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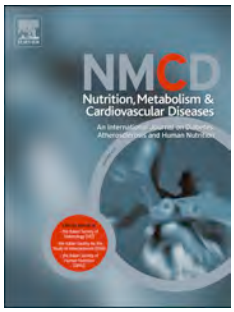

Cardiovascular Risk Biomarkers and Metabolically Unhealthy Status in Prepubertal Children: Comparison of Definitions

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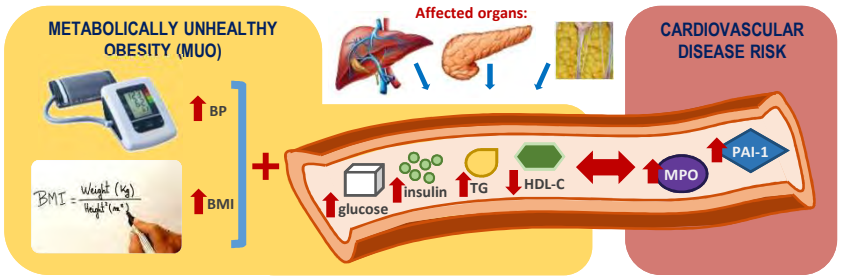

BMI: body mass index; BP: blood pressure; CVD: cardiovascular disease; HDL-C: high-density lipoprotein cholesterol; MPO: myeloperoxidase; PAI-1: plasminogen activator inhibitor 1; TG: triglycerides.

Cardiovascular Risk Biomarkers and Metabolically Unhealthy Status in Prepubertal Children: Comparison of Definitions

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Abstract

Background and aims. The early onset of cardio-metabolic abnormalities, known as metabolically unhealthy (MU) status, is highly associated with obesity and cardiovascular disease (CVD), as well as with increased morbidity and mortality later in life. Given the lack of a consensus MU classification for prepubertal children, we aimed to compare available MU definitions in terms of their association with CVD risk biomarkers.

Methods and results. A total of 930 prepubertal children (622 with overweight/obesity, 462 males) aged 5 to 10.9 years were recruited, anthropometric measures were taken and biomarkers were analyzed. Children were classified using eight MU definitions based on different cut-offs for blood pressure, triacylglycerides, high-density lipoprotein cholesterol, glucose and homeostasis model assessment for insulin resistance (HOMA-IR). MU prevalence in children with overweight/obesity ranged between 30% and 60% across definitions. Plasma concentrations of resistin, leptin, myeloperoxidase (MPO) and total plasminogen activator inhibitor 1 (tPAI-1) were higher, and those of adiponectin were lower, in MU compared to MH children with overweight/obesity. Linear regression analyses confirmed the contribution of MPO and tPAI-1 concentrations to MU status, with most significant results derived from definitions that use age and sex-specific criteria and that account for HOMA-IR.

Conclusion. Plasma concentrations of MPO and tPAI-1 are increased in prepubertal MU children irrespective of having normal-weight or overweight/obesity. Inclusion of age and sex-specific cut-offs for cardio-metabolic components as well as insulin resistance criteria increases the quality of MU definitions as seen by their stronger association with CVD biomarkers concentrations.

Keywords: cardiovascular disease, metabolic health, insulin resistance, childhood obesity.

Introduction

Childhood obesity is one of the most serious public health issues of the 21st century. Results from the most recent study in Spain indicated that 33% of children aged 6 to 9 years had overweight or obesity according to the International Obesity Task Force (IOFT) criteria [1]. Obesity in children is associated with an altered adipokine profile and a high risk of metabolic complications that may appear at very early ages [2, 3]. Moreover, the onset of obesity in early infancy is also associated with an increased risk of future cardio-metabolic disorders and premature mortality [4].

However, it has been shown that not all individuals with obesity exhibit metabolic disturbances. Obesity that is not associated with cardio-metabolic abnormalities is termed metabolically healthy (MH) obesity (MHO), whereas obesity with metabolic alterations is called metabolically unhealthy (MU) obesity (MUO). Notably, normal-weight individuals can also exhibit a MU profile, which also increases disease risk for this population, independently of obesity [5].

Children are commonly classified as MU when there is a state of insulin resistance (IR) or when one or more cardio-metabolic criteria, namely, hypertension, dyslipidemia, or altered glucose metabolism, which are features of the so-called metabolic syndrome, are present. However, there is no consensus definition to identify and classify MU children, which complicates the interpretation of available results [6].

To this date, increased concentrations of circulating CVD risk biomarkers have been observed in children [2, 3, 7] as well as in MU adults [8] with obesity. However, there is a need of studies assessing CVD risk in MU prepubertal children.

Therefore, the present study aimed to: 1) examine the prevalence of MU status in prepubertal Spanish children with and without overweight/obesity according to eight MU definitions, 2) analyze the differences in CVD risk biomarkers among children with MU status, with and

without overweight/obesity, and 3) assess the usefulness of available MU definitions based on their association with the identified CVD risk biomarkers.

Methods

Study Population

A total of 930 prepubertal children (308 normal-weight and 622 overweight/obesity, 462 males and 468 females) aged 5 to 10.9 years were recruited from primary care centers and schools in three Spanish cities (Cordoba, Santiago de Compostela, and Zaragoza). Inclusion criteria were: prepubertal stage (Tanner I) and absence of metabolic diseases. Exclusion criteria were: Tanner stage II to V and use of medications for blood pressure (BP), glucose, or lipid metabolism. Children were assessed as described in previous studies [3]. Parents or guardians were informed of the purpose and procedures of the study before written consents were obtained. The Ethics Committees of all participating institutions approved the study, which complied with the Declaration of Helsinki (Edinburgh 2000 revised).

Anthropometric Measures

Anthropometric parameters and systolic and diastolic BP (SBP and DBP, respectively) were measured and BMI was calculated (kg/m^2). Overweight and obesity were defined using age- and sex-specific BMI cut-off points, equivalent to adult values of 25 kg/m^2 for overweight and 30 kg/m^2 for obesity [9]. The Z-Score for BMI (BMI-z) was calculated as previously [2].

Biomarker Analysis

Blood samples were drawn after an overnight fast and routine analyses were performed at each participating hospital as previously [2]. Plasma insulin was analyzed using radioimmunoassay (CV: 2.6%) in an automatic microparticle analyzer (AxSYM; Abbott Laboratories, Chicago, Ill., USA) and homeostasis model assessment for insulin resistance (HOMA-IR) was calculated. Plasma aliquots were centrifuged and stored at -80°C for later analyses of active and total plasminogen activator inhibitor 1 (PAI-1), soluble intercellular adhesion molecule 1 (sICAM-1), soluble endothelial selectin (sE-selectin), soluble vascular

adhesion molecule 1 (sVCAM-1), matrix metalloproteinase 9 (MMP-9), high sensitivity C-reactive protein (hsCRP), tumor necrosis factor alpha (TNF-α), interleukins (IL) 6 and IL-8, and myeloperoxidase (MPO) [2, 10]. SE-selectin was measured in a subsample of the population due to changes in the commercial kit used for analyses. From these, candidate biomarkers of MU status were selected.

MH/MU Classification

Children were classified according to: A) five definitions based on metabolic syndrome components: Olza *et al.[11]*, Ahrens *et al.* [12], Li *et al.* [13], IDF [14] and Reinehr *et al.* [15], and B) three definitions based on HOMA-IR cut-offs: Olza *et al*. [11], Prince *et al*. [16], and Li *et al*. [13] (Table 1). The fulfillment of at least one criterion was indicative of MU status. We accessed all references and their corresponding charts or tables to classify the children, using the same cut-off points or percentiles as in the original studies.

Statistical Analysis

Results are expressed as the means ± SEM. Variables with a non-normal distribution were transformed. HOMA-IR, tPAI-1, MMP-9, and TNF-α were logarithmically transformed, and insulin, HDL-cholesterol (HDL-C) and sE-Selectin were square-root transformed. Biomarker circulating concentrations were compared among four groups: 1) MH normal-weight, 2) MU normal-weight, 3) MH overweight/obesity and 4) MU overweight/obesity, using a general linear model adjusted for age. Differences between pairs of means were assessed using a pairwise Bonferroni post-hoc test. An additional adjustment for BMI was conducted to discard findings exclusively due to increased adiposity.

The kappa (κ) coefficient evaluated agreement between MU definitions, considering a κ value of less than 0.2 as poor, 0.2 through 0.4 as marginal, 0.4 through 0.6 as moderate, 0.6 through 0.8 as strong, and above 0.8 as very strong [17].

For both tPAI-1 and MPO (dependent variables), their association with MU status (independent variable) was evaluated by backward-step linear regression for each definition, including age and gender in the analysis, stratifying by BMI status (normal-weight and overweight/obesity). $P<0.05$ was considered significant. Sample size calculations were conducted using a power of 80% (beta error = 0.20) and a confidence interval of 95% (alpha I error of 0.05), considering previously reported MPO concentrations in children, showing a 20-25% difference between children with obesity and normal-weight [2, 3]. A minimum sample size of 167 per group was obtained. All statistical analyses were performed using the Statistical Package for Social Science software (IBM SPSS 24, IBM Corporation, Somers, N.Y., USA).

Results

Table 2 shows the main characteristics of the children participating in the study according to gender. Some significant differences were observed for anthropometry, glucose, and lipid metabolism parameters.

Percentages of MUO children according to the studied definitions ranged between 14% and 70% and are shown in Figure 1. Data from the total population is shown because similar results were observed in males and females (Table S1). The highest proportions of MUO children were observed when the definitions of Olza *et al.*[11], Ahrens *et al.* [12] and Li *et al.* [13] were used, and the lowest proportions were observed with the definitions of IDF [14], Reinehr *et al.* [15] and with IR-based definitions. MU prevalence among normal-weight children ranged between 1.7% and 25% across definitions (Table S2). The highest Cohen's Kappa agreement was observed between Olza *et al.* [11], Ahrens *et al.* [12] and Li *et al.* [13] definitions (Table S3). Regarding IR-based definitions, the highest agreement was observed between Olza *et al*. [11]-IR and Li *et al.* [13]-IR definitions (Table S4).

Biomarker differences among the studied groups are shown in Table 3, with children classified according to Olza *et al.* MU definition [11]. Since findings were similar for both males and females, results are shown for the total population. Plasma concentrations of resistin were significantly higher in MU individuals compared to their MH counterparts, independently of BMI status. Leptin concentrations were also higher in MU individuals, although children with overweight/obesity displayed higher values than children with normal-weight. Plasma adiponectin showed lower concentrations in the MU overweight/obesity group compared to MH children. Concerning CVD risk biomarkers, MU children with overweight/obesity exhibited higher concentrations of tPAI-1 and MPO than MH individuals, even after an additional adjustment for BMI. Given their clear differences between MH and MU children, MPO and tPAI-1 were selected for successive analyses.

In children with overweight/obesity, linear regression analysis showed significant associations between MU status and the selected CVD biomarkers tPAI-1 and MPO, according to the definitions of Olza *et al.* [11], Ahrens *et al.* [12], Li *et al.* [13], Olza *et al.* [11]-IR and Li *et al.* [13]-IR. The observed associations in children with normal-weight were similar, although less significant.

Discussion

Available definitions of MUO for children share multiple criteria, but there is no consensus definition for the classification of children at risk of cardio-metabolic disturbances. To our knowledge, this is the first study to demonstrate an association between MU status and CVD risk biomarkers (tPAI-1 and MPO) in prepubertal children using different definitions.

Our first analysis showed that the classification of children as MH or MU varied across definitions, finding the double of MUO prevalence with the definitions of Olza *et al.* [11] or Ahrens *et al.* [12] than with those of IDF [14] or Reinehr *et al.* [15] (~65% vs. ~30%) (Figure 1). Previous studies revealed ~30 to 75% of MUO children and adolescents with obesity. However, none of these studies were performed exclusively in prepubertal children [6, 13]. Importantly, we also found up to a 25% prevalence of MU among children with normal-weight. The most likely reason for the variability of prevalence is the higher specificity of the criteria used within the metabolic syndrome-based classifications of Olza *et al.* [11] and Ahrens *et al.* [12], which account for age and gender in the assessment of TAG, HDL-C and BP. Moreover, these definitions also include a HOMA-IR cut-off, which could improve their quality of the classification given the association that exists between insulin resistance and other metabolic disturbances in MU individuals (Table 1). In contrast, the more general criteria in other classifications may lead to false negative results, lowering the prevalence. Finally, definitions solely based in HOMA-IR cut-offs revealed lower prevalence, with the definition of Prince *et al.* [16] showing the lowest prevalence given its higher HOMA-IR cut-off (Table 1). This finding is not surprising since HOMA-IR classifications miss MU children that fulfill other criteria.

The observed adipokine status agrees with previous findings in which MUO individuals display altered adipokine circulating levels derived from excessive adipose tissue [18]. We observed higher leptin and resistin and lower adiponectin concentrations in the MU overweight/obesity group compared to the MH groups. Similarly, leptin has been associated with MUO in children

and adolescents [19] and resistin has been observed to be increased in adolescents with obesity and metabolic syndrome [20] and in the presence of abdominal obesity [3] and fat mass [21] in children. However, to the best of our knowledge, our study is the first to describe the association between resistin and MH/MU status in prepubertal children. Concerning adiponectin, our findings confirm previous studies in which its levels were negatively associated with body fat, obesity-related metabolic abnormalities and MUO risk in children and adolescents [2, 3, 18, 19, 22]. Therefore, we show that the derangement of adipokine profile begins at prepubertal stage in MU children, principally through the alteration of resistin and adiponectin, and that this occurs beyond BMI status.

Regarding CVD risk biomarkers, they differed across MH and MU normal-weight and overweight/obesity groups of prepubertal children, highlighting tPAI-1 and MPO, which were increased in MU individuals. Moreover, the regression analysis confirmed the association between MU status and these biomarkers in children with overweight/obesity but also, to some extent, in those with normal-weight. PAI-1 was selected for successive analyses given its association with increased thrombosis and atherosclerosis risk [23, 24]. In addition, PAI-1 plasma concentrations have been observed to be increased in MUO adults [5, 8] and children with obesity [3] and metabolic syndrome [7]. The present study shows it is already increased in MU children independently of their BMI status. A possible explanation for the BMI-independent association between PAI-1 and MU status could be that circulating PAI-1 may be synthesized in the liver in addition to adipose tissue [23]. [As for MPO, a pro-oxidant enzyme released by leukocytes, it was also selected for the comparison of definitions given its previous association with endothelial function in children with obesity [25] as well as with other CVD risk and pro-inflammatory biomarkers [2, 26]. Altogether, the increase in PAI-1 and MPO concentrations in MU children with overweight/obesity as early as in the prepubertal stage indicates the importance of these molecules in the increased cardio-metabolic risk in children.

Other CVD risk biomarkers such as sE-Selectin, sICAM-1, sVCAM-1 and MMP-9 were increased in children with overweight/obesity but did not differ according to MU status, as previously observed in children [27], adolescents [28] and young adults [29]. The same was observed for pro-inflammatory biomarkers, as the increase of hsCRP, IL-6, and TNF-α concentrations was due to obesity, as previously shown [5, 8].

As for the adequacy of MU definitions to detect children presenting CVD risk biomarker derangement, prevalence and linear regression results highlight the importance of the applied cardio-metabolic criteria. The fact that classifications of Olza *et al.* [11] and Ahrens *et al.* [12] account for age and gender in the assessment of TAG, HDL-C and BP, arises as an essential aspect (Table 1). Moreover, the significant linear regression results derived from MU definitions exclusively based on HOMA-IR cut-offs, despite their lowest reported MU prevalence, support the importance of the inclusion of this criterion, as it increases the quality and power of the definition to find children with overweight/obesity with a MU status.

Although fat accumulation remains an independent risk factor for MU, our study demonstrated that MU status is also associated with an altered CVD risk biomarker profile in prepubertal children with either normal-weight or overweight/obesity. The limited associations observed in our study may be due to a situation in which the cardio-metabolic derangement is in progress, as well as to the prepubertal character of the population. Stronger associations with MUO will most likely become apparent at later stages in life, as indicated by previous studies in adolescents, young adults and older adults [8, 19, 29].

The present study has strengths and limitations. Strengths include the sample size, the prepubertal character of the population, avoiding puberty associated physiological, hormonal and metabolic alterations that may confound the observed results [15], the inclusion of normal-weight children and the exhaustive analysis of a wide variety of adipokines and CVD biomarkers. Limitations include the absence of a clinical correspondent of CVD, the

development of Olza *et al.* [11] definition within a population shared with the present study and the determination of sE-Selectin in a subsample of the population.

We found that adiponectin was decreased and leptin, resistin, tPAI-1, and MPO were increased in MU prepubertal children independently of their weight status. Moreover, we observed that MU definitions using sex- and age-specific criteria and accounting for HOMA-IR reveal the highest MU prevalence in children with overweight/obesity, as well as the strongest associations with CVD risk biomarkers. Therefore, these definitions should be used in further studies and clinical practice.

Acknowledgements

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Figure Legends

Figure 1 Prevalence of metabolically healthy and unhealthy prepubertal children with overweight/obesity according to different classification criteria. MHO: metabolically healthy overweight/obesity; MUO: metabolically unhealthy overweight/obesity.

Tables

Table 1 Definitions of metabolically unhealthy (MU) status in children compared in the present study.

Definition	Age range	Population weight status	Blood Pressure	TAG	HDL-C	Glucose	HOMA-IR
Olza *et al.* (11)	5-18 y	NW OW OB	SBP or DBP ≥90th percentile for age, sex, and height	>90th percentile for age, sex, and race	<10th percentile for age, sex, and race	≥100 mg/dL	> 2.5
Ahrens *et al.* (12)	2-11 y	NW OW OB	SBP or DBP ≥90th percentile for age, sex, and height	≥90th percentile for age and sex	≤ 10th percentile for age and sex	≥ 90th percentile for age and sex	≥ 90th percentile for age and sex
Li *et al.* (13)	6-11 y	OB	SBP or DBP ≥90th percentile for age, sex, and height	≥110 mg/dL	< 40 mg/dL	≥100 mg/dL	
IDF (14)	6-<16 y		SBP≥130 or DBP≥85	≥150 mg/dL	< 40 mg/dL	≥100 mg/dL	

Reinehr *et al.* (15)	11.6 ± 2.8 y	OB	BP > 95th percentile for age and sex	> 150 mg/dL	< 40 mg/dL	≥100 mg/dL	
Olza *et al.* (11)-IR	5-18 y	NW OW OB					> 2.5
Prince *et al.* (16)-IR	8-17 y	OW OB					≥ 3.16
Li *et al.* (13)-IR	6-18 y	OB					> 2.3

MU status was considered when at least one criterion was fulfilled. BP: blood pressure; DBP: diastolic blood pressure; HDL-C: HDL cholesterol; HOMA-IR: homeostasis model assessment for insulin resistance; NW: normal-weight; OB: obesity; OW: overweight; SBP: systolic blood pressure; TAG: triacylglycerides.

Table 2 General characteristics of the population included in the study

	Male (N= 462)	**Female (N=468)**
Anthropometry		
Age (y)	8.7 (1.4)	8.4 (1.5)*
Weight (kg)	40.3 (13.8)	38.8 (11.7)
Height (m)	1.3 (0.1)	1.3 (0.1)*
Overweight/obesity (%)	69.9	64.1
BMI (kg/m^2)	21.9 (5.5)	21.8 (4.9)
BMI-z	1.99 (2.24)	1.53 (1.60)*
Hip Circ (cm)	79.4 (11.8)	79.1 (9.9)
Waist/Hip C	0.91 (0.08)	0.90 (0.07)
WC (cm)	71.9 (15.9)	70.9 (13.7)
WC/Height	0.54 (0.10)	0.53 (0.09)
SBP (mm Hg)	102.8 (14.7)	103.9 (12.4)
DBP (mm Hg)	63.2 (11.6)	64.3 (10.8)
Glucose metabolism		
Glucose (mg/dl)	85 (7)	83 (7)*
Insulin (mU/l) $^{+}$	7.6 (6.0)	8.6 (5.9)*

	Male (N= 462)	Female (N=468)
HOMA-IR[+]	1.60 (1.37)	1.78 (1.28)*
Lipid metabolism		
CHOL (mg/dl)	170 (32)	169 (29)
TAG (mg/dl)	62 (32)	69 (36)*
HDL-C (mg/dl) [+]	59 (15)	55 (14)*
LDL-C (mg/dl)	97 (28)	98 (26)
HDL/LDL	0.69 (0.39)	0.64 (0.42)
APO A (mg/dl)	145 (31)	140 (29)*
APO B (mg/dl)	67 (19)	70 (18)*
APO B/LDL	0.72 (0.15)	0.73 (0.16)
APO A/APO B	2.30 (0.76)	2.11 (0.72)*

Data are mean (SD). * Significant differences (P<0.05) between genders. [+] P-values for transformed variables are based on transformed differences.

APO: apolipoprotein; BMI: body mass index; CHOL: cholesterol; DBP: diastolic blood pressure; HDL-C: HDL cholesterol; Hip Circ: Hip circumference; HOMA-IR: homeostatic model assessment-insulin resistance; LDL-C: LDL cholesterol; QUICKI: quantitative insulin sensitivity check index; SBP: systolic blood pressure; TAG: triacylglycerides; WC: waist circumference.

Table 3 Adipokines and CVD risk biomarkers of the studied population classified according to the metabolically unhealthy definition of Olza *et al.* (11)

	Normal-weight								**Overweight/Obesity**							
	MH			**MU**			**MH**			**MU**			**P (ANCOVA)**	
	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	Age	Age+BMI
Adipokines														
Adiponectin (mg/l)	191	24.76 ±	0.86[a]	57	20.93 ±	1.42[ab]	194	21.92 ±	0.91[a]	310	18.75 ±	0.55[b]	0.000	0.008
Resistin (µg/l)	191	12.25 ±	0.78[a]	57	15.87 ±	1.82[ab]	194	12.43 ±	0.68[a]	309	15.32 ±	0.58[b]	0.001	0.010
Leptin (µg/l)	189	3.61 ±	0.28[a]	54	4.65 ±	0.60[a]	193	12.73 ±	0.66[b]	314	19.79 ±	0.79[c]	0.000	0.022
L:A ratio	188	0.18 ±	0.02	54	0.29 ±	0.05	193	2.25 ±	1.16	310	1.39 ±	0.08	0.095	0.381
CVD risk biomarkers														
Total PAI-1 (µg/l)*	192	16.89 ±	0.81[a]	57	21.41 ±	2.07[ab]	192	22.77 ±	1.07[b]	311	26.99 ±	0.98[c]	0.000	0.011
Active PAI-1 (µg/l)	152	5.10 ±	0.32[a]	44	5.35 ±	0.82[a]	146	9.27 ±	0.65[b]	201	12.66 ±	0.76[c]	0.000	0.394

	Normal-weight								Overweight/Obesity							
	MH				MU				MH				MU			P (ANCOVA)	
	N	Mean		SEM	N	Mean		SEM	N	Mean		SEM	N	Mean	SEM	Age	Age+BMI
sE-Selectin (µg/l)*	40	72.02	±	14.25[a]	13	151.07	±	49.82[ab]	47	61.90	±	14.85[ab]	110	60.33	± 7.05[b]	0.000	0.389
sICAM-1 (mg/l)	192	0.15	±	0.01	57	0.15	±	0.01	191	0.16	±	0.01	311	0.17	± 0.01	0.185	0.931
sVCAM-1 (mg/l)	150	1.19	±	0.03[a]	44	1.25	±	0.04[ab]	146	1.10	±	0.03[bc]	199	1.06	± 0.02[c]	0.000	0.832
MMP-9 (µg/l)*	152	85.91	±	3.75	44	95.65	±	9.00	145	88.07	±	4.72	201	88.70	± 4.38	0.689	0.676
MPO (µg/l)	191	19.70	±	2.53[a]	57	34.66	±	6.05[ab]	194	19.85	±	1.77[a]	309	32.63	± 2.64[b]	0.000	0.001
hsCRP (mg/l)	205	1.44	±	0.54[a]	65	2.21	±	1.12[ab]	197	3.40	±	0.44[b]	322	3.29	± 0.21[b]	0.002	0.410
IL-6 (ng/l)	179	4.13	±	0.56	56	2.69	±	0.63	185	4.52	±	0.79	312	5.34	± 0.62	0.230	0.737
IL-8 (ng/l)	188	1.69	±	0.12[a]	58	1.65	±	0.17[ab]	190	2.10	±	0.20[ab]	310	2.42	± 0.16[b]	0.006	0.791
TNF-α (ng/l)*	192	3.06	±	0.12[ab]	58	2.92	±	0.25[a]	191	3.62	±	0.15[bc]	314	3.76	± 0.13[c]	0.000	0.614

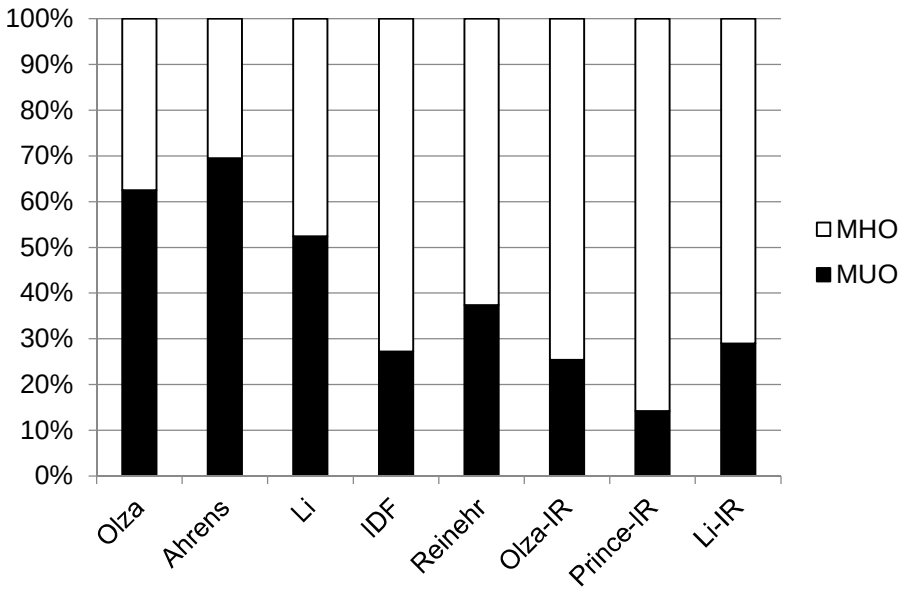
ANCOVA adjusted by age or age and BMI as indicated. Mean values within the same row not sharing the same superscript letter were significantly different by the Bonferroni post-hoc test. * P-values for transformed variables are based on transformed differences.

hsCRP: high sensitivity C reactive protein; IL-6: interleukin 6; IL-8: interleukin 8; L:A ratio: leptin:adiponectin ratio; MH: metabolically healthy; MMP-9: matrix metalloproteinase 9; MPO: myeloperoxidase; MU: metabolically unhealthy; N: number of samples; PAI-1: plasminogen activator inhibitor 1; SEM: standard error of the mean; sICAM-1: soluble intercellular adhesion molecule 1; sE-selectin: soluble endothelial selectin; sVCAM-1: soluble vascular adhesion molecule 1; TNF-α: tumor necrosis factor alpha.

Table 4 Association between metabolically unhealthy definitions and plasma concentrations of cardiovascular disease risk biomarkers in prepubertal children.

Classification	tPAI-1				MPO			
	Beta NW	P NW	Beta OW/OB	P OW/OB	Beta NW	P NW	Beta OW/OB	P OW/OB
Olza *et al.* (11)	0.118	0.068	0.137	**0.002**	0.174	**0.007**	0.158	**0.000**
Ahrens *et al.* (12)	0.143	**0.026**	0.176	**0.000**	0.133	**0.039**	0.120	**0.007**
Li *et al.* (13)	0.106	0.099	0.094	**0.035**	0.112	0.082	0.079	0.076
IDF (14)	0.105	0.098	0.032	0.469	-0.005	0.934	-0.027	0.542
Reinehr *et al.* (15)	0.108	0.093	0.079	0.077	0.078	0.227	0.085	0.057
Olza *et al.* (11)-IR	0.130	**0.038**	0.147	**0.001**	0.064	0.305	0.116	**0.010**
Prince *et al.* (16)-IR	0.148	**0.018**	0.120	**0.008**	0.086	0.173	0.042	0.348
Li *et al.* (13)-IR	0.120	0.055	0.173	**0.000**	0.110	0.079	0.109	**0.015**

Beta: standardized beta coefficient; IR: insulin resistance; NW: normal-weight; OW/OB: overweight/obesity; P: significance of the linear regression adjusted for age and sex; MPO: myeloperoxidase; tPAI-1: total plasminogen activator inhibitor 1.


100%
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Olza
Ahrens
Li
IDF
Reinehr
Olza-IR
Prince-IR
Li-IR
MHO
MUO

Highlights

- Prevalence of metabolically unhealthy (MU) children varies with definitions.
- Age and sex-specific MU definitions including HOMA-IR show higher MU prevalence.
- MU status is associated with higher concentrations of tPAI-1 and MPO.
- MU definitions considering HOMA-IR show stronger associations with MPO and tPAI-1.