Cardiorespiratory fitness and targeted proteomics involved in brain and cardiovascular health in children with overweight/obesity

Abel Plaza-Florido[1], Maria Rodriguez-Ayllon[2], Signe Altmäe[3,4], Francisco B. Ortega[1,5,6], Irene Esteban-Cornejo[1]

Running title

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Affiliations

[1] PROFITH "PROmoting FITness and Health through physical activity" research group, Sport and Health University Research Institute (iMUDS), Department of Physical and Sports Education, Faculty of Sport Sciences, University of Granada, Granada, Spain.

[2] Department of Epidemiology, Erasmus MC University Medical Center, Rotterdam, the Netherlands.

[3] Department of Biochemistry and Molecular Biology, Faculty of Sciences, University of Granada, Granada, Spain; Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain.

[4] Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institutet, and Division of Obstetrics and Gynecology, Karolinska Universitetssjukhuset, Stockholm, Sweden

[5] Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland.

[6] Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden.

*** Corresponding authors**: Abel Plaza-Florido (email: abeladrian@ugr.es) and Francisco B. Ortega (email: ortegaf@ugr.es)

Abel Plaza-Florido & Francisco B. Ortega: Department of Physical and Sports Education, Faculty of Sports Science, University of Granada, Granada, Spain. Carretera de Alfacar, s/n CP: 18071 Granada. Tlf: (+34) 958 24 43 53

E-mail addresses:

Abel Plaza-Florido: abeladrian@ugr.es

Maria Rodriguez-Ayllon: m.rodriguez@erasmusmc.nl

Signe Altmäe: signealtmae@ugr.es

Francisco B. Ortega: ortegaf@ugr.es

Irene Esteban-Cornejo: ireneesteban@ugr.es

ORCID of the author(s)

Abel Plaza-Florido: https://orcid.org/0000-0002-5374-3129

Maria Rodriguez-Ayllon: https://orcid.org/0000-0002-8267-0440

Signe Altmäe: https://orcid.org/0000-0002-0708-1865

Francisco B. Ortega: https://orcid.org/0000-0003-2001-1121

Irene Esteban-Cornejo: https://orcid.org/0000-0002-0027-1770

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ABSTRACT

Background/Objectives: Cardiorespiratory fitness (CRF) is inversely associated with cardiovascular disease (CVD) risk factors and brain health impairments. However, the molecular mechanisms linking CRF to health in children are poorly understood. We aimed to examine protein levels related to brain health and CVD in plasma of fit compared to unfit children with overweight/obesity (OW/OB).

Methods/Design: Eighty-seven children with OW/OB (10.08 ± 1.1 years, 59% boys) from the ActiveBrains project were included. CRF was measured by performing a treadmill test, and children were categorized into fit or unfit. Targeted proteomics in plasma was performed using Olink's proximity extension assay technology of Neurology panel in the whole sample and of Cardiovascular panel in a subsample.

Results: Sixteen proteins (PLXNB3, sFRP3, CLEC1B, RSPO1, Gal8, CLEC10A, GCP5, MDGA1, CTSC, LAT, IL4RA, PRSS27, CXCL1, Gal9, MERTK, and GT) were differentially expressed between fit and unfit children with OW/OB after adjusting for sex, maturational status, and body mass index. However, statistically significant differences disappeared after applying FDR correction.

Conclusion: Potential candidate proteins related to CRF levels in children with OW/OB were detected, being involved in several biological processes such as neurogenesis, immune/inflammatory response, signal transduction, platelet activation. Nevertheless, these preliminary findings should be confirmed or contrasted in future studies using larger sample sizes, longitudinal and experimental designs.

Keywords: exercise; omics; pediatrics; neurology; adiposity

Highlights

- The molecular mechanisms underlying the link of cardiorespiratory fitness (CRF) with cardiovascular and brain health in children with overweight/obesity (OW/OB) are poorly understood.
- Targeted proteomic analysis revealed differentially expressed proteins (PLXNB3, sFRP3, CLEC1B, RSPO1, Gal8, CLEC10A, GCP5, MDGA1, CTSC, LAT, IL4RA, PRSS27, CXCL1, Gal9, MERTK, and GT) in plasma of "Fit" compared to "Unfit" children with OW/OB. These proteins are involved in several biological processes such as immune/inflammatory response, neurogenesis, signal transduction, and cellular metabolic process.
- Longitudinal and experimental studies are warranted to reveal how improvements in CRF are related to changes in circulating levels of the abovementioned proteins and how they might reduce cardiovascular diseases risk factors and brain health impairments later in life.

1. Introduction

Childhood obesity is related to increased cardiovascular disease (CVD) risk factors (e.g., fasting glucose, triglycerides, blood pressure, atherosclerosis, inflammation), and negatively affects brain health (i.e., cognitive and brain development) (Reinert, Po'e, & Barkin, 2013; Skinner, Perrin, Moss, & Skelton, 2015). Both, CVD risk factors and brain health impairment share similar pathophysiology mechanisms, what has been called "the heart-brain connection" (Roger, 2017). Closely related to adiposity and body composition are physical fitness components, and particularly, cardiorespiratory fitness (CRF). Consistent evidence support the notion that CRF is a powerful marker of health in youth (Ortega, Ruiz, Castillo, & Sjöström, 2008). Higher CRF levels are inversely associated with CVD risk factors during childhood (even early subclinical atherosclerosis) (Garciá-Hermoso, Ramírez-Vélez, Garciá-Alonso, Alonso-Martínez, & Izquierdo, 2020), and later in adulthood (Högström, Nordström, & Nordström, 2014). Besides, CRF is positively associated with brain volume at different regions related to better academic performance (Esteban-Cornejo et al., 2017), and improved hippocampal connectivity and cognitive function (Esteban-Cornejo et al., 2021) in children with OW/OB.

CRF and OW/OB are both related to CVD risk factors and brain health outcomes in children. However, less is known regarding the molecular mechanisms relating CRF to cardiovascular and brain health in children with OW/OB. Interestingly, high-throughput technological provides the tools to explore the molecular mechanisms of CRF in children with OW/OB detecting thousands of molecules simultaneously (Plaza-Florido et al., 2021; Sanford et al., 2020). For that purpose, we applied targeted proteomic approach for search of cardiovascular and brain health biomarkes in different fitness

levels in childhood OW/OB by analyzing 184 proteins (92 brain health- and 92 CVD-related proteins) in plasma of fit compared to unfit children with OW/OB.

2. Methods

2.1 Participants and study design

This cross-sectional study included eighty-seven children with OW/OB (10.14 ± 1.3 years, 59% boys) with available data of proteins included in the Olink Neurology panel and CRF. The project (Clinical Trial: NCT02295072) was approved by the Committee for Research Involving Human Subjects at the University of Granada (Reference: 848, February 2014). Briefly, the inclusion criteria were: (i) children aged 8 – 11 years old with OW/OB using the World Obesity Federation cut-off points (Cole & Lobstein, 2012), (ii) not have neurological disorders that might have an impact on movement performance; (iii) the girls have not started menstruation; (iv) right-handed (differences in brain health between left- and right-handed may exist). All parents/legal guardians written informed consent following the Declaration of Helsinki and received the studys information.

2.2. Anthropometry, body composition and maturation

An electronic scale and a stadiometer (Seca Instruments, Germany, Ltd) were used to obtain weight, height, and body mass index (BMI) calculated as kg/m^2. Children were classified with OW/OB using the sex- and age-specific cut-off points (World Obesity Federation) (Cole & Lobstein, 2012). Body composition variables such as fat mass index (FMI) and lean mass index (LMI) were quantified using dual-energy X-ray absorptiometry (DXA, discovery densitometer from Hologic). Peak height velocity (PHV) reported the maturational status of children using validated algorithms for boys

and girls (Moore et al., 2015). PHV was calculated as follows for boys: −8.1 + (0.0070346 × (age x sitting height)) and girls: −7.7 + (0.0042232 × (age × height)).

2.3. Cardiorespiratory fitness

CRF (i.e., VO_2peak) assessment has been described previously (Plaza-Florido et al., 2021). Briefly, an incremental treadmill test (HP-Cosmos ergometer) was performed using a gas analyzer (General Electric Corporation). The slope started at 6% with grade increments of 1% every minute until volitional exhaustion, while the speed was constant during the exercise test (i.e., 4.8 km/h). CRF (i.e., VO_2peak) was reported relative to body mass (mL/kg/min). CRF levels higher than 42 ml/kg/min (for boys) and 35 ml/kg/min (for girls) relative to body mass were considered to classify children as "fit", while children with lower values were considered as "unfit". These health-related cut-off points for CRF relative to body mass were provided by a meta-analysis addressing the cardio-protective role of CRF in children (Ruiz et al., 2016).

2.4. Brain health- and CVD-related targeted proteomics

Blood collected in EDTA tubes (in the morning between 8-9 a.m. after 12h of fasting) was centrifuged at 1000×g for 10 min. Isolated plasma was stored at −80ºC. The 92 brain health-related and 92 CVD-related proteins were quantified in plasma (1 microliter) at the Olink laboratory in Uppsala using the PEA technology (Proseek Multiplex Neurology and Cardiovascular panel 96 × 96 reagents kit [Olink® Bioscience, Uppsala, Sweden]). Due to budget restrictions, proteins included in the Cardiovascular panel were measured in a subsample of 44 participants from the ActiveBrains project. This subsample was randomly selected using the SPSS software (version 21.0; Armonk, NY, USA). Subsequently, we verified that the subsample of 44 participants did not differ in key characteristics of this study, i.e., sex, age, maturation, or CRF, being therefore the

subsample representative of the complete sample. The PEA technology has been described in detail (https://www.olink.com/). Briefly, antibody pairs labeled with DNA oligonucleotides bind to the target protein in plasma. Then, oligonucleotides in proximity hybridize and are extended by a DNA polymerase. Finally, the new DNA sequence (specific for each protein) is detected and quantified using a microfluidic qPCR. Normalized protein expression values (NPX values) are presented as arbitrary units in the log2 scale. NPX values are calculated from Ct values and are interpreted in the opposite direction (i.e., higher NPX values show higher protein concentrations, while higher Ct values indicate lower concentration). Intra- and inter-assay coefficients of variations, detection limits, and specific information for each protein are reported on the manufacturer's website (https://www.olink.com/).

2.5 Statistical analyses

SPSS version 21.0 (IBM Corporation, NY, USA) was used for statistical analyses, while a threshold of $p < 0.05$ was considered statistically significant. Student t-test (continuous variables) and chi-square (categorical variables) tests were used to study the differences on sample characteristics between fit and unfit children with OW/OB. ANCOVA was performed to obtain adjusted mean differences on 92 brain health-related and 92 CVD-related proteins in plasma between fit and unfit children with OW/OB after including sex, maturational status (i.e., PHV), and BMI as confounders. In addition, we tested if previous analyses reported similar results after adjusting for adiposity (FMI) instead of BMI. Analyses were adjusted for multiple comparisons using false discovery rate (FDR) based on the Benjamini-Hochberg method (Benjamini & Hochberg, 1995).

3. Results

Characteristics of participants are shown in **Table 1**. In the fit group, 27% of participants were boys and 73% girls, while 79% were boys and 21% were girls in the unfit group. The fit group presented higher VO_2peak relative to body mass, lower body weight, BMI, FMI compared to the unfit group ($p < 0.05$). Descriptive information about the subsample of 44 children with data for CVD-related proteins is presented in **Supplementary Table 1**.

Concerning 92 brain health-related proteins (N = 87; 34 Fit vs. 53 Unfit), 1 protein (MAPT) was below the limit of detection across all the plasma samples and was not included in statistical analyses. Ten proteins were differentially expressed between study groups. Specifically, 9 proteins were down-regulated (PLXNB3, sFRP3, CLEC1B, RSPO1, Gal8, GCP5, MDGA1, CTSC, LAT; adjusted mean differences ranged from -1.00 to -0.23 NPX values, $p <0.05$) and 1 up-regulated (CLEC10A; adjusted mean differences 0.28 NPX values , $p<0.05$), in fit children compared to unfit children after adjusting by sex, maturation (i.e., PHV), and BMI (**Table 2; Figure 1, Panel A**). These proteins were involved in diverse diseases categories: neurological, cardiovascular, cancer, and inflammatory and biological processes: immune/inflammatory response, neurogenesis, signal transduction, and cellular metabolic process (**Table 2**). However, when applying the multiple testing correction, the expression level of these proteins did not reach significance level.

Regarding 92 CVD-related proteins (N = 44; 19 Fit vs. 25 Unfit), 6 proteins were differentially expressed. Specifically, 3 proteins were down-regulated (PRSS27, CXCL1, Gal9; adjusted mean differences ranged from -1.04 to -0.21 NPX values, $p<0.05$) and 3 up-regulated (GT, IL4RA, MERTK; adjusted mean differences ranged from 0.19 to 0.54 NPX values, $p < 0.05$) in fit children compared to unfit children after adjusting by sex, maturation (i.e., PHV), and BMI (**Table 3; Figure 1**, **Panel B**). These proteins were

involved in diverse diseases categories: cardiovascular, pulmonary, and inflammatory, among others and biological processes: cell adhesion, immune response, and inflammatory response, among others (**Table 3**). The abovementioned proteins were differentially expressed between fitness levels groups adjusting for FMI instead of BMI (Supplementary Tables **2** and **3**). The protein expression levels, however, were not statistically significant after performing multiple hypothesis testing correction (FDR > 0.05). The non-significant results for 81 brain health- and 86 CVD-related proteins are presented in **Supplementary Tables 4 and 5** (all $p > 0.05$).

4. Discussion

This study indicates that a number of cardiovascular and brain health biomarkers might be differentially expressed in plasma of fit compared to unfit children with OW/OB. Specifically, from neurology panel, 9 biomarkers were down-regulated (PLXNB3, sFRP3, CLEC1B, RSPO1, Gal8, GCP5, MDGA1, CTSC, LAT) and 1 up-regulated (CLEC10A); and from cardiovascular panel, 3 were down-regulated (PRSS27, CXCL1, Gal9) and 3 up-regulated (GT, IL4RA, MERTK). These proteins were involved in several diseases categories (e.g., neurological, cardiovascular, inflammatory, pulmonary) and biological process (e.g., neurogenesis, signal transduction, immune function, inflammatory response). Thus, these findings provide novel plasma biomarkers related to CRF levels in children with OW/OB. However, statistically significant associations disappeared after performing multiple corrections, and therefore these findings should be interpreted as preliminary and confirmed or contrasted in future studies involving larger sample size, longitudinal designs, and randomized clinical trials.

Interestingly, our fit and unfit groups showed a mean difference of 4.98 mL/kg/min in VO_2peak relative to body mass. In this regard, differences of 1.75 mL/kg/min of VO_2peak relative to body mass have been considered relevant from a clinical point of view in adults (Bonafiglia et al., 2021). Importantly, an increase of 0.38 ml/kg/min in VO_2peak has been considered clinically important for the reduction of body fat percentage (2.30%) in children with OW/OB (García-Hermoso et al., 2020). Thus, the different patterns of protein expression between fit and unfit children may help to understand better the molecular mechanisms relating CRF to health in children with OW/OB. To our knowledge, no previous studies have investigated the relationship between childhood OW/OB and fitness with protein biomarkers reported in the current study. Thus, Several proteins (i.e., differentially expressed in fit compared to unfit children) are discussed in the context of existing knowledge linking these protein biomarkers to human diseases in adults, animal experiments, or cell cultures. We will discuss proteins presenting higher mean differences between fit and unfit groups, and interesting proteins with possible implications for brain and cardiovascular health. The biological process and disease categories of 16 differentially expressed proteins between fit and unfit groups were presented in Tables **2** and **3**.

LAT and CXCL1 proteins (from Neurology and Cardiovascular_panels) showed the highest mean differences (1.00 and -1.04 NPX values respectively; A 1 NPX value difference means a doubling of protein concentration) between fit and unfit groups (downregulated in plasma of fit compared to unfit children with OW/OB). LAT (Linker for activation of T-cells family member 1) is part of the T-cell receptor complex (TCR). It works as an integrator node of several signaling pathways regulating T cell activation (Bartelt & Houtman, 2013). In this context, obesity is characterized by chronic "over-activation" of the immune system, which is reflected by altered T cell activity and

infiltration in adipose tissue contributing to systemic low-grade chronic inflammation (Wang, Wang, & Xu, 2021). Thus, we could hypothesize that lower levels of LAT in plasma of fit compared to unfit children with OW/OB could be indicative of a lower chronic "over-activation" of the immune system. In the brain health context, an experiment in the zebrafish model showed that LAT could impact early neurogenesis (Loviglio et al., 2017). LAT suppression was associated with increasing brain cells number and size. Conversely, LAT overexpression was associated with decreased cell proliferation in the brain and microcephaly in zebrafish (Loviglio et al., 2017). CXCL1 (C-X-C Motif Chemokine Ligand 1) is involved in the immune- inflammatory response (i.e., contribute to attracting immune cells into injury sites) (Wang et al., 2018), and higher CXCL1 levels in plasma were associated with more adiposity in young adults (Klevebro et al., 2021). Childhood obesity is characterized by a systemic low-grade inflammation that is related to a higher risk of CVD (Barton, 2012; Ortega, Lavie, & Blair, 2016). In this context, CXCL1 has been considered a pro-inflammatory chemokine and its neutralization has been proposed as a therapeutic target for CVD treatment (Wang et al., 2018). Interestingly, regular physical activity, which can increase CRF levels, decreased CXCL1 levels in serum of rodents (Jablonski et al., 2020). Our findings suggest that CRF could contribute to reduced CXCL1 levels in plasma of children with OW/OB.

CLEC1B and sFRP3 were down-regulated proteins in fit children, showing higher mean differences between the study groups. Increased CLEC1B levels in plasma have been related to a higher risk of coronary artery disease (Fei et al., 2020). In addition, a study of proteomics biomarkers discovery in CVD showed that CLEC1B was one of the most important predictors of subclinical atherosclerosis in humans (Mosley et al., 2018). Importantly, childhood obesity can contribute to the development of coronary artery disease and atherosclerosis during adulthood (Barton, 2012). To note, secreted frizzled-

related proteins (sFRPs) regulate the Wnt signaling pathway involved in hypertrophy cardiac growth and remodeling (Bergmann, 2010). In this regard, childhood obesity has been associated with hypertrophy and remodelling of the heart (Jing et al., 2016), while higher plasma sFRP3 has been related to a higher risk of CVD mortality in middle-aged adults (Askevold et al., 2014). Decreased CLEC1B and sFRP3 levels in plasma of fit children with OW/OB could contribute to a healthier cardiovascular profile in children with OW/OB, compared to their unfit peers. Mechanistic studies and randomized clinical trial are needed to reveal the specific molecular mechanisms and the clinical relevance of the abovementioned associations. In the neurological context, sFRP3 reduction contributes to faster new neuron development influencing neurogenesis in the hippocampus of mice (Jang et al., 2013). Interestingly, neuronal activation induced by voluntary running reduced sFRP3 expression in the hippocampus of mice (specifically in the dentate gyrus) (Jang et al., 2013). Thus, downregulation of CLEC1B and sFRP3 proteins in fit compared to unfit children indicates these biomarkers' promising role in linking CRF to brain and cardiovascular health in children with OW/OB.

Galectins is a family of proteins involved in CVD and brain health (Barake, Soza, & González, 2020; van der Hoeven et al., 2016). In our study, two different galectins, Gal9 and Gal8, were downregulated in fit compared to unfit children with OW/OB. Childhood obesity can lead to type 2 diabetes and coronary artery disease during adulthood (Fang et al., 2019). Interestingly, patients with type 2 diabetes and coronary artery disease showed higher Gal9 in serum compared to controls (Ozturk et al., 2015). Besides, body mass index (indicator used to define obesity) has been positively associated with Gal9 in plasma of adults (Pang et al., 2021). In the neurological context, Gal9 is highly expressed in microglia and astrocytes (Barake et al., 2020), while higher Gal9 concentrations in cerebrospinal fluid were positively associated with cognitive decline in

adults with human immunodeficiency virus (HIV) (Premeaux et al., 2019). Besides, serum Gal9 levels were higher in patients with Alzheimer's disease and mild cognitive impairment compared to controls (Wang, Niu, Yue, Fu, & Wang, 2019), and were negatively associated with global cognitive function (Wang et al., 2019). Regarding Gal8, this protein can activate human platelets playing a role in thrombosis/inflammation (Romaniuk et al., 2010), while this protein was upregulated in tumoral endothelial cells (Bidon-Wagner & Le Pennec, 2002). In contrast, Gal8 may play a neuroprotective role in the brain (Barake et al., 2020; Pardo et al., 2019). In cell culture, Gal8 contributes to the survival of hippocampal neurons under stress conditions found in neurocognitive diseases (e.g., oxidative stress, glutamate-induced excitotoxicity, nutrient deficits) (Pardo et al., 2019). Besides, Gal8 knock-out rodents reported higher apoptosis in the hippocampus compared to wild-type (Pardo et al., 2019). Nevertheless, more research is needed to understand the health implications and molecular mechanisms underlying the lower levels of Gal9 and Gal8 in plasma of fit compared to unfit children with OW/OB.

Several limitations should be considered. First, causality cannot be assumed due to the cross-sectional study design. Second, proteins included in the Cardiovascular panel were quantified in a subsample of 44 participants. Third, our sample size was relatively small, and most of the children in the unfit group were boys (8 of 53) with OB (41 of 53) while most of the children in the fit group were girls (25 of 34) with OW (although better balanced, 19 of 34), which could have influenced the analysis. Nevertheless, the ANCOVA analysis was controlled by sex, maturation (PHV), and BMI to attenuate the potential confounding role in this analysis. Fifth, the estimation of maturity status through somatic methods has an error of 1 year around the estimation (Moore et al., 2015). Otherwise, CRF was objectively quantified with a gas analyzer using adapted treadmill protocol for children with OW/OB.

Regarding the strength of the associations reported in our study, we showed that several proteins (i.e., PLXNB3, sFRP3, CLEC1B, RSPO1, Gal8, CLEC10A, GCP5, MDGA1, CTSC, LAT, IL4RA, PRSS27, CXCL1, Gal9, MERTK, and GT) presented adjusted mean differences ranging from -1.04 to 1.00 NPX values (a 1 NPX value difference means a doubling of protein concentration) between fit and unfit children with OW/OB. Thus, the differences in protein expression between fitness groups could be important; however, the lack of studies linking these proteins to childhood obesity and other health-related outcomes and the cross-sectional study design preclude highlighting the biological/clinical relevance of the associations reported in our study. Future studies using longitudinal designs and randomized controlled trials should note how fitness changes over time are related to the protein biomarkers levels detected in our study.

5. Conclusion

The differential protein expression detected in fit compared to unfit children with OW/OB contribute to identify novel brain health- and CVD-related biomarkers (i.e., PLXNB3, sFRP3, CLEC1B, RSPO1, Gal8, CLEC10A, GCP5, MDGA1, CTSC, LAT, IL4RA, PRSS27, CXCL1, Gal9, MERTK, and GT) associated with CRF levels in children with OW/OB. Adjusted mean differences between fit and unfit children with OW/OB ranged from -1.04 to 1.00 NPX values (A 1 NPX value difference means a doubling of protein concentration). However, these findings should be interpreted with caution due to the cross-sectional study design and the relatively small sample size, with significant differences not persisting after multiple correction. Cross-sectional studies using larger sample sizes and randomized controlled trials are needed to test the impact of exercise interventions on CRF and these brain health- and CVD-related proteins.

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.

Supplementary information

The article contains supplementary information online at European Journal of Sport Science website

Data and Resource Availability

We did not obtain children's parents consent to widely share the data nor was it included in the IRB protocol.

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Table 1. Characteristics of the participants

Variables	Total sample n=87*	Fit children n=34	Unfit children n=53	P value
Sex, age, and maturational status				
Boys, n (%)	51 (59%)	9 (27%)	42 (79%)	**<0.001**
Age (years)	10.08 ± 1.12	9.86 ± 1.14	10.22 ± 1.10	0.15
Years from PHV	-2.25 ± 0.97	-2.02 ± 0.91	-2.39 ± 0.98	0.08
BMI group by Cole *et al.*				
Overweight	31 (36%)	19 (56%)	12 (23%)	**0.003**
Obesity	56 (64%)	15 (44%)	41 (77%)	
Anthropometry				
Weight (kg)	55.34 ± 10.30	51.57 ± 10.08	57.77 ± 11.35	**0.01**
Height (cm)	144.01 ± 8.60	143.53 ± 9.63	144.31 ± 7.96	0.68
Waist circumference (cm)	89.50 ± 9.84	85.27 ± 8.83	92.22 ± 9.57	**0.001**
BMI (kg/m^2)	26.45 ± 3.55	24.81 ± 2.65	27.50 ± 3.67	**<0.001**
FMI (kg/m^2)	11.51 ± 2.81	10.29 ± 2.13	12.29 ± 2.92	**<0.001**
LMI (kg/m^2)	13.92 ± 1.40	13.46 ± 1.33	14.21 ± 1.38	**0.02**
Cardiorespiratory fitness				
VO_2peak relative to body mass (ml/kg/min)	37.56 ± 4.55	40.59 ± 4.26	35.61 ± 3.59	**<0.001**

Data presented as unadjusted mean ± SD, and as number and frequency. BMI: Body mass-index, FMI: Fat mass-index, LMI: lean mass-index, PHV: Peak height velocity. BW: Body weight. Bold numbers show $P < 0.05$* Brain health-related proteins were measured in the complete sample (34 Fit vs. 53 unfit), while cardiovascular disease-related proteins were quantified in a subsample of 44 participants (19 Fit vs. 25 Unfit; descriptive Supplementary Table 1).

Table 2. Differentially expressed proteins, analysed with Olink Neurology Panel between Fit and Unfit children with overweight/obesity.

Fit vs Unfit - Neurologic panel (main study simple, N = 87)											
	Fit children (N = 34)			Unfit children (N = 53)			Difference between groups				
	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	p-value	FDR
CLEC10A	5.84	5.70	5.99	5.56	5.44	5.69	0.28	0.07	0.50	0.01	0.17
CLEC1B	9.70	9.26	10.14	10.52	10.14	10.89	-0.82	-1.47	-0.17	0.02	0.17
CTSC	2.76	2.57	2.96	3.12	2.96	3.28	-0.35	-0.64	-0.07	0.01	0.17
gal8	5.62	5.26	5.97	6.21	5.91	6.51	-0.59	-1.11	-0.07	0.03	0.28
GCP5	5.21	4.97	5.45	5.67	5.47	5.87	-0.46	-0.82	-0.10	0.01	0.17
LAT	5.49	4.95	6.02	6.49	6.04	6.94	-1.00	-1.79	-0.22	0.01	0.17
MDGA1	4.85	4.59	5.10	5.23	5.01	5.45	-0.39	-0.76	-0.01	0.04	0.41
PLXNB3	4.12	3.84	4.41	4.66	4.42	4.90	-0.54	-0.96	-0.12	0.01	0.17
RSPO1	1.86	1.74	1.97	2.08	1.98	2.18	-0.23	-0.40	-0.06	0.01	0.17
sFRP3	3.05	2.75	3.36	3.67	3.41	3.92	-0.61	-1.06	-0.17	0.01	0.17

	Disease area	Biological process
CLEC10A	Cardiovascular, neurological, infectious, cancer	Immune response
CLEC1B	Cardiovascular, skeletal, renal, metabolic, digestive, cancer	Cell differentiation, signal transduction
CTSC	Cardiovascular, metabolic, cancer, cutaneous	Cell death, cellular metabolic process, immune response, proteolysis
gal8	Neurological, inflammatory, cancer	Other GO terms
GCP5	Cardiovascular, renal, neurological, metabolic, digestive, cancer	Cellular metabolic process
LAT	Renal, neurological, inflammatory, infectious, cancer	Cell adhesion, cellular metabolic process, immune response, MAPK cascade, signal transduction

MDGA1	Neurological	Cell differentiation, neurogenesis
PLXNB3	Neurological, cancer	Axon development, axon guidance, cell adhesion, cell differentiation, neurogenesis, signal transduction
RSPO1	Digestive, cancer, cutaneous	Cellular metabolic process, signal transduction
sFRP3	Skeletal, cancer	Cell death, cell differentiation, cell growth, signal transduction

ANCOVA analyses adjusted by sex, maturational status (i.e., peak height velocity) and body mass index. Data are presented using NPX (Normalized Protein eXpression) values. A 1 NPX value difference means a doubling of protein concentration.

CI: Confidence intervals, CLEC10A: C-type lectin domain family 10 member A, CLEC1B: C-type lectin domain family 1 member B, CTSC: Dipeptidyl peptidase 1, FDR: False discovery rate, Gal-8: Galectin-8, GCP5: Glypican-5, GO: Gene ontology, LAT: Linker for activation of T-cells family member 1, MDGA1: MAM domain-containing glycosylphosphatidylinositol anchor protein 1, PLXNB3: Plexin-B3, RSPO1: R-spondin-1, sFRP3: Secreted frizzled-related protein 3.

Table 3. Differentially expressed proteins, analysed with Olink Cardiovascular panel between Fit and Unfit children with overweight/obesity .

Fit vs Unfit - Cardiovascular panel (randomly selected subsample, 50% of main study sample, N = 44)											
	Fit children (N = 19)			Unfit children (N = 25)			Difference between groups				
	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	p-value	FDR
CXCL1	8.58	7.88	9.27	9.61	9.05	10.18	-1.04	-2.01	-0.07	0.04	0.67
Gal9	7.96	7.84	8.09	8.18	8.08	8.28	-0.21	-0.39	-0.04	0.02	0.49
GT	3.02	2.67	3.36	2.48	2.20	2.75	0.54	0.06	1.02	0.03	0.62
IL4RA	2.35	2.21	2.49	2.16	2.05	2.27	0.19	0.00	0.39	0.04	0.67
MERTK	6.28	6.12	6.44	5.99	5.87	6.12	0.29	0.07	0.51	0.01	0.49
PRSS27	8.79	8.52	9.06	9.25	9.03	9.47	-0.46	-0.83	-0.09	0.02	0.49
	Disease area						Biological process				
CXCL1	Cardiovascular, Pulmonary, inflammatory, hepatic, digestive, skeletal						Immune response, inflammatory response				
Gal9	Skeletal, neurological, Hemic and Lymphatic, cancer						Cell adhesion, immune response, inflammatory response, MAPK cascade				
GT	Metabolic, digestive, cancer						Catabolic process				
IL4RA	Cardiovascular, Pulmonary, neurological, inflammatory, infectious, cutaneous, cancer						Cell adhesion, immune response, inflammatory response				
MERTK	Cardiovascular, Pulmonary, neurological, inflammatory, digestive						Cell adhesion, coagulation, platelet activation, wound healing				
PRSS27	Cardiovascular, cancer						Other GO terms				

ANCOVA analyses adjusted by sex, maturational status (i.e., peak height velocity) and body mass index. Data are presented using NPX (Normalized Protein eXpression) values. A 1 NPX value difference means a doubling of protein concentration.

CI: Confidence intervals, CXCL1: C-X-C motif chemokine 1, FDR: False discovery rate, Gal-9: Galectin-9, GO: Gene ontology, GT: Gastrotropin, IL-4RA: Interleukin-4 receptor subunit alpha, MERTK: Tyrosine-protein kinase Mer, PRSS27: Serine protease 27

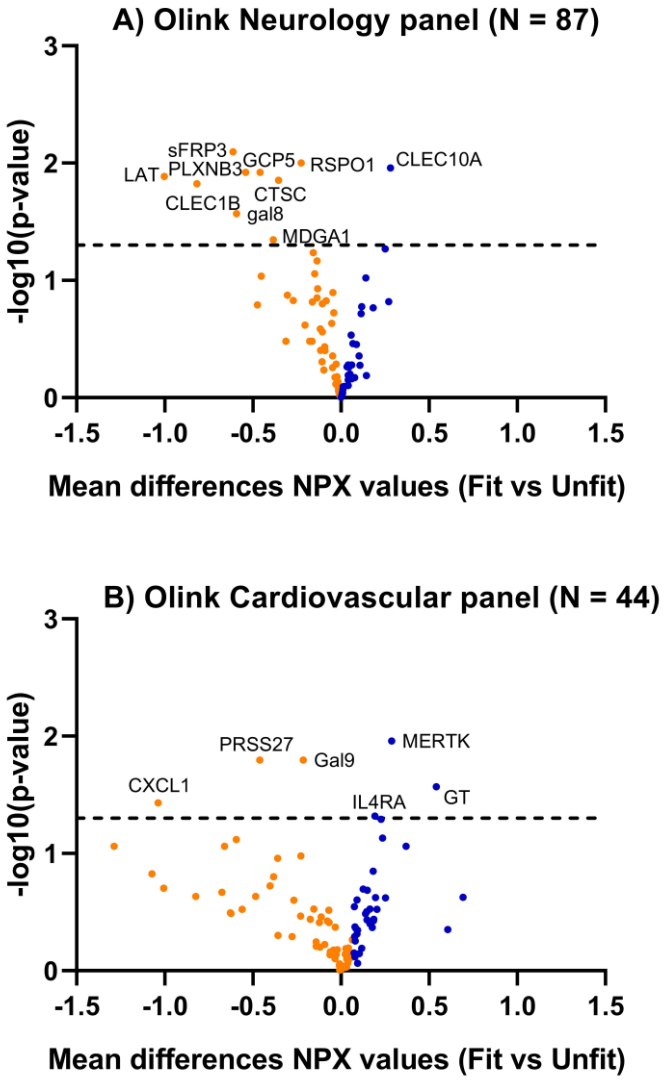

Figure 1. Volcano plot shows the 16 differential expressed proteins (10 Neurology panel [A] and 6 Cardiovascular panel [B]) between fit and unfit children with overweight/obesity controlling for sex, maturational status (i.e., peak height velocity), and body mass index. Up-regulated proteins are highlighted in orange, while down-regulated proteins are in blue. The x-axis reflects the NPX values mean differences (i.e., A 1 NPX value difference means a doubling of protein concentration), while the y-axis indicates statistical significance $p < 0.05$, which is $-\log10 > 1.30$ in the horizontal dashed line

Supplementary Table 1. Characteristics of the participants included in the analyses focused on the Cardiovascular protein panel (i.e. sub-sample).

Variables	Total sample n=44*	Fit children n=19	Unfit children n=25	P value
Sex, age, and maturational status				
Boys, n (%)	23 (52%)	5 (26%)	18 (72%)	**0.003**
Age (years)	10.23 ± 1.08	10.20 ± 1.17	10.25 ± 1.03	0.88
Years from PHV	-2.05 ± 0.97	-1.69 ± 0.88	-2.32 ± 0.95	**0.03**
BMI group by Cole *et al.*				
Overweight	17 (39%)	9 (47%)	8 (32%)	0.300
Obesity	27 (61%)	10 (53%)	17 (68%)	
Body composition and anthropometry				
Weight (kg)	56.23 ± 10.87	54.73 ± 10.92	57.36 ± 10.91	0.43
Height (cm)	145.37 ± 8.55	146.91 ± 9.93	144.21 ± 7.33	0.31
Waist circumference (cm)	89.21 ± 9.67	87.24 ± 9.19	90.71 ± 9.94	0.24
BMI (kg/m^2)	26.41 ± 3.55	25.10 ± 2.56	27.41 ± 3.91	**0.02**
FMI (kg/m^2)	11.70 ± 2.96	10.50 ± 2.14	12.62 ± 3.21	**0.02**
LMI (kg/m^2)	13.71 ± 1.44	13.57 ± 1.49	13.82 ± 1.42	0.57
Cardiorespiratory fitness				
VO2peak relative to BW (ml/Kg/min)	37.93 ± 5.23	41.10 ± 4.90	35.53 ± 4.14	**<0.001**

Data presented as unadjusted mean ± SD, and as number and frequency. BMI: Body mass-index, FMI: Fat mass index, LMI: lean mass index, PHV: Peak height velocity. BW: Body weight, LMI: Lean mass index. Bold numbers show $P < 0.05$ * Brain health-related proteins were measured in the complete sample (34 Fit vs. 53 unfit), while cardiovascular disease-related proteins were quantified in a subsample of 44 participants (19 Fit vs. 25 Unfit).

Supplementary Table 2. Differentially expressed proteins, analysed with Olink Neurology Panel between Fit and Unfit children with overweight/obesity (fat mass index was included as a confounder instead of body mass index).

Fit vs Unfit - Neurologic panel (main study simple, N = 87)											
	Fit children (N = 34)			Unfit children (N = 53)			Difference between groups				
	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	p-value	FDR
CLEC10A	5.85	5.69	6.00	5.55	5.44	5.67	0.30	0.08	0.52	0.01	0.19
CLEC1B	9.66	9.19	10.14	10.49	10.13	10.84	-0.82	-1.49	-0.15	0.02	0.19
CTSC	2.76	2.57	2.96	3.12	2.96	3.28	-0.36	-0.65	-0.07	0.02	0.19
gal8	5.67	5.19	5.95	6.20	5.92	6.49	-0.63	-1.17	-0.10	0.02	0.22
GCP5	5.18	4.91	5.43	5.62	5.43	5.82	-0.45	-0.82	-0.09	0.02	0.19
LAT	5.44	4.87	6.01	6.46	6.03	6.88	-1.02	-1.82	-0.22	0.01	0.19
MDGA1	4.80	4.53	5.07	5.23	5.03	5.44	-0.43	-0.82	-0.05	0.03	0.26
PLXNB3	4.09	3.79	4.39	4.66	4.43	4.88	-0.57	-0.99	-0.14	0.01	0.19
RSPO1	1.84	1.72	1.97	2.08	1.98	2.17	-0.23	-0.41	-0.06	0.01	0.19
sFRP3	2.99	2.67	3.32	3.67	3.43	3.91	-0.68	-1.13	-0.22	0.01	0.19
	Disease area						Biological process				
CLEC10A	Cardiovascular, neurological, infectious, cancer						Immune response				
CLEC1B	Cardiovascular, skeletal, renal, metabolic, digestive, cancer						Cell differentiation, signal transduction				
CTSC	Cardiovascular, metabolic, cancer, cutaneous						Cell death, cellular metabolic process, immune response, proteolysis				
gal8	Neurological, inflammatory, cancer						Other GO terms				
GCP5	Cardiovascular, renal, neurological, metabolic, digestive, cancer						Cellular metabolic process				

LAT	Renal, neurological, inflammatory, infectious, cancer	Cell adhesion, cellular metabolic process, immune response, MAPK cascade, signal transduction
MDGA1	Neurological	Cell differentiation, neurogenesis
PLXNB3	Neurological, cancer	Axon development, axon guidance, cell adhesion, cell differentiation, neurogenesis, signal transduction
RSPO1	Digestive, cancer, cutaneous	Cellular metabolic process, signal transduction
sFRP3	Skeletal, cancer	Cell death, cell differentiation, cell growth, signal transduction

ANCOVA analyses adjusted by sex, maturational status (i.e., peak height velocity) and fat mass index. Data are presented using NPX (Normalized Protein eXpression) values. A 1 NPX value difference means a doubling of protein concentration.

CI: Confidence intervals, CLEC10A: C-type lectin domain family 10 member A, CLEC1B: C-type lectin domain family 1 member B, CTSC: Dipeptidyl peptidase 1, FDR: False discovery rate, Gal-8: Galectin-8, GCP5: Glypican-5, GO: Gene ontology, LAT: Linker for activation of T-cells family member 1, MDGA1: MAM domain-containing glycosylphosphatidylinositol anchor protein 1, PLXNB3: Plexin-B3, RSPO1: R-spondin-1, sFRP3: Secreted frizzled-related protein 3.

Supplementary Table 3. Differentially expressed proteins, analysed with Olink Cardiovascular panel between Fit and Unfit children with overweight/obesity (fat mass index was included as a confounder instead of body mass index).

Fit vs Unfit - Cardiovascular panel (randomly selected subsample, 50% of main study sample, N = 44)											
	Fit children (N = 19)			Unfit children (N = 25)			Difference between groups				
	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	p-value	FDR
CXCL1	8.53	7.85	9.22	9.52	8.94	10.10	-0.98	-1.99	0.02	0.05	0.57
Gal9	7.97	7.85	8.09	8.17	8.07	8.28	-0.20	-0.38	-0.03	0.03	0.57
GT	2.97	2.63	3.31	2.43	2.14	2.71	0.54	0.05	1.04	0.03	0.57
IL4RA	2.36	2.22	2.49	2.15	2.04	2.26	0.21	0.01	0.41	0.04	0.57
MERTK	6.27	6.12	6.43	5.98	5.85	6.11	0.29	0.06	0.52	0.01	0.57
PRSS27	8.76	8.50	9.02	9.24	9.02	9.46	-0.48	-0.86	-0.09	0.02	0.57
	Disease area						Biological process				
CXCL1	Cardiovascular, Pulmonary, inflammatory, hepatic, digestive, skeletal						Immune response, inflammatory response				
Gal9	Skeletal, neurological, Hemic and Lymphatic, cancer						Cell adhesion, immune response, inflammatory response, MAPK cascade				
GT	Metabolic, digestive, cancer						Catabolic process				
IL4RA	Cardiovascular, Pulmonary, neurological, inflammatory, infectious, cutaneous, cancer						Cell adhesion, immune response, inflammatory response				
MERTK	Cardiovascular, Pulmonary, neurological, inflammatory, digestive						Cell adhesion, coagulation, platelet activation, wound healing				
PRSS27	Cardiovascular, cancer						Other GO terms				

ANCOVA analyses adjusted by sex, maturational status (i.e., peak height velocity) and fat mass index. Data are presented using NPX (Normalized Protein eXpression) values. A 1 NPX value difference means a doubling of protein concentration.

CI: Confidence intervals, CXCL1: C-X-C motif chemokine 1, FDR: False discovery rate, Gal-9: Galectin-9, GO: Gene ontology, GT: Gastrotropin, IL-4RA: Interleukin-4 receptor subunit alpha, MERTK: Tyrosine-protein kinase Mer, PRSS27: Serine protease 27

Supplementary Table 4. The list of 81 proteins from the Neurology panel, non-differentially expressed between Fit and Unfit children with overweight/obesity.

Fit vs Unfit - Neurologic panel (main study simple, N = 87)											
	Fit children (N = 34)			Unfit children (N = 53)			Difference between groups			p-value	FDR
	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI		
MSR1	7.08	6.91	7.26	6.83	6.69	6.98	0.25	-0.01	0.51	0.05	0.45
JAMB	8.23	8.12	8.34	8.39	8.29	8.48	-0.16	-0.32	0.01	0.06	0.45
CLM6	5.76	5.66	5.86	5.89	5.81	5.98	-0.14	-0.28	0.01	0.07	0.48
N2DL2	3.78	3.66	3.89	3.93	3.83	4.02	-0.15	-0.32	0.02	0.09	0.55
Alpha2MRAP	7.94	7.58	8.30	8.39	8.09	8.69	-0.45	-0.98	0.08	0.09	0.55
BMP4	4.54	4.43	4.65	4.40	4.30	4.49	0.14	-0.03	0.31	0.10	0.55
CD200R1	5.12	5.01	5.23	5.25	5.15	5.34	-0.13	-0.30	0.03	0.12	0.56
NBL1	5.59	5.55	5.63	5.63	5.60	5.67	-0.05	-0.11	0.01	0.13	0.56
GMCSFRalpha	5.99	5.72	6.26	6.30	6.07	6.53	-0.31	-0.71	0.10	0.13	0.56
ADAM22	4.88	4.75	5.00	5.01	4.91	5.12	-0.14	-0.32	0.05	0.14	0.56
IL5Ralpha	3.18	2.92	3.43	3.45	3.23	3.66	-0.27	-0.64	0.10	0.15	0.56
CTSS	5.58	5.50	5.66	5.67	5.60	5.73	-0.09	-0.20	0.03	0.15	0.56
HAGH	5.69	5.44	5.94	5.42	5.21	5.63	0.27	-0.10	0.64	0.15	0.56
CADM3	4.81	4.65	4.96	4.97	4.84	5.10	-0.16	-0.39	0.06	0.15	0.56
CD200	7.01	6.91	7.11	7.11	7.03	7.20	-0.11	-0.26	0.04	0.16	0.56
MANF	6.39	5.93	6.84	6.86	6.48	7.24	-0.47	-1.14	0.20	0.16	0.56
TNFRSF12A	5.39	5.27	5.50	5.27	5.17	5.36	0.12	-0.05	0.29	0.17	0.56
NCDase	4.27	4.09	4.45	4.09	3.94	4.24	0.18	-0.08	0.44	0.17	0.56
NrCAM	10.13	10.09	10.17	10.17	10.14	10.21	-0.04	-0.10	0.02	0.19	0.59
Siglec9	5.19	5.07	5.30	5.07	4.97	5.17	0.12	-0.06	0.29	0.19	0.59

THY1	10.53	10.48	10.59	10.59	10.54	10.63	-0.05	-0.14	0.03	0.23	0.69
GCSF	3.64	3.40	3.87	3.84	3.64	4.04	-0.20	-0.55	0.14	0.24	0.69
Dkk4	2.34	2.20	2.48	2.46	2.34	2.58	-0.12	-0.32	0.09	0.26	0.73
EDA2R	3.61	3.48	3.74	3.72	3.61	3.83	-0.11	-0.30	0.09	0.27	0.74
DDR1	7.81	7.73	7.88	7.75	7.69	7.81	0.06	-0.05	0.16	0.29	0.75
NAAA	3.63	3.40	3.85	3.79	3.60	3.98	-0.16	-0.50	0.17	0.33	0.78
NMNAT1	4.09	3.65	4.52	4.40	4.03	4.76	-0.31	-0.95	0.32	0.33	0.78
KYNU	9.03	8.78	9.27	9.20	9.00	9.41	-0.18	-0.54	0.18	0.33	0.78
PDGFRalpha	5.89	5.80	5.99	5.82	5.74	5.90	0.07	-0.07	0.21	0.35	0.79
SMPD1	4.65	4.52	4.77	4.56	4.45	4.66	0.09	-0.10	0.27	0.35	0.79
UNC5C	5.05	4.91	5.19	5.15	5.03	5.26	-0.09	-0.30	0.11	0.37	0.81
DRAXIN	4.21	4.03	4.39	4.33	4.17	4.48	-0.12	-0.39	0.15	0.40	0.83
VWC2	6.34	6.19	6.48	6.25	6.13	6.37	0.09	-0.12	0.30	0.40	0.83
SIGLEC1	6.64	6.46	6.82	6.53	6.38	6.69	0.10	-0.16	0.37	0.44	0.88
CPM	7.04	6.95	7.12	7.09	7.02	7.16	-0.05	-0.17	0.08	0.44	0.88
GZMA	6.38	6.17	6.60	6.49	6.31	6.67	-0.11	-0.42	0.21	0.50	0.93
NTRK2	7.15	7.09	7.21	7.18	7.13	7.23	-0.03	-0.11	0.06	0.52	0.93
CDH3	8.12	7.99	8.25	8.06	7.95	8.17	0.06	-0.13	0.25	0.53	0.93
SPOCK1	2.88	2.80	2.97	2.84	2.77	2.91	0.04	-0.09	0.17	0.53	0.93
CLM1	6.40	6.17	6.63	6.29	6.10	6.49	0.11	-0.23	0.45	0.53	0.93
SCARB2	4.17	4.07	4.28	4.13	4.04	4.21	0.05	-0.11	0.21	0.53	0.93
FLRT2	3.27	3.19	3.34	3.23	3.17	3.30	0.03	-0.08	0.14	0.54	0.93
RGMB	6.31	6.21	6.41	6.27	6.18	6.35	0.04	-0.10	0.19	0.55	0.93
EPHB6	4.96	4.86	5.07	5.01	4.92	5.10	-0.05	-0.21	0.11	0.55	0.93
NEP	3.03	2.79	3.27	3.13	2.93	3.33	-0.10	-0.45	0.25	0.58	0.95
TNR	5.01	4.87	5.15	4.96	4.84	5.08	0.05	-0.16	0.26	0.63	0.99
LAYN	5.78	5.66	5.90	5.74	5.64	5.84	0.04	-0.13	0.21	0.64	0.99
LAIR2	5.02	4.59	5.44	4.87	4.52	5.23	0.14	-0.48	0.77	0.65	0.99

TNFRSF21	8.59	8.52	8.66	8.62	8.56	8.68	-0.02	-0.13	0.08	0.66	0.99
EZR	5.10	4.99	5.21	5.13	5.04	5.22	-0.03	-0.19	0.13	0.67	0.99
CPA2	10.02	9.78	10.27	9.95	9.74	10.15	0.08	-0.29	0.44	0.67	0.99
IL12	9.14	8.93	9.34	9.08	8.91	9.25	0.06	-0.24	0.36	0.69	0.99
GDF8	4.24	4.10	4.38	4.20	4.08	4.32	0.04	-0.17	0.25	0.70	0.99
EFNA4	3.54	3.46	3.62	3.56	3.49	3.62	-0.02	-0.14	0.10	0.73	0.99
MATN3	12.95	12.88	13.02	12.97	12.91	13.03	-0.02	-0.12	0.09	0.73	0.99
WFIKKN1	3.73	3.60	3.86	3.76	3.65	3.87	-0.03	-0.22	0.16	0.76	0.99
SCARF2	7.14	7.04	7.24	7.16	7.08	7.24	-0.02	-0.17	0.12	0.76	0.99
CRTAM	6.08	5.87	6.28	6.04	5.86	6.21	0.04	-0.26	0.34	0.79	0.99
LXN	2.26	2.17	2.36	2.24	2.16	2.33	0.02	-0.12	0.16	0.80	0.99
GDNF	1.35	1.26	1.45	1.37	1.29	1.45	-0.02	-0.16	0.12	0.80	0.99
CDH6	5.42	5.35	5.49	5.41	5.35	5.47	0.01	-0.09	0.12	0.81	0.99
SCARA5	9.28	9.20	9.36	9.27	9.20	9.34	0.01	-0.11	0.13	0.85	0.99
CD38	6.01	5.92	6.10	6.02	5.94	6.09	-0.01	-0.14	0.12	0.86	0.99
BCAN	5.15	5.04	5.26	5.17	5.07	5.26	-0.01	-0.18	0.15	0.87	0.99
NRP2	8.97	8.94	9.01	8.98	8.95	9.01	-0.01	-0.06	0.05	0.87	0.99
NCAN	9.86	9.79	9.93	9.87	9.81	9.92	-0.01	-0.11	0.09	0.88	0.99
PRTG	7.19	7.07	7.31	7.20	7.10	7.30	-0.01	-0.19	0.16	0.89	0.99
GFRalpha1	7.59	7.50	7.69	7.59	7.51	7.66	0.01	-0.13	0.15	0.90	0.99
SMOC2	8.88	8.76	9.01	8.89	8.79	9.00	-0.01	-0.20	0.18	0.91	0.99
SKR3	6.50	6.41	6.59	6.49	6.41	6.57	0.01	-0.13	0.14	0.91	0.99
PVR	8.87	8.76	8.98	8.86	8.77	8.96	0.01	-0.15	0.17	0.91	0.99
RGMA	11.07	10.98	11.16	11.07	11.00	11.15	-0.01	-0.14	0.13	0.92	0.99
TMPRSS5	3.33	3.23	3.43	3.32	3.23	3.40	0.01	-0.14	0.16	0.92	0.99
GDNFRalpha3	5.56	5.46	5.66	5.57	5.48	5.65	-0.01	-0.16	0.15	0.94	0.99
NTRK3	7.51	7.43	7.59	7.51	7.45	7.58	0.00	-0.12	0.11	0.94	0.99
FcRL2	5.46	5.30	5.61	5.46	5.33	5.60	-0.01	-0.24	0.23	0.95	0.99

ADAM23	4.98	4.85	5.11	4.97	4.86	5.08	0.01	-0.19	0.20	0.95	0.99
CNTN5	6.28	6.13	6.44	6.28	6.15	6.41	0.00	-0.22	0.23	0.97	0.99
ROBO2	7.05	6.94	7.16	7.06	6.96	7.15	0.00	-0.16	0.16	0.98	0.99
PLXNB1	1.96	1.86	2.06	1.96	1.88	2.04	0.00	-0.14	0.15	0.99	0.99
BetaNGF	1.31	1.23	1.39	1.31	1.24	1.38	0.00	-0.12	0.12	0.99	0.99

ANCOVA analyses adjusted by sex, maturational status (i.e., peak height velocity) and body mass index. Data are presented using NPX (Normalized Protein eXpression) values. A 1 NPX value difference means a doubling of protein concentration. CI: Confidence intervals, FDR: False discovery rate. The full name of all proteins is available in Olink website: https://www.olink.com/products-services/target/neurology-panel/

Supplementary Table 5. The list of 86 proteins from the Cardiovascular panel, non-differentially expressed between Fit and Unfit children with overweight/obesity.

Fit vs Unfit - Cardiovascular panel (randomly selected subsample, 50% of main study simple, N = 44)											
	Fit children (N = 19)			Unfit children (N = 25)			Difference between groups				
	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	Adjusted Mean	Lower limit 95% CI	Upper limit 95% CI	p-value	FDR
BNP	1.56	1.40	1.73	1.33	1.20	1.47	0.23	0.00	0.46	0.05	0.67
PTX3	4.06	3.88	4.25	3.83	3.67	3.98	0.24	-0.02	0.50	0.07	0.67
Dkk1_I	8.38	7.90	8.85	8.97	8.59	9.36	-0.59	-1.25	0.07	0.08	0.67
CD40L	3.86	2.79	4.93	5.15	4.28	6.01	-1.29	-2.77	0.20	0.09	0.67
SLAMF7	3.62	3.31	3.93	3.25	3.00	3.50	0.37	-0.06	0.80	0.09	0.67
HBEGF	4.81	4.26	5.35	5.47	5.02	5.91	-0.66	-1.42	0.10	0.09	0.67
VEGFD	7.49	7.29	7.69	7.72	7.56	7.88	-0.23	-0.51	0.05	0.11	0.72
PAR1	8.40	8.08	8.72	8.76	8.50	9.02	-0.36	-0.80	0.09	0.11	0.72
IL27	5.55	5.37	5.73	5.37	5.22	5.51	0.18	-0.06	0.43	0.14	0.74
DECR1	4.37	3.31	5.42	5.44	4.58	6.30	-1.07	-2.55	0.40	0.15	0.74
CCL3	6.48	6.10	6.87	6.86	6.55	7.18	-0.38	-0.92	0.16	0.16	0.74
BMP6	4.02	3.59	4.46	4.43	4.07	4.78	-0.40	-1.01	0.21	0.19	0.74
ITGB1BP2	3.23	2.11	4.34	4.23	3.33	5.14	-1.01	-2.56	0.55	0.20	0.74
TIE2	7.57	7.43	7.71	7.44	7.33	7.56	0.13	-0.07	0.32	0.20	0.74
IL1RL2	4.29	4.12	4.46	4.14	4.00	4.28	0.15	-0.09	0.38	0.21	0.74
CCL17	8.15	7.38	8.93	8.83	8.20	9.46	-0.68	-1.76	0.41	0.22	0.74
SERPINA12	2.52	1.94	3.10	3.00	2.53	3.47	-0.48	-1.29	0.32	0.23	0.74
NEMO	5.68	4.69	6.67	6.51	5.71	7.31	-0.83	-2.20	0.55	0.23	0.74

FGF21	6.46	5.62	7.30	5.77	5.09	6.45	0.69	-0.47	1.86	0.24	0.74
TGM2	9.30	9.06	9.53	9.10	8.91	9.29	0.20	-0.13	0.52	0.24	0.74
CEACAM8	4.87	4.56	5.17	4.61	4.36	4.86	0.25	-0.18	0.68	0.24	0.74
hOSCAR	10.94	10.83	11.05	10.85	10.76	10.94	0.09	-0.07	0.24	0.25	0.74
CD84	4.80	4.47	5.14	5.07	4.80	5.34	-0.27	-0.73	0.20	0.25	0.74
MARCO	7.03	6.93	7.13	6.95	6.87	7.03	0.08	-0.07	0.22	0.29	0.74
LEP	7.22	7.01	7.43	7.37	7.20	7.54	-0.15	-0.45	0.14	0.30	0.74
ACE2	3.00	2.77	3.23	2.84	2.65	3.02	0.16	-0.15	0.48	0.30	0.74
ANGPT1	8.30	7.53	9.07	8.86	8.23	9.49	-0.56	-1.64	0.52	0.30	0.74
IL18	9.14	8.85	9.42	8.93	8.70	9.16	0.20	-0.19	0.60	0.30	0.74
THBS2	5.55	5.45	5.64	5.62	5.54	5.69	-0.07	-0.20	0.07	0.31	0.74
REN	6.30	6.09	6.51	6.16	5.99	6.32	0.15	-0.14	0.43	0.31	0.74
PDGFsubunit_B	8.40	7.49	9.30	9.02	8.29	9.76	-0.63	-1.88	0.63	0.32	0.74
PARP1	3.97	3.06	4.88	4.60	3.86	5.34	-0.63	-1.89	0.64	0.32	0.74
TNFRSF13B	9.56	9.36	9.76	9.42	9.26	9.59	0.14	-0.14	0.42	0.33	0.74
KIM1	7.10	6.75	7.45	7.33	7.05	7.61	-0.23	-0.71	0.25	0.34	0.74
CD4	4.88	4.70	5.05	4.99	4.85	5.13	-0.11	-0.35	0.13	0.35	0.74
XCL1	4.89	4.59	5.18	4.70	4.46	4.94	0.19	-0.22	0.60	0.36	0.74
SORT1	8.14	7.87	8.42	8.32	8.09	8.54	-0.17	-0.56	0.21	0.37	0.74
PDL2	3.71	3.48	3.94	3.56	3.37	3.75	0.15	-0.18	0.47	0.37	0.74
GDF2	9.65	9.35	9.95	9.47	9.23	9.71	0.18	-0.23	0.60	0.37	0.74
DCN	4.07	3.95	4.20	4.15	4.05	4.25	-0.08	-0.25	0.10	0.38	0.74
RAGE	13.54	13.34	13.74	13.66	13.50	13.83	-0.12	-0.41	0.16	0.39	0.74
PSGL1	4.29	4.18	4.40	4.36	4.27	4.45	-0.07	-0.22	0.09	0.39	0.74
TRAILR2	6.28	6.01	6.56	6.12	5.90	6.35	0.16	-0.22	0.55	0.40	0.74
IL17D	2.50	2.35	2.64	2.42	2.30	2.53	0.08	-0.12	0.28	0.42	0.75
SOD2	10.04	9.99	10.10	10.08	10.03	10.12	-0.03	-0.11	0.05	0.43	0.75
FABP2	8.63	8.31	8.95	8.45	8.19	8.71	0.18	-0.27	0.63	0.43	0.75

TF	5.80	5.65	5.96	5.72	5.59	5.84	0.08	-0.13	0.30	0.44	0.75
GH	7.46	6.32	8.60	6.86	5.93	7.78	0.61	-0.98	2.19	0.45	0.75
HO1	11.60	11.42	11.78	11.51	11.36	11.65	0.09	-0.15	0.34	0.45	0.75
PRSS8	8.80	8.61	8.98	8.71	8.56	8.86	0.09	-0.17	0.35	0.49	0.80
STK4	2.83	2.06	3.59	3.18	2.57	3.80	-0.36	-1.42	0.70	0.50	0.80
SCF	9.60	9.43	9.77	9.52	9.39	9.66	0.08	-0.16	0.31	0.51	0.80
IL16	6.62	6.12	7.12	6.85	6.45	7.25	-0.23	-0.92	0.47	0.51	0.80
PGF	7.52	7.36	7.67	7.45	7.32	7.58	0.07	-0.15	0.28	0.55	0.84
TNFRSF10A	2.93	2.74	3.13	2.85	2.69	3.01	0.08	-0.19	0.35	0.56	0.84
GIF	6.79	6.44	7.14	6.93	6.64	7.21	-0.14	-0.63	0.35	0.57	0.84
MMP7	9.37	9.12	9.63	9.47	9.26	9.67	-0.09	-0.44	0.26	0.60	0.86
IL1ra	5.81	5.40	6.21	5.95	5.62	6.28	-0.14	-0.71	0.43	0.62	0.86
THPO	3.82	3.46	4.18	3.94	3.65	4.23	-0.12	-0.62	0.38	0.63	0.86
BOC	4.34	4.22	4.47	4.30	4.20	4.41	0.04	-0.14	0.22	0.64	0.86
GLO1	6.44	6.07	6.81	6.32	6.02	6.62	0.12	-0.40	0.63	0.65	0.86
AMBP	7.43	7.34	7.52	7.40	7.33	7.47	0.03	-0.10	0.15	0.65	0.86
ADAMTS13	5.45	5.38	5.52	5.47	5.41	5.53	-0.02	-0.12	0.08	0.66	0.86
LPL	10.09	9.95	10.24	10.14	10.02	10.26	-0.04	-0.25	0.16	0.67	0.86
VSIG2	3.60	3.38	3.82	3.66	3.48	3.84	-0.06	-0.37	0.25	0.69	0.86
FGF23	3.05	2.91	3.19	3.01	2.90	3.13	0.04	-0.15	0.23	0.70	0.86
IgGFc_receptorIIb	3.77	3.49	4.06	3.70	3.47	3.93	0.07	-0.32	0.47	0.71	0.86
TNFRSF11A	5.92	5.70	6.14	5.98	5.80	6.16	-0.06	-0.36	0.25	0.72	0.86
HSP27	9.85	9.44	10.27	9.75	9.41	10.09	0.11	-0.47	0.68	0.72	0.86
SPON2	8.26	8.15	8.36	8.23	8.15	8.31	0.03	-0.12	0.17	0.72	0.86
CTSL1	7.12	7.00	7.23	7.14	7.05	7.24	-0.03	-0.19	0.14	0.73	0.86
IDUA	5.99	5.74	6.25	6.05	5.85	6.26	-0.06	-0.42	0.30	0.73	0.86
CTRC	9.86	9.49	10.23	9.78	9.48	10.08	0.08	-0.44	0.60	0.76	0.88
PAPPA	3.36	3.07	3.66	3.30	3.06	3.54	0.06	-0.35	0.47	0.76	0.88

AGRP	5.00	4.82	5.19	5.04	4.89	5.19	-0.04	-0.30	0.23	0.79	0.89
ADM	8.24	8.06	8.42	8.21	8.07	8.35	0.03	-0.21	0.28	0.79	0.89
MMP12	7.73	7.47	7.98	7.69	7.48	7.89	0.04	-0.31	0.39	0.82	0.91
HAOX1	5.55	4.77	6.33	5.46	4.82	6.09	0.09	-0.99	1.18	0.86	0.94
IL6	3.17	2.85	3.49	3.14	2.88	3.40	0.04	-0.41	0.48	0.87	0.94
PIgR	5.74	5.67	5.80	5.74	5.69	5.80	-0.01	-0.10	0.09	0.87	0.94
PRELP	7.76	7.65	7.86	7.76	7.68	7.85	-0.01	-0.15	0.14	0.90	0.96
FS	11.05	10.79	11.31	11.03	10.82	11.24	0.02	-0.34	0.38	0.92	0.96
CA5A	2.57	2.08	3.05	2.54	2.14	2.93	0.03	-0.65	0.71	0.93	0.96
LOX1	7.37	7.03	7.71	7.35	7.08	7.63	0.02	-0.45	0.49	0.94	0.96
TM	9.99	9.81	10.16	9.99	9.85	10.13	0.00	-0.25	0.24	0.98	0.98
SRC	5.92	5.31	6.52	5.93	5.44	6.42	-0.01	-0.85	0.83	0.98	0.98

ANCOVA analyses adjusted by sex, maturational status (i.e., peak height velocity) and body mass index. Data are presented using NPX (Normalized Protein eXpression) values. A 1 NPX value difference means a doubling of protein concentration. CI: Confidence intervals, FDR: False discovery rate. The full name of all proteins is available in Olink website: https://www.olink.com/products-services/target/cardiovascular-ii-panel/