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Sexually dimorphic metal alterations in childhood obesity are modulated by a complex interplay between inflammation, insulin, and sex hormones

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Abstract

Although growing evidence points to a pivotal role of perturbed metal homeostasis in childhood obesity, sexual dimorphisms in this association have rarely been investigated. In this study, we applied multi-elemental analysis to plasma and erythrocyte samples from an observational cohort comprising children with obesity, with and without insulin resistance, and healthy control children. Furthermore, a wide number of variables related to carbohydrate and lipid metabolism, inflammation, and sex hormones were also determined. Children with obesity, regardless of sex and insulin resistance status, showed increased plasma copper-to-zinc ratios. More interestingly, obesity-related erythroid alterations were found to be sex-dependent, with increased contents of iron, zinc, and copper being exclusively detected among female subjects. Our findings suggest that a sexually dimorphic hormonal dysregulation in response to a pathological cascade involving inflammatory processes and hyperinsulinemia could be the main trigger of this female-specific intracellular sequestration of trace elements. Therefore, the present study highlights the relevance of genotypic sex as a susceptibility factor influencing the pathogenic events behind childhood obesity, thereby opening the door to develop sex-personalized approaches in the context of precision medicine.

KEYWORDS
childhood obesity, inflammation, insulin, sex, trace elements

1 | INTRODUCTION

The prevalence of overweight and obesity currently accounts for approximately a 30% of the population worldwide, reaching pandemic levels according to the World Health Organization. Adverse health consequences of excess body weight are particularly relevant at pediatric age, being associated with higher predisposition to develop a myriad of non-communicable diseases, including metabolic and cardiovascular diseases, liver complications, asthma, psychological comorbidities, and many other disorders, which have become a major public health problem.[1] In this line, the "Global Burden of Disease Study" evaluated 84 behavioral, environmental, occupational, and metabolic risks in 2017, and concluded that high body mass index (BMI) is the risk factor that has experienced the greatest increase in exposure since 1990, being among the top five risk factors in terms of attributable deaths.[2]

Genotypic sex may influence the susceptibility of developing a wide range of metabolic, autoimmune, neurological, and cardiovascular diseases, leading to different prevalence depending on sex. In particular, sexual dimorphisms have proven to affect many of the metabolic complications behind obesity. On the one hand, men tend to produce energy through free fatty acid oxidation at rest, whereas women are prone to incorporate them into triacylglycerols, thereby promoting fat storage.[3] As a result, obesity prevalence is higher among females, but male-sex is paradoxically more susceptible to develop obesity-related comorbidities. This is in great part due to sex differences in fat accumulation, which in turn have large metabolic repercussions. Men usually have higher content of visceral adipose tissue (VAT), whilst subcutaneous adipose tissue (SAT) is the predominant fat depot in women.[4] Hormones are crucial in this sex-dependent susceptibility to obesity and related complications, since estrogens are the main elicitors of this differential adipose tissue distribution and exert a protective effect in women.[5] Furthermore, as VAT is known to have twice the amount of pro-inflammatory macrophages than SAT, healthy and metabolically ill male subjects are typically characterized by higher levels of inflammatory markers and lower capacity of inflammation resolution when compared to female counterparts.[6]

Trace elements play a multitude of essential roles in biological systems, participating in the antioxidant defense, electron and oxygen transport, hormonal regulation, immune response, or synthesis of DNA, RNA, proteins, and fatty acids, among other processes.[7–9] Considering their ubiquitous nature, adipose tissue-driven metabolic perturbations occurring in obesity are expected to be reflected in altered metal homeostasis. In this respect, a few authors have previously investigated the association between childhood obesity, metabolic risk factors, and circulating blood concentrations of various metal and metalloid elements.[10–16] Recently, we described metal disturbances to be closely inter-related with the characteristic pathogenic events behind childhood obesity and insulin resistance (IR), namely chronic low-grade inflammation, exacerbated oxidative stress, impaired insulin-mediated carbohydrate metabolism, and dyslipidemia.[17] However, the potential involvement of sex in this perturbed metal homeostasis remains largely unexplored. Only a few observational studies have previously considered the potential impact of sex on childhood obesity-related metal alterations, all of which reported similar results within boys and girls (e.g., decreased blood levels of Fe, Mg, Zn; increased blood levels of Cu).[11,12] However, Błażewicz et al. interestingly found that the sex criterion may influence the association between metal contents and the BMI of children (e.g., negative correlation between BMI and blood Zn, plasma Cu, and urinary Fe among girls; positive correlation between BMI and plasma Co among boys).[11] Other authors have demonstrated that diet-induced obesity disrupts trace element homeostasis in various brain regions of mice in a sex-specific manner.[18,19] Furthermore, there is increasing evidence suggesting that adverse health effects triggered by the exposure to toxic heavy metals (e.g., cadmium, lead) may differ depending on sex, which could be attributed to differences in kinetics, mode of action, or susceptibility.[20]

In this work, we studied the influence of sex in modulating metal metabolism in childhood obesity and IR. To this end, multi-elemental analysis was applied to plasma and erythrocyte samples from a sex-stratified observational cohort comprising children with obesity and IR (ObIR+), children with obesity without IR (ObIR-), and healthy control children (CNT).

2 | EXPERIMENTAL PROCEDURES

2.1 | Study population and sample collection

Prepubertal children (Tanner Stage I) of both sexes, aged between 6 and 10 years, were recruited at "Hospital Universitario Puerta del Mar" (Cádiz, Spain), including children with obesity and IR (ObIR+), children with obesity without IR (ObIR-), and healthy control children (CNT), as described elsewhere.[21] Briefly, obesity was diagnosed when children presented a BMI over two standard deviations above the mean of the reference population, adjusted for sex and

age.[22] Furthermore, children with obesity underwent an oral glucose tolerance test (OGTT) to diagnose concomitant IR when meeting at least one of the following criteria: (i) homeostasis model assessment of insulin resistance (HOMA-IR) score above 3.5, (ii) fasting insulin levels above 15 mU/L, (iii) insulin levels above 75 mU/L at 120 min of the OGTT, (iv) insulin levels above 150 mU/L at any time point of the OGTT.[23] For the control group, healthy non-obese children needing a blood test for other medical reasons (e.g., routine health monitoring) were also enrolled. Subjects with other known chronic systemic diseases or suffering of acute infectious processes were not eligible for the study.

Venous blood samples were collected after overnight fasting in the morning using BD Vacutainer tubes and Advance vacuum system. Tubes were centrifuged at 1500*g* for 10 min at 4°C to obtain the plasma, and the resulting pellets were then washed three times with cold saline solution (9 g/L NaCl, 4°C) to obtain the erythrocyte fraction by centrifuging at 1500*g* for 5 min at 4°C. Plasma and erythrocyte samples were aliquoted and stored at −80°C until analysis. The study was performed in accordance with the principles contained in the Declaration of Helsinki. The Ethical Committee of "Hospital Universitario Puerta del Mar" (Cádiz, Spain) approved the study protocol (Ref. PI22/01899), and all participants and/or legal guardians provided written informed consent.

2.2 | Anthropometric and biochemical variables

Anthropometric variables, including height, weight, waist circumference (WC), and BMI were evaluated by pediatric endocrinologists. Blood levels of glucose (Glc), insulin (Ins), lipids (i.e., total cholesterol, TC; high-density lipoprotein cholesterol, HDL-C; low-density lipoprotein cholesterol, LDL-C; triglycerides, TG), C-reactive protein (CRP), and sex hormones (i.e., luteinizing hormone, LH; follicle-stimulating hormone, FSH; 17β-estradiol, E2; testosterone, T; androstenedione, A4; dehydroepiandrosterone sulfate, DHEAS; progesterone, P; cortisol, CORT) were determined using an Alinity automatic analyzer (Abbot, Spain). White blood cell count measurements were performed in an automated hematology analyzer and subsequently employed to compute various inflammatory indices, namely the systemic immune inflammation index (SII), the systemic inflammation response index (SIRI), the aggregate index of systemic inflammation (AISI), the platelet-to-lymphocyte ratio (PLR), the monocyte-to-lymphocyte ratio (MLR), and the neutrophil-to-lymphocyte ratio (NLR). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by applying the following formula: HOMA-IR = (Glc × Ins) × 0.055/22.5.

2.3 | Multi-elemental analysis of plasma and erythrocyte samples

Trace elements were determined by diluting aliquots of 150 μL of plasma, or 50 μL of the erythrocyte fraction, to a final volume of 3 mL using an alkaline solution containing 2% 1-butanol (w/v), 0.05% EDTA (w/v), 0.05% Triton X-100 (w/v), 1% NH_4OH (w/v), and 1 μg/L rhodium (internal standard).[24] Samples were filtered through 0.45 μm pore size hydrophilic PTFE filters before analysis. Then, multi-elemental analysis was performed in an Agilent 7900 inductively coupled plasma mass spectrometer (ICP-MS) equipped with collision/reaction cell system and with nickel sampling and skimmer cones (Agilent Technologies). High-purity grade helium (>99.999%) was employed as the collision gas, and the ICP-MS working conditions were set as follows[25]: sampling depth, 7 mm; forward power, 1550 W; plasma gas, 15 L/min; auxiliary gas, 1 L/min; carrier gas, 1 L/min; make-up gas, 0.10 L/min; collision gas, 5 mL/min. The isotopes monitored were ^{52}Cr, ^{53}Cr, ^{55}Mn, ^{56}Fe, ^{57}Fe, ^{59}Co, ^{63}Cu, ^{66}Zn, ^{77}Se, ^{78}Se, ^{82}Se, ^{95}Mo, ^{98}Mo, and ^{103}Rh, using a dwell time of 0.3 s per isotope. Multi-elemental calibration curves were prepared within the concentration range 0.5–2500 μg/L, containing 1 μg/L rhodium as the internal standard.

2.4 | Statistical analysis

Multi-elemental data pre-processing and statistical analysis were performed in the MetaboAnalyst 5.0 web tool (https://www.metaboanalyst.ca/), as follows. After removing variables with more than 20% missing values, the kNN algorithm was applied to impute the remaining missing values. Then, the data were log transformed and Pareto scaled. Analysis of variance (ANOVA) with Fisher LSD post hoc test and Student's *t*-test were employed to look for within-sex and between-sex differences, respectively. Furthermore, Pearson's correlations were computed between metal levels and biochemical variables, including parameters related to glucose and lipid metabolism, inflammation, and sex hormones. *p*-Values below 0.05 were considered as statistically significant.

3 | RESULTS

The study participants (N = 74) were on average 9.0 years-old and 56.8% were male (Table 1). Evidently,

TABLE 1 Demographic, anthropometric, and biochemical data of the study participants stratified according to sex and obesity status.

	Male subjects				Female subjects				*p*-value (*t*-test)
	CNT	ObRI-	ObIR+	*p*-value (ANOVA)	CNT	ObRI-	ObIR+	*p*-value (ANOVA)	
Demographic and anthropometric variables									
N	16	9	17	-	11	7	14	-	-
Age (years)	8.5 ± 1.6	9.8 ± 1.8	9.3 ± 1.4	NS	8.6 ± 1.8	8.2 ± 1.1	9.4 ± 1.0	NS	NS
Weight (kg)	28.0 ± 5.7^{a}	63.7 ± 12.2^{b}	62.6 ± 10.3^{b}	$1.2{\cdot}10^{-13}$	26.9 ± 3.9^{a}	45.6 ± 11.1^{b}	56.2 ± 9.8^{c}	$1.4{\cdot}10^{-8}$	NS
Body mass index (BMI, kg/m^2)	16.8 ± 1.5^{a}	30.5 ± 3.3^{b}	31.1 ± 4.3^{b,*}	$8.1{\cdot}10^{-9}$	16.2 ± 1.4^{a}	25.4 ± 2.8^{b}	27.4 ± 3.5^{b,*}	$2.4{\cdot}10^{-10}$	$1.5{\cdot}10^{-2}$
Waist circumference (WC, cm)	58.5 ± 6.5^{a}	100.6 ± 10.9 ^{b}	99.3 ± 10.7^{b}	$1.1{\cdot}10^{-2}$	58.3 ± 4.1^{a}	83.66 ± 9.8^{b}	88.5 ± 6.7^{b}	$1.8{\cdot}10^{-4}$	NS
Carbohydrate and lipid metabolism									
Glucose (Glc, mg/dL)	83.9 ± 3.8	84.2 ± 5.0	88.1 ± 8.4	NS	84.5 ± 5.6	83.6 ± 3.8	85.2 ± 8.5	NS	NS
Insulin (Ins, μU/mL)	4.1 ± 2.1^{a}	13.4 ± 3.0^{b}	22.9 ± 11.3^{c}	$4.5{\cdot}10^{-7}$	5.1 ± 2.1^{a}	10.5 ± 4.3^{b}	16.8 ± 7.0^{c}	$1.8{\cdot}10^{-5}$	NS
HOMA-IR	0.8 ± 0.4^{a}	2.7 ± 0.7^{b}	5.1 ± 3.0^{c}	$3.5{\cdot}10^{-6}$	1.1 ± 0.4^{a}	2.2 ± 0.9^{b}	3.7 ± 1.8^{c}	$6.5{\cdot}10^{-5}$	NS
Total cholesterol (TC, mg/dL)	159.1 ± 25.1	169.8 ± 14.0	149.9 ± 24.1	NS	176.3 ± 26.3	150.6 ± 38.0	173.4 ± 42.9	NS	NS
High-density lipoprotein cholesterol (HDL-C, mg/dL)	65.6 ± 14.4^{a}	47.7 ± 12.3^{b}	41.8 ± 6.0^{b}	$8.9{\cdot}10^{-4}$	65.2 ± 13.5^{a}	46.0 ± 3.4^{b}	46.1 ± 9.4^{b}	$4.0{\cdot}10^{-2}$	NS
Low-density lipoprotein cholesterol (LDL-C, mg/dL)	85.3 ± 19.0	102.0 ± 8.5	89.4 ± 20.7	NS	104.4 ± 25.0	92.5 ± 37.2	111.5 ± 39.8	NS	NS
Triglycerides (TG, mg/dL)	45.8 ± 18.0^{a}	93.1 ± 36.4^{b}	93.9 ± 51.9^{b}	$4.7{\cdot}10^{-3}$	51.6 ± 21.3^{a}	60.3 ± 20.4^{a}	87.5 ± 53.7^{b}	$1.9{\cdot}10^{-2}$	NS
Inflammatory markers									
C-reactive protein (CRP, mg/dL)	0.5 ± 0.3	3.2 ± 1.7	3.5 ± 2.9	NS	0.6 ± 0.4	2.6 ± 1.1	3.4 ± 3.4	NS	NS
Systemic immune inflammation index (SII)	373.1 ± 214.2^{a}	412.4 ± 173.6^{ab}	596.4 ± 350.7^{b,*}	$2.8{\cdot}10^{-2}$	210.8 ± 56.9^{a}	358.4 ± 160.5^{b}	393.6 ± 143.4^{b,*}	$1.1{\cdot}10^{-2}$	$2.1{\cdot}10^{-2}$
Systemic inflammation response index (SIRI)	0.63 ± 0.29^{a}	0.77 ± 0.30^{ab}	1.3 ± 1.1^{b}	$2.2{\cdot}10^{-2}$	0.40 ± 0.18^{a}	0.67 ± 0.38 ^{b}	0.69 ± 0.27^{b}	$4.5{\cdot}10^{-2}$	NS
Aggregate index of systemic inflammation (AISI)	208.0 ± 116.9^{a}	273.0 ± 132.0^{ab}	436.5 ± 366.3^{b,*}	$2.2{\cdot}10^{-2}$	110.5 ± 47.6^{a}	213.7 ± 131.4 ^{b}	205.1 ± 88.1^{b,*}	$3.3{\cdot}10^{-2}$	$4.5{\cdot}10^{-2}$
Platelet-to-lymphocyte ratio (PLR)	138.8 ± 60.4	111.8 ± 32.2	134.6 ± 57.4	NS	95.6 ± 26.8	138.8 ± 42.7	111.7 ± 36.3	NS	NS
Monocyte-to-lymphocyte ratio (MLR)	0.23 ± 0.077	0.21 ± 0.050	0.27 ± 0.087*	NS	0.17 ± 0.028	0.20 ± 0.063	0.21 ± 0.062*	NS	$7.7{\cdot}10^{-3}$
Neutrophil-to-lymphocyte ratio (NLR)	1.14 ± 0.51^{a}	1.74 ± 0.31^{b}	1.78 ± 0.99 ^{b,*}	$1.5{\cdot}10^{-2}$	0.77 ± 0.23^{a}	1.38 ± 0.78^{b}	1.34 ± 0.40^{b,*}	$1.4{\cdot}10^{-2}$	$4.9{\cdot}10^{-2}$
Sex hormones									
Follicle-stimulating hormone (FSH, mU/mL)	1.1 ± 0.5	1.1 ± 0.6	1.1 ± 0.9	NS	1.4 ± 0.8	1.3 ± 0.9	2.1 ± 0.9	NS	NS
Luteinizing hormone (LH, mU/mL)	0.14 ± 0.04	0.11 ± 0.02	0.18 ± 0.11	NS	0.12 ± 0.01^{a}	0.12 ± 0.01^{a}	0.22 ± 0.14^{b}	$4.5{\cdot}10^{-2}$	NS
Testosterone (T, ng/dL)	8.83 ± 4.64 ^{a}	13.6 ± 3.2 ^{b}	13.8 ± 2.59 ^{b}	$6.2{\cdot}10^{-3}$	7.6 ± 4.4 ^{a}	11.3 ± 3.1 ^{ab}	18.4 ± 6.0 ^{b}	$1.2{\cdot}10^{-3}$	NS
Androstenedione (A4, ng/mL)	0.60 ± 0.12	0.61 ± 0.26	0.74 ± 0.60*	NS	0.78 ± 0.35^{a}	0.58 ± 0.46^{a}	1.82 ± 0.41^{b,*}	$1.7{\cdot}10^{-2}$	$1.5{\cdot}10^{-2}$
Dehydroepiandrosterone sulfate (DHEAS, μg/mL)	91.9 ± 30.3	90.5 ± 33.6	119.2 ± 82.9*	NS	97.6 ± 46.9^{a}	103.0 ± 33.7^{a}	186.1 ± 61.8^{b,*}	$4.5{\cdot}10^{-2}$	$4.9{\cdot}10^{-2}$
Cortisol (CORT, μg/dL)	7.0 ± 2.1^{a}	6.4 ± 6.3^{a}	11.8 ± 5.4^{b}	$2.7{\cdot}10^{-2}$	7.4 ± 0.7^{a}	10.9 ± 6.3^{b}	10.3 ± 4.9^{b}	$1.7{\cdot}10^{-2}$	NS

Note: Results are expressed as mean ± standard deviation. Superscript letters within each row indicate within-sex significant differences between groups that are marked with different letters, according to ANOVA and subsequent post hoc Fisher LSD test.

Abbreviation: NS, non-significant.

*Denotes between-sex significant differences according to the Student's *t*-test.

obesity-related anthropometric variables (i.e., BMI, weight, WC) were higher in children with obesity compared to healthy controls, regardless of sex and the IR status, and this increase was sharpened among ObIR+ boys with respect to their female counterparts. Male and female children with obesity also showed elevated fasting plasma levels of insulin and HOMA-IR scores, especially whom presenting concomitant IR. Additionally, obesity was associated with lower HDL-C and increased TG contents within both sex groups, but IR did not influence these dyslipidemia factors. As expected, a pro-inflammatory status was observed in children with obesity, with raised white blood cell-based inflammatory indices (i.e., SII, SIRI, ASIS, NLR) and CRP levels (this latter without reaching statistical significance) in the ObIR- and ObIR+ groups. This obesity-related increase of inflammatory markers was more pronounced among males (statistically significant for ObIR+ subjects), as previously reported.[6] Similarly, hormones also experienced sex-dependent perturbations in our study population. Girls with obesity had elevated levels of luteinizing hormone, testosterone, androstenedione, dehydroepiandrosterone sulfate, and cortisol, regardless of the concomitant presence or absence of IR. In contrast, this increment of circulating sex hormones was attenuated among boys with obesity, who only showed greater levels of testosterone and cortisol. 17β-Estradiol and progesterone were undetectable in our study participants, in line with previous evidence suggesting that conventional clinical assays lack the required sensitivity to detect the low concentrations typically found in prepubertal children blood.[26] Finally, multi-elemental analysis of plasma and erythrocyte samples revealed an altered biodistribution of various essential trace elements in childhood obesity. In plasma, increased copper and decreased zinc levels were found among male and female children with obesity, with and without IR, compared to controls (Table 2). On the other hand, we observed a female-specific raise of the erythroid contents of iron, copper, and zinc, but no significant elemental differences were detected within the male group (Table 3).

To better understand the plausible inter-relationships between the above-mentioned multi-elemental alterations and other obesity-related biochemical parameters, data were subjected to correlation analysis. We observed a strong positive association between multiple markers related to hyperinsulinemia, dyslipidemia, inflammation, and abnormal hormonal regulation within both female (Figure 1A) and male (Figure 1B) subjects, which could suggest the existence of shared molecular mechanisms behind these pathogenic events occurring in childhood obesity. Furthermore, these biochemical variables were found to correlate with metal contents in a sex- and tissue-dependent manner. In plasma, copper was positively associated with several inflammatory markers regardless of sex, whereas zinc levels showed a consistent negative correlation with inflammatory and insulinemia-related variables (Figure 1C,D). Furthermore, a female-specific negative association was observed between plasmatic zinc and sex hormones (LH, T). On the other hand, we interestingly found that most of these variables were consistently and positively associated with erythroid metal contents exclusively among female subjects (Figure 1E), whereas only a few inflammatory markers were significantly correlated with metal levels within the male group (Figure 1F).

4 | DISCUSSION

Although sex is known to influence the susceptibility of developing childhood obesity, sex-specific heterogeneities

TABLE 2 Plasma concentrations of trace elements in the three study groups (i.e., CNT, ObIR-, ObIR+) stratified according to sex.

	Male subjects				Female subjects			
	CNT	**ObRI-**	**ObIR+**	***p*-value**	**CNT**	**ObRI-**	**ObIR+**	***p*-value**
Copper	1297.2 ± 176.3^{a}	1588.3 ± 202.4^{b}	1477.9 ± 191.4^{b}	$1.8{\cdot}10^{-3}$	1292.4 ± 178.2^{a}	1454.5 ± 56.8^{b}	1484.5 ± 138.3^{b}	$9.4{\cdot}10^{-3}$
Zinc	853.4 ± 210.2^{a}	725.1 ± 64.1^{b}	681.8 ± 125.8^{b}	$1.2{\cdot}10^{-2}$	792.8 ± 93.1^{a}	725.3 ± 78.2^{b}	711.8 ± 63.3^{b}	$4.6{\cdot}10^{-2}$
Iron	695.5 ± 227.6	745.5 ± 211.3	720.0 ± 325.3	NS	780.0 ± 224.0	795.5 ± 236.3	649.7 ± 177.6	NS
Selenium	134.8 ± 17.3	123.1 ± 12.1	123.4 ± 14.9	NS	132.1 ± 14.1	122.9 ± 21	129.4 ± 25.6	NS
Manganese	3.8 ± 0.9	4.0 ± 0.9	3.5 ± 0.6	NS	4.6 ± 1.1	4.2 ± 1.1	3.7 ± 1.7	NS
Molybdenum	2.7 ± 0.7	2.7 ± 0.6	2.6 ± 1.0	NS	2.8 ± 0.6	3.0 ± 0.9	3.0 ± 1.0	NS
Chromium	7.9 ± 5.7	7.6 ± 4.7	5.9 ± 4.3	NS	6.1 ± 3.2	6.2 ± 2.5	4.8 ± 3.4	NS
Cobalt	1.9 ± 1.6	1.6 ± 0.3	1.9 ± 1.7	NS	1.5 ± 0.3	1.5 ± 0.4	1.6 ± 0.2	NS

Note: Results are expressed as mean ± standard deviation (μg/L). Superscript letters within each row indicate within-sex significant differences between groups that are marked with different letters, according to ANOVA and subsequent post hoc Fisher LSD test.
Abbreviation: NS, non-significant.

TABLE 3 Erythroid concentrations of trace elements in the three study groups (i.e., CNT, ObIR-, ObIR+) stratified according to sex.

	Male subjects				Female subjects			
	CNT	**ObRI-**	**ObIR+**	***p*-value**	**CNT**	**ObRI-**	**ObIR+**	***p*-value**
Copper	582.9 ± 101.4	584.5 ± 103.7	602.5 ± 114.1	NS	523.0 ± 68.0[a]	582.5 ± 101.2[b]	611.4 ± 62.9[b]	$4.1{\cdot}10^{-2}$
Zinc	9856.1 ± 1926.0	9478.6 ± 1792.6	9426.2 ± 2109.4	NS	8558.9 ± 1702.1[a]	10082.7 ± 626.6[b]	9727.7 ± 586.9[b]	$4.5{\cdot}10^{-2}$
Iron	760885.7 ± 6,523,804	778078.9 ± 104156.6	723643.1 ± 75174.8	NS	722598.9 ± 63720.4[a]	821810.6 ± 33169.7[b]	824075.4 ± 39473.4[b]	$3.8{\cdot}10^{-4}$
Selenium	153.5 ± 28.7	140.6 ± 33.1	155.9 ± 58.8	NS	153.7 ± 28.3	153.3 ± 26.5	166.5 ± 37.3	NS
Manganese	16.4 ± 11.9	13.8 ± 5.7	16.5 ± 10.9	NS	20.1 ± 18.7	25.6 ± 15.0	18.6 ± 20.0	NS
Molybdenum	23.1 ± 3.9	26.3 ± 5.4	19.8 ± 4.8	NS	23.9 ± 6.5	27.9 ± 6.1	22.5 ± 3.7	NS

Note: Results are expressed as mean ± standard deviation (μg/L). Superscript letters within each row indicate within-sex significant differences between groups that are marked with different letters, according to ANOVA and subsequent post hoc Fisher LSD test.
Abbreviation: NS, non-significant.

in the association between excess body weight and metal homeostasis have rarely been investigated. Thus, further research is crucial to better understand the involvement of sex-dependent factors in the characteristic metal alterations behind childhood obesity and its comorbidities.

Herein, we observed increased plasma copper levels, along with decreased zinc, among children with obesity compared to non-obese controls, regardless of sex and IR status of the subjects (Table 2). These findings concur with previous studies reporting raised copper-to-zinc ratios in childhood obesity,[13,14,17] which could be explained by the mutual antagonism between these two trace elements within a complex crosstalk involving inflammatory processes, exacerbated oxidative stress, and leptin resistance.[27,28] On the one hand, the characteristic chronic low-grade inflammation state that occurs in childhood obesity could be the most likely driver of these metal perturbations, since the secretion of pro-inflammatory cytokines by adipocytes is known to alter the expression of metal transporters, thereby inducing intracellular copper efflux and zinc uptake, and consequently altering their biodistribution within the organism. This increment of circulating copper (a redox-active metal that may generate free radical species) and decreased zinc levels (a component of many antioxidant enzymes) may in turn provoke oxidative stress. Finally, low blood zinc increases leptin concentrations by down-regulating zinc alpha-2 glycoprotein expression, thereby exacerbating inflammation in a vicious circle. This hypothesis linking higher Cu/Zn with inflammation was further corroborated by correlation analysis, with several inflammatory markers (i.e., CRP, white blood cell-based inflammatory indices) being consistently associated with the levels of plasmatic copper (positive correlation) and zinc (negative correlation) within both sex groups (Figure 1C,D). Moreover, decreased plasma zinc levels were also found to be negatively correlated with increased fasting plasma insulin and/or increased HOMA-IR scores among male and female subjects. This points to the important repercussions that inflammatory-mediated depletion of circulating zinc levels may have in the development of IR and compensatory hyperinsulinemia, as this micronutrient plays essential roles in the production, storage, and action of insulin.[29]

More interestingly, and unlike the results reported for plasma, multi-elemental analysis of erythrocyte samples revealed sex-dimorphic metal alterations, with increased erythroid content of iron, zinc, and copper among female subjects with obesity, whereas no differences were found for any of the trace elements under investigation within the male group (Table 3). This sequestration of metals inside the erythroid cytosolic fraction could be majorly provoked by the above-mentioned altered expression of metal transporters that is triggered by inflammation. However, we observed here that boys have a sharpened obesity-related raise of inflammatory markers compared to girls (Table 1), which apparently contradicts this rationale. As explained above, sex-dependent inflammatory response has been attributed to differential adipose tissue distribution (i.e., macrophage-rich VAT in males vs. SAT in females).[4] In contrast, different investigations have indicated that IR is more prevalent in prepubertal girls, but switches to become higher in boys along sexual maturation.[30] This close inter-relationship between adiposity, inflammation, insulin action, and sex hormones was corroborated in our correlation analyses (Figure 1A,B). Nevertheless, we interestingly found that all these variables were consistently associated with erythroid metal contents exclusively among female subjects (Figure 1E), whereas only a few inflammatory markers were

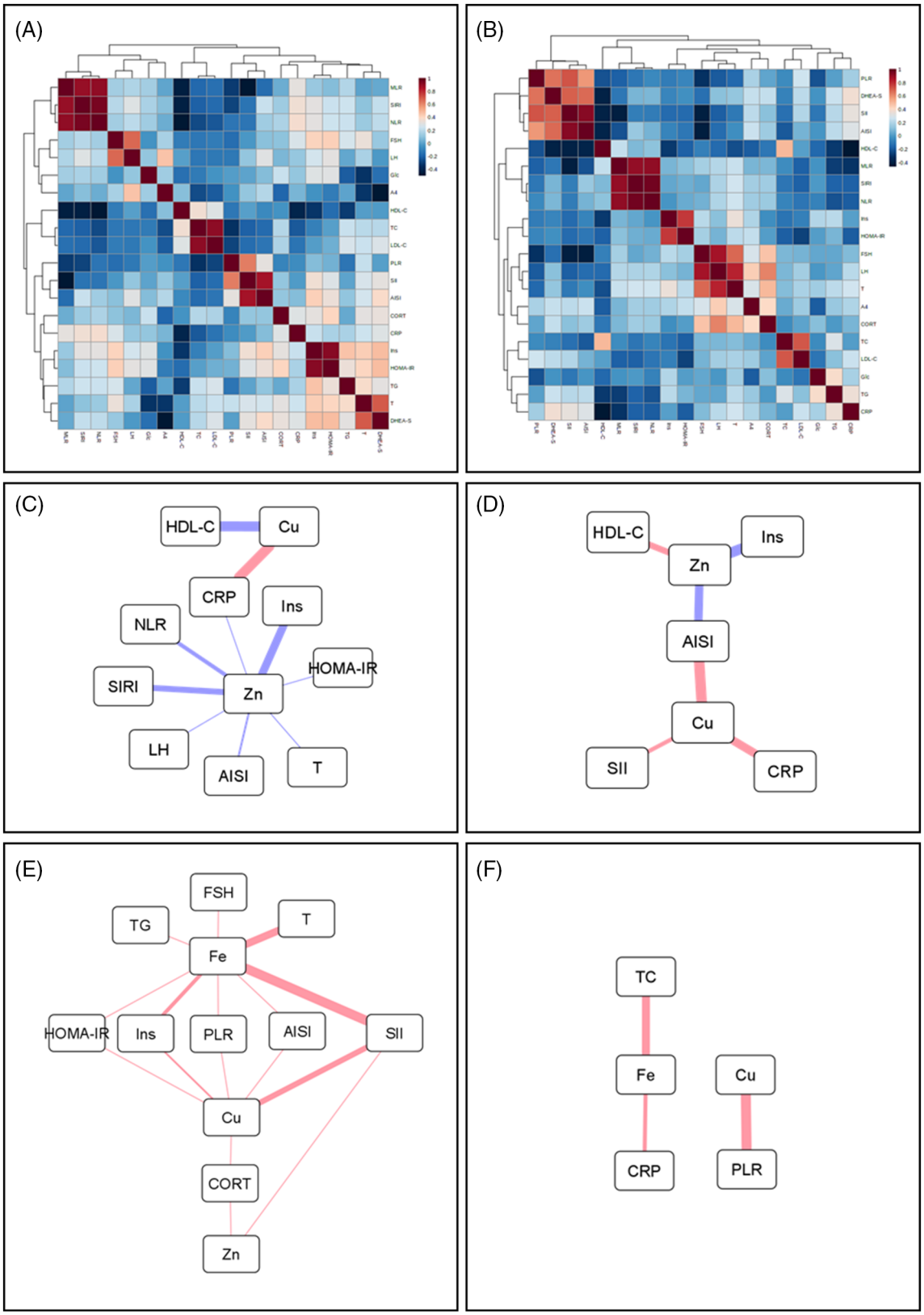

FIGURE 1 Results from Pearson's correlation analyses. Correlation matrices between different obesity-related biochemical variables (i.e., carbohydrate and lipid metabolism, inflammation, sex hormones) within females (A) and males (B); network representation of the correlations between biochemical variables and plasma metal levels within females (C) and males (D); network representation of the correlations between biochemical variables and erythroid metal levels within females (E) and males (F). In network representations, positive and negative correlations are represented as red and blue lines, respectively (the thicker the line, the stronger the correlation).

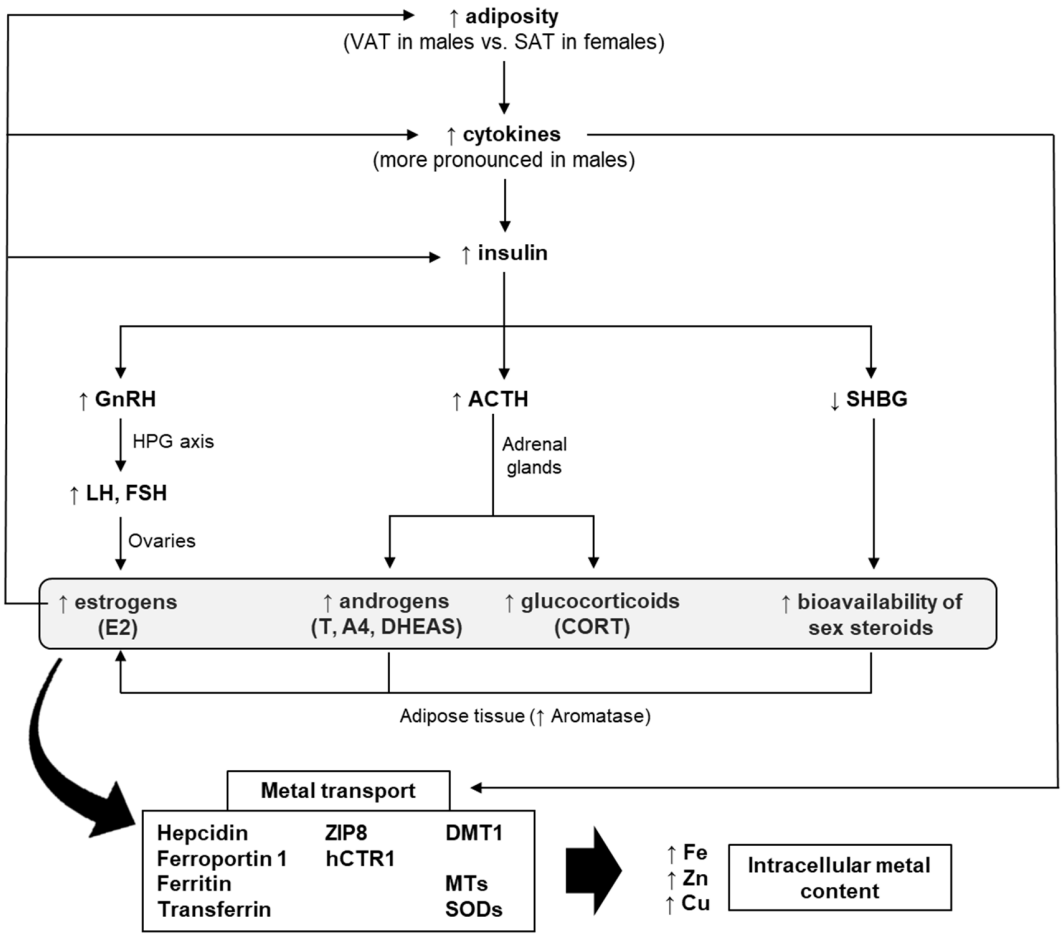

FIGURE 2 Schematic representation of the plausible sex-dependent mechanisms behind childhood obesity-related alterations in adiposity, inflammation, insulin metabolism, sex hormone regulation, and metal homeostasis.

significantly correlated with metal levels within the male group (Figure 1F). Accordingly, this could point to a central role of the interplay between inflammation, insulin, and sex hormones in the female-specific metal alterations that we observed in erythrocytes.

In this respect, it is worth mentioning that inflammation and hyperinsulinemia in girls can boost the production of sex hormones through different mechanisms, as schematized in Figure 2.[31,32] On the one hand, insulin promotes the secretion of the gonadotropin-releasing hormone (GnRH), which subsequently stimulates the release of LH and FSH in the hypothalamic–pituitary-gonadal (HPG) axis, and finally triggers estrogen biosynthesis in ovaries. Alternatively, insulin is also known to enhance the metabolism of androgens and glucocorticoids that is mediated by the adrenocorticotropic hormone (ACTH) in adrenal glands. Another mechanism contributing to release circulating hormones in obesity is associated with the reduction of sex hormone-binding globulin (SHBG) levels, which consequently increases the bioavailability of testosterone and other sex steroids. Within this complex meshwork of regulation processes, it should also be noted that aromatase, which enzymatically converts androgens into estrogens, is highly expressed in adipose tissue. Conversely, data on sex hormone levels in male obesity are scarce and more confusing. In pubertal adolescents and adults, the above-mentioned over-expression of aromatase has been associated with decreased testosterone-to-estradiol ratios in obese subjects, which may suppress GnRH and ultimately inhibit gonadal testosterone release.[33] However, few specific data regarding gonadotropins and sex steroids are available for pre-pubertal boys, although growing evidence suggests that, similarly to girls, they also show increased androgen production, probably due to enhanced adrenal activity.[31,34] In this framework, the exacerbated release of sex hormones occurring in obesity may in turn bidirectionally modulate lipid metabolism, inflammation, and insulin secretion in a sexually dimorphic manner,[4,35] since estrogens are more biologically active than androgens, thereby creating a vicious cycle (Figure 2). Thus, this is finally reflected in a sex-dependent increase of circulating blood

concentrations of a myriad of sex hormones (i.e., estrogens, androgens, glucocorticoids), which is expected to be much more pronounced among girls, in line with our results (Table 1).

Within this cascade of pathogenic events, we hypothesize that the sexually dimorphic over-production of inflammatory mediators and hormones could be, at least in part, responsible for the female-specific metal sequestration observed in erythrocytes from obese subjects (Table 3), especially taking into consideration that sex steroids and cytokines are known to influence intracellular metal transport in different ways (Figure 2). A wide number of studies have demonstrated that sex hormones can regulate iron metabolism by altering the expression of related proteins, such as hepcidin, ferroportin 1, ferritin, and transferrin, in a variety of cells.[36–40] Indeed, their administration has been associated with increased intracellular iron incorporation into MCF-7 cells[39] and erythrocytes,[40] in line with our findings. Other authors have reported that sex hormones can also induce an altered modulation of zinc (e.g., zinc transporter ZIP8) and copper (e.g., human copper transporter 1 hCTR1) transporters.[41,42] Similarly, 17β-estradiol treatment has been proven to up-regulate the expression of the divalent metal transporter 1 (DMT1),[36,42] which participates in the cellular uptake of iron and other divalent trace elements, including zinc and copper. In this respect, Rytz et al. reported that women with metabolic syndrome have higher ferric-reducing ability than healthy subjects, which would facilitate DMT1-mediated assimilation of iron.[43] Complementarily to this enhanced metal transport, some studies have demonstrated that sex steroids can also induce the synthesis and increase the activity of proteins that participate in handling intracellular metals to avoid cytotoxicity, such as metallothioneins[44] and superoxide dismutases.[45] On the other hand, the secretion of pro-inflammatory cytokines by adipocytes in obesity is also known to alter the expression of multiple protein transporters of zinc,[27] copper,[28] and iron.[46] Altogether, this existing molecular evidence supports our rationale that sex hormones and inflammation could act as pivotal elicitors of the female-specific erythroid accumulation of trace elements occurring in childhood obesity, thereby suggesting that girls might possess specific cellular mechanisms to control metal homeostasis. As a clarifying example of sex-driven differences in metal metabolism, it is well-known that females have a much higher capacity to extrude potentially toxic calcium from their body than males.[47] This could in turn explain why metal contents in hair samples are usually higher in female subjects, plausibly as a protective mechanism to remove metal species from the circulation with the aim of avoiding pathological consequences.[48] Therefore, we propose that sex-specific hormonal regulation in response to inflammation and hyperinsulinemia could confer female children with obesity a specific defensive mechanism against the harmful effects that circulant metals may have. Although not supported with experimental data, as we solely analyzed blood samples, we hypothesize that this altered metal transport might affect other cellular compartments besides erythrocytes, since disturbances related to inflammation and sex hormones in childhood obesity have a systemic nature.

The main strengths of our study lie in the use of a well-characterized cohort, comprising children with obesity and IR (ObIR+), children with obesity without IR (ObIR-), and healthy control children (CNT). Unlike other previously published works on the role of metals in childhood obesity,[10–15] we here considered the concomitant presence or absence of IR to differentiate between metabolically healthy (ObIR-) and unhealthy (ObIR+) subjects. Furthermore, the population exclusively consisted of prepubertal children (Tanner Stage I), which considerably minimizes inter-individual variability factors (e.g., hormonal regulation) and thereby facilitates the identification of robust associations between childhood obesity and metal alterations. The comprehensive biochemical characterization of the study participants, including multi-elemental analysis, assessment of carbohydrate and lipid metabolism, and determination of sex hormones and inflammatory markers, enabled us to holistically decipher the plausible mechanisms behind our results. However, it is also worth mentioning that the stratification of the study population according to sex inherently limited the statistical power of the stratified analyses, which makes necessary future studies in larger and independent cohorts to validate our findings. Another important limitation was the impossibility of detecting some sex hormones (e.g., 17β-estradiol) because of sensitivity issues. Because of the observational nature of the study design, further research is needed to better understand this interplay between metals, inflammation, insulin, and sex hormones, as well as to elucidate the molecular mechanisms underlying these observations. The study of other tissues would enable corroborating the systemic nature of this altered metal biodistribution.

5 | CONCLUSIONS

In summary, we describe here for the first time the existence of sexually dimorphic erythroid multi-elemental alterations in childhood obesity. Our findings suggest that genotypic sex may influence the susceptibility to develop obesity at very early ages, before the puberty-induced raise of hormones, which have been proposed

before as the major contributors to sexual dimorphisms. This indeed reinforces that sex hormones could not be the unique drivers of these sex-dependent disturbances, but rather a gear participating in complex molecular mechanisms that already begin in the genesis of the disease. In particular, we found elevated plasma copper-to-zinc ratios in male and female children with obesity, but a female-specific sequestration of iron, zinc, and copper was observed within the erythroid cytosolic fraction. Our results point to sex-dependent inter-relationships between metal homeostasis, inflammatory processes, hyperinsulinemia, and enhanced production of sex hormones as pivotal elicitors of these pathological perturbations. Therefore, these findings highlight the importance of addressing inter-individual variabilities as potential risk factors contributing to the susceptibility of developing obesity at pediatric age.

AUTHOR CONTRIBUTIONS

Conceptualization: Raúl González-Domínguez. *Data curation*: Álvaro González-Domínguez and Raúl González-Domínguez. *Formal analysis*: Álvaro González-Domínguez, María Millán-Martínez and Raúl González-Domínguez. *Funding acquisition*: Raúl González-Domínguez and Alfonso María Lechuga-Sancho; *Investigation*: Álvaro González-Domínguez, María Millán-Martínez, Jesús Domínguez-Riscart, Alfonso María Lechuga-Sancho, and Raúl González-Domínguez. *Methodology*: María Millán-Martínez and Raúl González-Domínguez. *Project administration*: Raúl González-Domínguez. *Resources*: Raúl González-Domínguez and María Millán-Martínez *Supervision*: Raúl González-Domínguez. *Roles/Writing—original draft*: Álvaro González-Domínguez and Raúl González-Domínguez. *Writing—review & editing*: Álvaro González-Domínguez, María Millán-Martínez, Jesús Domínguez-Riscart, María Millán-Martínez and Raúl González-Domínguez. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

Datasets are available from the corresponding author on reasonable request.

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REFERENCES

1. Di Cesare M, Sorić M, Bovet P, Miranda JJ, Bhutta Z, Stevens GA, et al. The epidemiological burden of obesity in childhood: a worldwide epidemic requiring urgent action. BMC Med. 2019;17:212.
2. GBD 2017 Risk Factor Collaborators. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392:1923–94.
3. Tramunt B, Smati S, Grandgeorge N, Lenfant F, Arnal J-F, Montagner A, et al. Sex differences in metabolic regulation and diabetes susceptibility. Diabetologia. 2020;63:453–61.
4. Chang E, Varghese M, Singer K. Gender and sex differences in adipose tissue. Curr Diab Rep. 2018;18:69.
5. Leeners B, Geary N, Tobler PN, Asarian L. Ovarian hormones and obesity. Hum Reprod Update. 2017;23:300–21.
6. Varghese M, Song J, Singer K. Age and sex: impact on adipose tissue metabolism and inflammation. Mech Ageing Dev. 2021;199:111563.
7. Stevenson MJ, Uyeda KS, Harder NHO, Heffern MC. Metal-dependent hormone function: the emerging interdisciplinary field of metalloendocrinology. Metallomics. 2019;11:85–110.
8. Wang C, Zhang R, Wei X, Lv M, Jiang Z. Metalloimmunology: the metal ion-controlled immunity. Adv Immunol. 2020;145:187–241.
9. Zoroddu MA, Aaseth J, Crisponi G, Medici S, Peana M, Nurchi VM. The essential metals for humans: a brief overview. J Inorg Biochem. 2019;195:120–9.
10. Yalçın SS, Fırat MÇ, Tosun E, Yalçın S. A possible etiological factor in obesity: element status in blood and tooth of overweight versus normal-weight children. Int J Environ Health Res. 2018;29:1–13.
11. Błażewicz A, Klatka M, Astel A, Partyka M, Kocjan R. Differences in trace metal concentrations (Co, Cu, Fe, Mn, Zn, Cd, and Ni) in whole blood, plasma, and urine of obese and nonobese children. Biol Trace Elem Res. 2013;155:190–200.
12. Thillan K, Lanerolle P, Thoradeniya T, Samaranayake D, Chandrajith R, Wickramasinghe P. Micronutrient status and associated factors of adiposity in primary school children with normal and high body fat in Colombo municipal area, Sri Lanka. BMC Pediatr. 2021;21:14.
13. Fan Y, Zhang C, Bu J. Relationship between selected serum metallic elements and obesity in children and adolescent in the U.S. Nutrients. 2017;9:104.
14. Azab SFA, Saleh SH, Elsaeed WF, Elshafie MA, Sherief LM, Esh AMH. Serum trace elements in obese Egyptian children: a case-control study. Ital J Pediatr. 2014;40:20.

15. Tascilar ME, Ozgen IT, Abaci A, Serdar M, Aykut O. Trace elements in obese Turkish children. Biol Trace Elem Res. 2011; 143:188–95.
16. González-Domínguez Á, Domínguez-Riscart J, Millán-Martínez M, Lechuga-Sancho AM, González-Domínguez R. Exploring the association between circulating trace elements, metabolic risk factors, and the adherence to a Mediterranean diet among children and adolescents with obesity. Front Public Health. 2023;10:1016819.
17. González-Domínguez Á, Millán-Martínez M, Domínguez-Riscart J, Mateos RM, Lechuga-Sancho AM, González-Domínguez R. Altered metal homeostasis associates with inflammation, oxidative stress, impaired glucose metabolism, and dyslipidemia in the crosstalk between childhood obesity and insulin resistance. Antioxidants. 2022;11:2439.
18. Totten MS, Pierce DM, Erikson KM. The influence of sex and strain on trace element dysregulation in the brain due to diet-induced obesity. J Trace Elem Med Biol. 2021;63:126661.
19. Totten MS, Pierce DM, Erikson KM. Diet-induced obesity disrupts trace element homeostasis and gene expression in the olfactory bulb. Nutrients. 2020;12:3909.
20. Vahter M, Akesson A, Lidén C, Ceccatelli S, Berglund M. Gender differences in the disposition and toxicity of metals. Environ Res. 2007;104:85–95.
21. González-Domínguez Á, Visiedo F, Domínguez-Riscart J, Ruiz-Mateos B, Saez-Benito A, Lechuga-Sancho AM, et al. Blunted reducing power generation in erythrocytes contributes to oxidative stress in prepubertal obese children with insulin resistance. Antioxidants. 2021;10:244.
22. Hernández M, Castellet J, Narvaiza JL, Rincón JM, Ruiz I, Sánchez E, et al. Curvas y tablas de crecimiento. Instituto de Investigación sobre Crecimiento y Desarrollo, Fundación Faustino Orbegozo. 2018, Madrid: Editorial Garsi.
23. Eyzaguirre F, Mericq V. Insulin resistance markers in children. Horm Res Paediatr. 2009;71:65–74.
24. Lu Y, Kippler M, Harari F, Grander M, Palm B, Nordqvist H, et al. Alkali dilution of blood samples for high throughput ICP-MS analysis-comparison with acid digestion. Clin Biochem. 2015;48:140–7.
25. González-Domínguez A, Millán-Martínez M, Sánchez-Rodas D, Lechuga-Sancho AM, González-Domínguez R. Characterization of the plasmatic and erythroid multi-elemental biodistribution in childhood obesity using a high-throughput method for size-fractionation of metal species. In: González-Domínguez R, editor. Mass spectrometry for metabolomics. Methods in molecular biology. New York: Humana; 2023. p. 123–32.
26. Bay K, Andersson AM, Skakkebaek NE. Estradiol levels in prepubertal boys and girls--analytical challenges. Int J Androl. 2004;27:266–73.
27. Gu K, Xiang W, Zhang Y, Sun K, Jiang X. The association between serum zinc level and overweight/obesity: a meta-analysis. Eur J Nutr. 2019;58:2971–82.
28. Gu K, Li X, Xiang W, Jiang X. The relationship between serum copper and overweight/obesity: a meta-analysis. Biol Trace Elem Res. 2020;194:336–47.
29. Barra NG, Anhê FF, Cavallari JF, Singh AM, Chan DY, Schertzer JD. Micronutrients impact the gut microbiota and blood glucose. J Endocrinol. 2021;250:R1–R21.
30. Jeffery SC, Hosking J, Jeffery AN, Murphy MJ, Voss LD, Wilkin TJ, et al. Insulin resistance is higher in prepubertal girls but switches to become higher in boys at age 16: a cohort study (EarlyBird 57). Pediatr Diabetes. 2018;19:223–30.
31. Solorzano CMB, McCartney CR. Obesity and the pubertal transition in girls and boys. Reproduction. 2010;140:399–410.
32. Baraskar K, Thakur P, Shrivastava R, Shrivastava VK. Female obesity: association with endocrine disruption and reproductive dysfunction. Obes Med. 2021;28:100375.
33. Kelly DM, Jones TH. Testosterone and obesity. Obes Rev. 2015; 16:581–606.
34. Vandewalle S, De Schepper J, Kaufman JM. Androgens and obesity in male adolescents. Curr Opin Endocrinol Diabetes Obes. 2015;22:230–7.
35. Ortiz-Huidobro RI, Velasco M, Larqué C, Escalona R, Hiriart M. Molecular insulin actions are sexually dimorphic in lipid metabolism. Front Endocrinol. 2021;12:690484.
36. Qu Y, Li N, Xu M, Zhang D, Xie J, Wang J. Estrogen upregulates iron transporters and iron storage protein through hypoxia inducible factor 1 alpha activation mediated by estrogen receptor β and G protein estrogen receptor in BV2 microglia cells. Neurochem Res. 2022;47:3659–69.
37. Prakash C, Mishra M, Kumar P, Kumar V, Sharma D. Dehydroepiandrosterone alleviates oxidative stress and apoptosis in iron-induced epilepsy via activation of Nrf2/ARE signal pathway. Brain Res Bull. 2019;153:181–90.
38. Pluta D, Franik G, Blukacz Ł, Kowalczyk K, Witkowska A, Wysocka M, et al. The correlation between the concentration of hepcidin in serum and the occurrence of insulin resistance and hyperandrogenemia in women with polycystic ovary syndrome. Eur Rev Med Pharmacol Sci. 2018;22: 7379–84.
39. Bajbouj K, Shafarin J, Abdalla MY, Ahmad IM, Hamad M. Estrogen-induced disruption of intracellular iron metabolism leads to oxidative stress, membrane damage, and cell cycle arrest in MCF-7 cells. Tumour Biol. 2017;39:10104283177 26184.
40. Guo W, Bachman E, Li M, Roy CN, Blusztajn J, Wong S, et al. Testosterone administration inhibits hepcidin transcription and is associated with increased iron incorporation into red blood cells. Aging Cell. 2013;12:280–91.
41. Zang ZS, Xu YM, Lau ATY. Molecular and pathophysiological aspects of metal ion uptake by the zinc transporter ZIP8 (SLC39A8). Toxicol Res. 2016;5:987–1002.
42. Arredondo M, Núñez H, López G, Pizarro F, Ayala M, Araya M. Influence of estrogens on copper indicators: in vivo and in vitro studies. Biol Trace Elem Res. 2010;134:252–64.
43. Rytz CL, Pialoux V, Mura M, Martin A, Hogan DB, Hill MD, et al. Impact of aerobic exercise, sex, and metabolic syndrome on markers of oxidative stress: results from the brain in motion study. J Appl Physiol. 2020;128:748–56.
44. Ljubojević M, Orct T, Micek V, Karaica D, Jurasović J, Breljak D, et al. Sex-dependent expression of metallothioneins MT1 and MT2 and concentrations of trace elements in rat liver and kidney tissues: effect of gonadectomy. J Trace Elem Med Biol. 2019;53:98–108.
45. Unfer TC, Maurer LH, Kemerich DM, Figueiredo CG, Duarte MMF, Gelain DP, et al. Non-genomic, direct modulatory effect of 17β-estradiol, progesterone and their synthetic

derivatives on the activity of human erythrocyte CuZn superoxide dismutase. Free Radic Res. 2013;47:219–32.
46. González-Domínguez Á, Visiedo-García FM, Domínguez-Riscart J, González-Domínguez R, Mateos RM, Lechuga-Sancho AM. Iron metabolism in obesity and metabolic syndrome. Int J Mol Sci. 2020;21:5529.
47. De Loof A. The essence of female–male physiological dimorphism: differential Ca2+−homeostasis enabled by the interplay between farnesol-like endogenous sesquiterpenoids and sex-steroids? The calcigender paradigm. Gen Comp Endocrinol. 2015;211:131–46.
48. Peña-Fernández A, del Carmen L-BM, González-Muñoz MJ. Effects of sex on the levels of metals and metalloids in the hair of a group of healthy Spanish adolescents (13 to 16 years old). Environ Sci Pollut Res. 2017;24:23666–78.

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