes) and plasma (Vacutainer® Hemogard™ tubes, containing potassium salt of ethylenediamine tetra-acetic acid—EDTA —as an anticoagulant). The resulting serum and plasma aliquots were stored at −80°C until analyses. Targeted proteomics analysis was described elsewhere.[19,20] Briefly, the quantification of 92 neurological-related proteins was performed on plasma samples (1 μL) at the Olink laboratory in Uppsala, utilizing the Proximity Extension Assay (PEA) technology (Proseek Multiplex Neurological panel 96 reagents kit [Olink® Bioscience, Uppsala, Sweden]). Detailed information about the PEA technology can be found at https://www.olink.com. Shortly, antibody pairs labelled with DNA oligonucleotides bind to the target proteins in the plasma sample. The proximal oligonucleotides then hybridize and are extended by a DNA polymerase. Subsequently, the resulting new DNA sequence, specific to each protein, is detected and quantified using microfluidic qPCR. Normalized protein expression values (NPX values) are presented as arbitrary units in logarithmic scale ($\log_2$). Detailed information about intra- and inter-assay coefficients of variation, detection limits and specific characteristics of each protein can be found on the manufacturer's website (https://olink.com). MAPT protein was omitted from the statistical analysis as it was undetectable in all plasma samples due to being below the limit of detection.

2.4 | Confounders

Sex, peak height velocity (PHV),[21] parental education level, BMI and cardiorespiratory fitness (CRF) were potential confounders.[17,19] Parental education level was assessed using a self-report questionnaire completed by the parents. The responses from both parents were combined and categorized into three groups: neither parent had a university degree, one parent had a university degree and both parents had a university degree. Body weight and height were measured using an electronic scale and stadiometer (Seca Instruments, Germany, Ltd), respectively. BMI was calculated as weight divided by height squared (kg/m^2). The fat mass index (FMI) was calculated/estimated from dual-energy X-ray absorptiometry (DXA) measurements using the Hologic Discovery densitometer. CRF (i.e., VO_2peak relative to body weight [ml/kg/min]) was assessed during an incremental treadmill test using a metabolic cart (CPX Ultima CardiO2, Medical Graphics), as we described in our previous study.[20]

2.5 | Statistical analyses

R (version 4.2.3; R Foundation for Statistical Computing) was used for statistical analyses and graphic design, while a threshold of $p < 0.05$ was considered statistically significant. Diastolic blood pressure and HDL-cholesterol were winsorized to limit the influence of extreme values. In short, the winsorization method replaces extreme high/low values for the closest (highest/lowest) valid values. Analysis of covariance (ANCOVA) was used to test mean β differences in the neurological-related proteins between MHO and MUO, after adjusting for the following covariates: sex, PHV, parental education, BMI and CRF. Furthermore, linear regression models were used to study the associations between individual MS markers and circulating neurological-related proteins. All β values included in the results section are standardized. Additionally, we tested if similar results were reported after adjusting for adiposity (FMI) instead of BMI. Due to the number of comparisons (91 comparisons per predictor), analyses were adjusted by a false discovery rate (FDR) based on the Benjamini-Hochberg method[22] using the "p.adjust" function in R.

3 | RESULTS

Table 1 shows the descriptive characteristics of the 84 children (44 MHO and 40 MUO) included in this study. Of eighty-four participants, 61% were boys and 39% were girls, while 38% presented OW and 62% OB. Children with MHO had lower weight, BMI, FMI, PHV and triglycerides and higher CRF and HDL-Cholesterol compared with their MUO peers ($p < 0.05$; Table 1).

Figure 1 shows differentially concentration levels of proteins between MHO and MUO groups. Specifically, four proteins were found to be significant (MANF, NRP2, CD38 and LAIR2; β differences ranged from −0.395 to −0.282, $p < 0.05$) in the OW/OB sample (Figure 1, panel A) while only two proteins remained statistically significant (CD38, LAIR2; β differences went from −0.345 to −0.333, $p < 0.05$) in sample including only children with OB (Figure 1, panel B) (ANCOVA models disclosure available in Supplementary file 1). These proteins play a crucial role in various cardiometabolic categories, including neurological, cardiovascular and inflammatory conditions, as well as biological processes such as angiogenesis, cellular metabolism and neuron projection development (as shown in Table 2). However, all these significant differences disappeared after FDR correction (FDR ≥0.05).

Figure 2 displays multiple linear regressions between individual MS markers and the 91 neurological-related circulating proteins.

TABLE 1 Descriptive characteristics of the sample.

	All		*Metabolic Healthy (MHO)*		*Metabolic Unhealthy (MUO)*		*p*
	n		*n*		*n*		
Physical characteristics	84		44		40		
Girls, *n* (%)	31	39.3	11	25.0	22	55.0	**0.010**
Age (years)		10.09 ± 1.13		10.04 ± 1.18		10.14 ± 1.08	0.712
Weight (kg)		55.12 ± 11.25		51.75 ± 10.87		58.83 ± 10.59	**0.003**
Height (cm)		144.10 ± 8.66		143.18 ± 9.18		145.10 ± 8.05	0.313
Years from peak height velocity offset		−2.26 ± 0.97		−2.50 ± 0.95		−2.00 ± 0.94	**0.018**
Body mass index (kg/m^2)		26.31 ± 3.50		24.96 ± 2.83		27.79 ± 3.59	**<0.001**
Fat mass index (kg/m^2)		11.40 ± 2.77		10.40 ± 2.15		12.50 ± 2.97	**<0.001**
Cardiorespiratory fitness (mL/kg/min)[a]		37.67 ± 4.55		39.26 ± 4.02		35.91 ± 4.50	**0.001**
Body mass index category (%)							**0.029**
Overweight	23	37.7	17	38.6	6	15.0	
Obesity	61	62.3	27	61.4	34	85.0	
Parental education university level (%)							0.096
None of the parents	54	64.3	25	56.8	29	72.5	
One of the two parents	16	19.1	8	22.9	8	15.7	
Both parents	14	16.6	11	20.0	3	15.7	
Cardiometabolic health							
HDL-cholesterol (mg/dL)		50.85 ± 11.17		56.98 ± 9.67		44.11 ± 8.58	**<0.001**
Triglycerides (mg/dL)		95.63 ± 48.13		72.00 ± 19.59		121.62 ± 56.43	**<0.001**
Fasting glucose (mg/dL)		86.52 ± 6.99		85.64 ± 5.82		87.50 ± 8.04	0.224
Waist circumference (cm)		89.10 ± 9.58		86.33 ± 8.87		92.16 ± 9.51	0.005
Blood pressure							
Systolic (mmHg)		99.46 ± 13.33		96.93 ± 10.63		102.25 ± 15.44	0.068
Diastolic (mmHg)		56.79 ± 13.57		55.00 ± 11.36		58.75 ± 15.56	0.208

Note: Values are mean ± SD or percentages.

[a]Cardiorespiratory fitness (VO_2peak relative to body mass) was assessed with an incremental treadmill test and it was conducted with the use of a gas analyser. Bold numbers indicate $p < 0.05$.

HDL-cholesterol exhibited a negative association with 13 proteins where CD38, LAYN, JAMB and EDA2R persisted after FDR correction (Figure 2, panel A). Interestingly, triglycerides displayed a positive link with 22 proteins, and after FDR correction, CD38, CPM, IL12, KYNU, MSR1 and SMOC2 retained significant (Figure 2, panel B). Fasting glucose, diastolic and systolic blood pressure and waist circumference unveiled diverse protein associations but were not statistically significant after applying the FDR correction (FDR ≥0.05; Figure 2, panels C, D, E and F; linear models' disclosure available in Supplementary file 2). Noteworthy, the nine proteins (CD38, CPM, EDA2R, IL12, JAMB, KYNU, LAYN, MSR1, SMOC2) associated with HDL-cholesterol and/or triglycerides remained statistically significant after performing the FDR correction and are involved in various disease categories, including neurological, cardiovascular, cancer and inflammatory conditions, as well as biological processes such as maintenance of the blood–brain barrier, NAD biosynthetic process, amyloid-beta clearance and regulation of cholesterol and angiogenesis (see Table 3). Similar results were found adjusting for adiposity (i.e., FMI) instead of BMI (Supplementary File 3).

4 | DISCUSSION

This study shows that children with MHO present distinct neurological-related circulating proteins concentration in plasma compared with children with MUO phenotype. Specifically, four proteins exhibited lower concentration (MANF, NRP2, CD38 and LAIR2) in children with MHO compared with MUO. However, statistically significant differences disappeared after applying FDR correction, and therefore, these findings should be interpreted with caution. On the other hand, the MS markers were associated with thirty-five proteins in children with OW/OB. Particularly, after FDR correction, nine proteins (CD38, CPM, EDA2R, IL12, JAMB, KYNU, LAYN, MSR1 and SMOC2) remained statistically significant (FDR <0.05). These proteins are involved in several disease categories (e.g., neurological, cardiovascular and inflammation) and biological processes (e.g., angiogenesis, regulation of immune function and cholesterol transport, maintenance of blood–brain barrier and insulin secretion). The general biological information about the abovementioned proteins is reported in Tables 2 and 3.

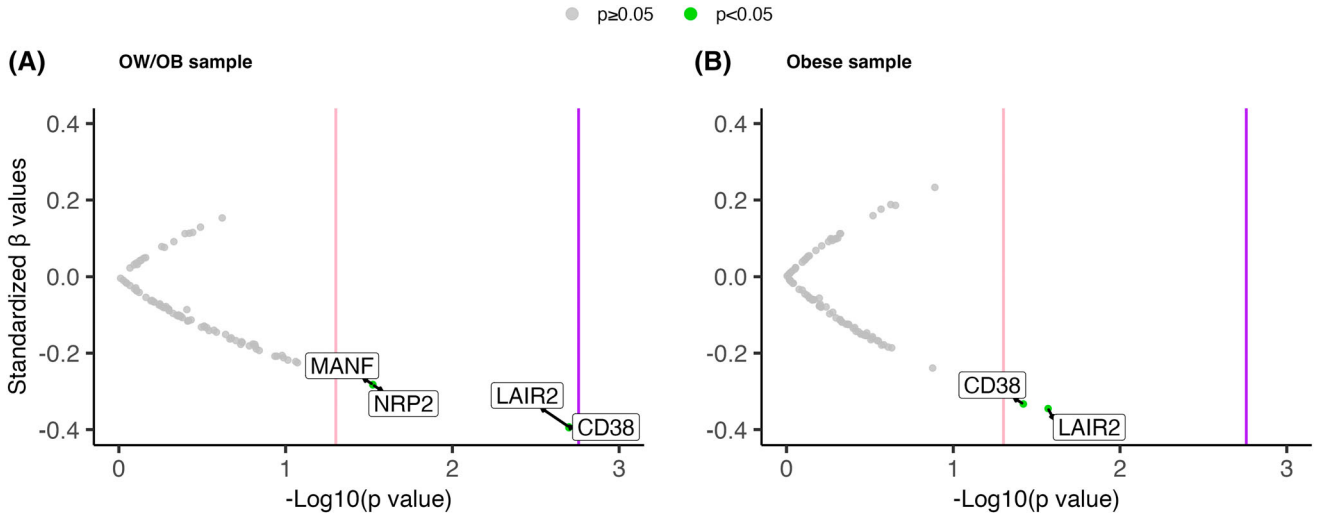

FIGURE 1 Volcano plots show standardized β differences of means for neurological-related proteins from the Olink neurological assay between the metabolic healthy obesity (MHO) versus metabolic unhealthy obesity (MUO) phenotypes in children with overweight/obesity (Panel A; $n = 84$) and between MHO versus MUO children with obesity (Panel B; $n = 61$). The figure displays proteins that were significantly different ($p < 0.05$) in green and non-significantly different between study groups ($p \geq 0.05$) in grey. The x-axis displays p values, with a solid pink line used as a cut-point for statistical significance ($p < 0.05$) and a solid violet line as a cut-point for false discovery rate (FDR <0.05). The y-axis indicates differences between MHO and MUO groups in standardized β differences of means. Analyses of covariance (ANCOVA) were adjusted by sex, peak height velocity (PHV), parental education, body mass index (BMI) and cardiorespiratory fitness (CRF).

TABLE 2 Molecular functions, biological processes and disease areas of the differential plasma levels of proteins related to brain health between metabolic healthy and metabolic unhealthy children with overweight/obesity.

Protein name	Main function	Molecular function	Biological process	Disease area
CD38	It is involved in NAD catalysis and intracellular calcium signalling.	NAD^+ nucleosidase activity Identical protein binding Transferase activity	Regulation of B cell proliferation Regulation of cell growth Regulation of insulin secretion	Cardiovascular/ Neurological/ Aging
LAIR2	It may play roles in both inhibition of collagen-induced platelet aggregation and vessel formation during placental implantation.	Collagen binding	Regulation of T cell	Immunological
MANF	Enhances spontaneous, as well as evoked, GABAergic inhibitory postsynaptic currents in dopaminergic neurons	Growth factor activity RNA binding Sulfatide binding	Dopaminergic neuron differentiation Neuron projection development Regulation of response to endoplasmic reticulum stress	Neurological
NRP2	High affinity receptor for semaphorins 3C, 3F, VEGF-165 and VEGF-145 isoforms of VEGF, and the PLGF-2 isoform of PGF.	Cytokine binding Heparine binding Semaphorin receptor activity	Angiogenesis Axon guidance Regulation of post-synapse organization	Neurological

Note: The information was gathered from the GeneCards (https://www.genecards.org) and UniProtKB website (https://www.uniprot.org).
Abbreviations: cADPR, cyclic ADP-ribose; CD38, ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1; GABA, Gamma-aminobutyric acid; LAIR2, Leukocyte-associated immunoglobulin-like receptor 2; MANF, Mesencephalic astrocyte-derived neurotrophic factor; NRP2, Neuropilin-2; PGF, Placental growth factor; VEGF, Vascular endothelial growth factor.

We will review proteins that consistently exhibited associations with various predictor variables, such as CD38, which have withstood FDR correction. Additionally, we will discuss proteins, such as MSR1, that have demonstrated a significant impact on brain and cardiovascular health in previous studies.[7,19] From this perspective, the most relevant neurological-related proteins found in the study are discussed in the context of current knowledge linking these proteins to human diseases in adults or animal experiments. Notably, plasma levels of CD38 were significantly lower in children with MHO compared with the MUO phenotype, and it was consistently significant

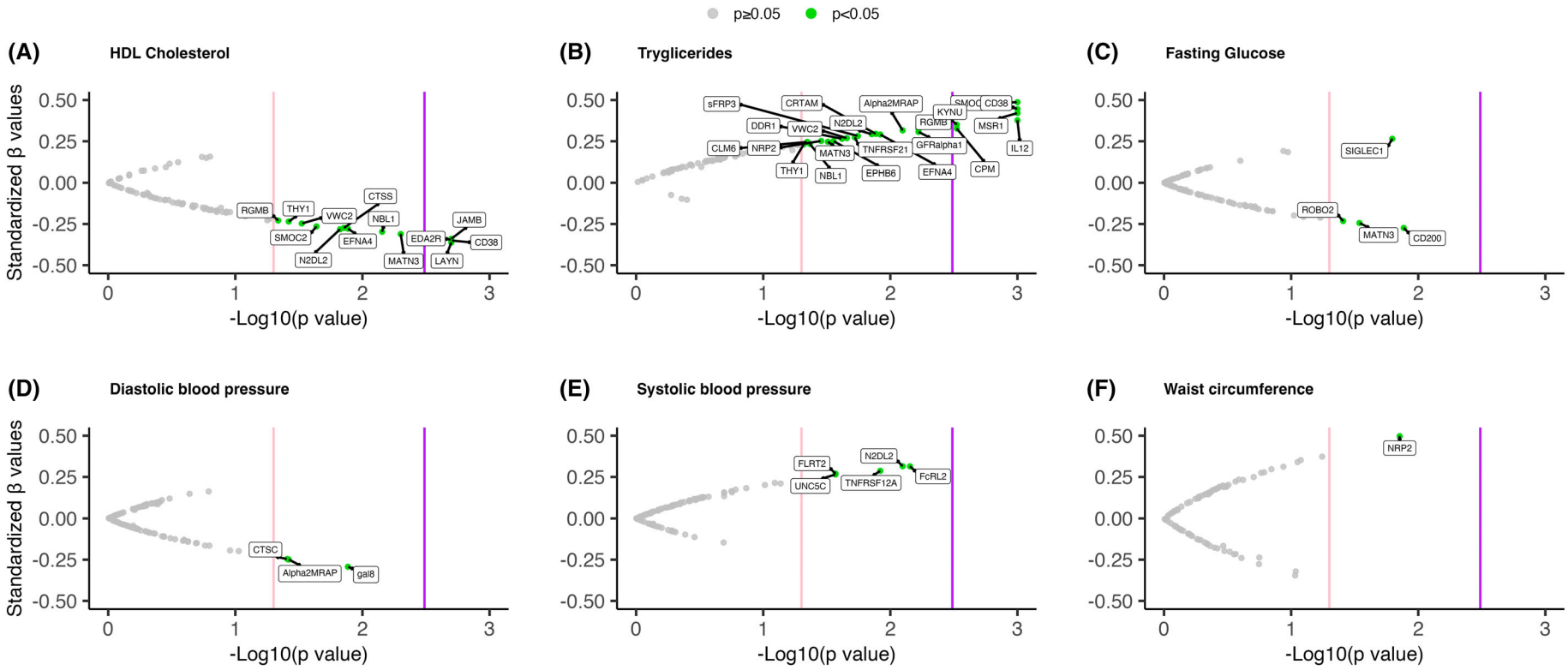

FIGURE 2 Volcano plots show the standardized β values from linear regression models between Olink neurological-related proteins and the different factors of the metabolic syndrome in children with overweight/obesity ($n = 84$). The figure shows proteins that were significantly different ($p < 0.05$) in green and non-significantly different between study groups ($p \geq 0.05$) in grey. The x-axis displays p values, with a solid pink line used as a cut-point for statistical significance ($p < 0.05$) and a solid violet line as a cut-point for false discovery rate (FDR <0.05). The y-axis indicates differences between MHO and MUO groups in standardized β values. Linear regression models are adjusted by sex, peak height velocity (PHV), parental education, body mass index (BMI) and cardiorespiratory fitness (CRF).

TABLE 3 Molecular functions, biological processes and disease areas of the differential plasma levels of proteins (FDR <0.05) related to brain health regarding the different factors of the metabolic syndrome in children with overweight/obesity.

Protein name	Main function	Molecular function	Biological process	Disease area
CD38[a]	It is involved in NAD catalysis and intracellular calcium signalling	NAD^+ nucleosidase activity Identical protein binding Transferase activity	Regulation of B cell proliferation Regulation of cell growth Regulation of insulin secretion	Cardiovascular/ Neurological/ Aging
CPM	It is believed to play important roles in the control of peptide hormone and growth factor activity at the cell surface, and in the membrane-localized degradation of extracellular proteins	Carboxypeptidase activity Metallocarboxypeptidase activity Zinc ion binding	Anatomical structure morphogenesis Peptide metabolic process Protein processing	Immunological/ Innate response
EDA2R	Mediates the activation of the NF-kappa-B and JNK pathways.	Signalling receptor activity Tumour necrosis factor receptor activity	Positive regulation of I-kappaB kinase/NF-kappaB signalling Positive regulation of JNK cascade Positive regulation of NF-kappaB transcription factor activity	Immunological/ Cancer
IL12	Induces the production of interferon-gamma (IFN-gamma), favours the differentiation of T-helper 1 (Th1) cells and is an important link between innate resistance and adaptive immunity.	Growth factor activity Cytokine activity Protein heterodimerization activity	Cellular response to virus Immune response Regulation of natural killer cell activation	Immunological/ Innate response
JAMB	Plays a role in homing and mobilization of haematopoietic stem and progenitor cells within the bone marrow. Also functions as an inhibitory somatodendritic cue that prevents the myelination of non-axonal parts of neurons.	Integrin binding	Maintenance of blood–brain barrier Regulation of myelination Lymphocyte aggregation	Neurological/ Innate response
KYNU	Catalyses the cleavage of L-kynurenine (L-Kyn) and L-3-hydroxykynurenine (L-3OHKyn) into anthranilic acid (AA) and 3-hydroxyanthranilic acid (3-OHAA), respectively.	Pyridoxal phosphate binding Protein homodimerization activity Kynureninase activity	NAD biosynthetic process Response to type II interferon Tryptophan catabolic process to kynurenine	Locomotor/ Cardiovascular/ Aging
LAYN	Receptor for hyaluronate.	Carbohydrate binding Hyaluronic acid binding	Unknown	Immunological
MSR1	Membrane glycoproteins implicated in the pathologic deposition of cholesterol in arterial walls during atherogenesis.	Amyloid-beta binding Scavenger receptor activity Low-density lipoprotein particle binding	Amyloid-beta clearance Cholesterol transport Regulation of cholesterol storage	Cardiovascular/ Neurological
SMOC2	Promotes matrix assembly and cell adhesiveness. Also, it can stimulate endothelial cell proliferation, migration, as well as angiogenesis.	Calcium ion binding Heparin binding Extracellular matrix binding	Regulation of angiogenesis Regulation of vascular endothelial growth factor signalling pathway Regulation of fibroblast growth factor receptor signalling pathway	Neurological

Note: The information was gathered from the GeneCards (https://www.genecards.org) and UniProtKB website (https://www.uniprot.org).
Abbreviations: cADPR, cyclic ADP-ribose; CD38, ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1; CPM, Carboxypeptidase M; EDA2R, Tumour necrosis factor receptor superfamily member 27; IL12, Interleukin-12; JAMB, Junctional adhesion molecule B; KYNU, Kynureninase; LAYN, Layilin; MSR1, Macrophage scavenger receptor types I and II; SMOC2, SPARC-related modular calcium-binding protein 2.
[a]More than one statistically significant association with metabolic syndrome markers.

either in children with OB or OW/OB, increasing the robustness of the findings. Moreover, CD38 was the only neurological-related protein associated with more than one MS marker (specifically triglycerides and HDL-cholesterol) after FDR correction.

CD38, also known as ADP-ribosyl cyclase or cyclic ADP-ribose hydrolase 1, is a single-pass type II membrane protein involved in nicotinamide adenine dinucleotide (NAD^+) catalysis and intracellular calcium ions (Ca^{2+}) signalling. CD38 is key into the regulation of intracellular calcium levels either in cardiovascular (i.e., regulating the contraction and relaxation of the vascular smooth muscle and regulating myocardial ischaemia-reperfusion and cardiac hypertrophy[23–25]) and brain function (i.e., regulation of gene expression and synaptic vesicle

release in neurons and oligodendrocyte progenitor cell development and myelination[26–28]). Remarkably, the levels of free intracellular Ca^{2+} directly impact both cardiovascular and brain health by the activation of the ryanodine receptor 2, promotion of Ca^{2+} release from lysosomes via two-pore channels, induction of extracellular Ca^{2+} by the transient receptor potential M2, voltage mediation of Ca^{2+} channels in grey and white matter and excitability through Ca^{2+} waves.[29–34] On the other hand, CD38 has been intimately associated with NAD^+ age-related modulation decline and inflammation.[35] Interestingly, Escande et al. demonstrated that mice deficient in CD38 or subjected to the CD38 inhibitor apigenin exhibited increased levels of NAD^+ and were shielded from the harmful effects of a high-fat diet in mice.[36] Camacho-Pereira et al. indicated that old CD38 knockout mice maintain NAD^+ levels and mitochondrial function.[35] Tarragó et al. demonstrated that inhibiting CD38 prevents the decline of NAD^+, enhances glucose tolerance, improves physical function and exercise capacity and boosts cardiac function in mice.[37] In this sense, Barbosa et al. revealed that boosting intracellular NAD^+ levels in tissues (such as liver, muscle, brain and heart) protects against obesity, MS, aging and type 2 diabetes in mice.[38,39] Curiously, elevated expression and activity of CD38 are related to a decrease in NAD^+ levels, leading to cellular dysfunction and suggesting an essential role during inflammation.[37] Therefore, high CD38 levels seem to be associated with metabolic dysfunction, accelerated aging and detrimental effects on cardiovascular and brain health. Our study shows that better cardiometabolic health status in children with OW/OB is associated with lower levels of CD38 protein in plasma.

The macrophage scavenger receptor types I and II (MSR1) plasma protein levels were positively associated with triglycerides levels in children with OW/OB. MSR1 contributes to the pathologic accumulation of cholesterol in arterial walls during the development of atherosclerosis.[40] Even though it is preferentially expressed in macrophages, MSR1 is also expressed in vascular smooth muscle cells, endothelial cells, microglia and astrocytes.[41] Particularly, this protein plays a pivotal role in clearing infectious agents and toxic molecules, including amyloid-beta protein, damage-associated molecular patterns (DAMPs) and modified lipids such as oxidized low-density lipoprotein (oxLDL).[42–44] Interestingly, MSR1-mediated phagocytosis triggers both pro- and anti-inflammatory responses, demonstrating that could exert both protective and potentially harmful effects in organs.[41] Higher concentrations of MSR1 contribute to the development of atherosclerosis by engaging with modified lipoproteins and participating in macrophage activation, leading to the secretion of chemotactic factors and inflammatory cytokines.[41] In relation to brain health, higher levels of MSR1 contribute to poor neurovascular health specifically inducing microglia-tissue-specific macrophages of the brain to adopt the M1 pro-inflammatory phenotype.[45] Particularly, "normal" concentration values of MSR1 on microglial cells facilitate the clearance of β-amyloid and prevent amyloid build-up in the brain.[46] Indeed, MSR1 levels were found to be increased in activated microglia and within the brains of individuals diagnosed with Alzheimer's disease, linking an increased concentration with a detrimental role in brain health.[47] Importantly, a randomized controlled trial showed that a 20-week concurrent exercise intervention (aerobic and strength training) decreased MSR1 levels in plasma from children with OW/OB[7]. Here, we observe an association between increased MSR1 in plasma and higher levels of triglycerides, backing up the hypothesis that MSR1 levels are related to worsened cardiometabolic and brain health.

These findings, yet promising, must be viewed with some caution. First, considering the cross-sectional study design and exploratory approach, it is not possible to assume causality. Second, our relatively small sample ($n = 84$) implies less power, and therefore, additional proteins might be identified as relevant in future studies with larger sample sizes. Third, Olink's neurology-related panel quantifies 92 proteins, whereas other methods (e.g., mass spectrometry) could provide a more robust analysis and allow for the measurement of a broader range of proteins. Finally, the cut-off values employed in our study were specific for 12-year-old individuals, as this age group closely aligns with the 9- to 11-year-old age range. However, the robustness of these cut-off values matches those recommended for adults by the International Diabetes Federation and the Adult Treatment Panel, with adjustments made based on age and gender to align with youth growth curves.[4,11] Despite these limitations, we performed targeted proteomics analysis using PEA technology to investigate the differences in protein concentrations for 92 proteins between the MHO vs MUO phenotype in children. In addition to that, VO_2 peak was measured using a gold standard method in an adapted protocol of treadmill for children with OW/OB.

5 | CONCLUSION

We detected that the concentration of four proteins (MANF, NRP2, CD38 and LAIR2) (FDR ≥0.05) were different between children with MHO and MUO phenotypes, which are involved in neurology, immunology and cardiovascular pathways. Among MS markers, triglycerides and HDL-cholesterol were associated with nine proteins (CD38, CPM, EDA2R, IL12, JAMB, KYNU, LAYN, MSR1 and SMOC2; FDR <0.05). Remarkably, CD38 was the protein that showed the most consistent association with cardiometabolic health. Overall, our findings identified potential novel neurological-related proteins in plasma linking cardiometabolic health status with brain health in children with OW/OB. Future studies involving larger sample sizes are warranted to corroborate or contrast the robustness of our findings.

AUTHOR CONTRIBUTIONS

Msc Marcos Olvera-Rojas had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: Olvera-Rojas, Plaza-Florido, Solis-Urra, Osuna-Prieto, Ortega. Acquisition, analysis, or interpretation of data: Olvera-Rojas, Plaza-Florido, Solis-Urra, Ortega. Drafting of the manuscript: Olvera-Rojas (wrote the first draft), Plaza-Florido, Solis-Urra, Osuna-Prieto, Ortega. Critical revision of the manuscript for important intellectual content: Plaza-Florido, Solis-Urra, Osuna-Prieto, Ortega. Statistical analysis: Olvera-Rojas, Plaza-Florido, Solis-Urra. Obtained funding: Ortega.

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CONFLICT OF INTEREST STATEMENT

The authors affirm that the study was carried out without any commercial or financial associations that could be perceived as a potential conflict of interest.

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