Received: 23 February 2021 | Revised: 10 April 2021 | Accepted: 26 April 2021

DOI: 10.1111/ijpo.12817

ORIGINAL RESEARCH

Pediatric OBESITY WILEY

Impaired metabolic health over-time and high abdominal fat are prospectively associated with high-sensitivity C-reactive protein in children: The IDEFICS study

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Funding information
Ministerio de Ciencia e Innovación, Grant/Award Number: FJCI-2017-34967; Sixth Framework Programme, Grant/Award Number: 016181 (FOOD)

Summary

Background: Metabolic risk and inflammatory state have an early life onset and are associated with future diseases.

Objectives: To assess the association between metabolic syndrome (MetS) and metabolic health with high-sensitive C-reactive protein (hsCRP), cross-sectionally and longitudinally, in children.

Methods: 2913 European children (2-10 years) from eight countries from the IDEFICS study were investigated. Data were collected at baseline and 2 years later (follow-up). A MetS z-score was computed with waist circumference (WC), insulin resistance index, blood pressure, high-density lipoprotein cholesterol and triglycerides. Metabolically unhealthy (MU) status was assessed. Multi-level linear and logistic regressions were performed.

Results: Among the MetS markers, WC was more consistently associated with hsCRP cross-sectional and prospectively. Baseline MetS score was significantly associated with greater risk of high hsCRP at follow-up and with prevalence and incidence of hsCRP. Those children who became MU overtime were significantly ($P < .05$) associated with future higher levels of hsCRP, independently of weight status at baseline.

Conclusions: Transition over time to a MU state was associated with higher levels of hsCRP at follow-up, independent of weight status at baseline. Screening of metabolic factors and routine measurement of WC are needed to prevent inflammatory status and related chronic diseases in children.

KEYWORDS
abdominal fat, children, Europe, inflammation, metabolic health, metabolic syndrome

1 | INTRODUCTION

Metabolic syndrome (MetS) is a cluster of several cardio-metabolic risk factors such as central or total adiposity, hyperglycaemia, dyslipidaemia and elevated blood pressure,[1,2] and it has been associated with inflammation in the carotid arteries.[3] The inflammatory mechanisms induce endothelial dysfunction and increase adhesion molecule expression, playing a key role in the onset, development and settlement of the lesion.[4–7]

Among all the inflammatory biomarkers, high-sensitivity C-reactive protein (hsCRP) is widely used in epidemiological studies, and it has been related to carotid intima-media thickness (cIMT)[5] even in children.[6] Noteworthy, adults with MetS are more likely to have elevated concentrations of inflammation markers.[8,9] In this sense, it has been suggested that both MetS and an inflammatory state could coexist as they seem to be triggered by similar factors, particularly by adipose tissue.[10] For that reason, there is no consensus in the literature regarding the direction of the association given that it seems to be found in both ways, that is, MetS as the cause of inflammation and vice versa.[11] Previous studies have shown that the presence of MetS in childhood has been related to the risk of cardio-metabolic disorders later in life[12,13] and with subclinical atherosclerosis in children.[14,15] Additionally, those with MetS in childhood showed higher risk of having high cIMT and diabetes mellitus type 2 (T2DM) as adults.[12] Thus, MetS in childhood can predict adverse levels of cardiovascular risk factors in the future.[16] and this could be due to its association with inflammation. Recently, it has been suggested that MetS is better identified by a continuous score which has shown associations with insulin resistance, inflammation, endothelial damage and cardiovascular diseases (CVD).[17] In 2014, Ahrens et al[18] proposed a definition for MetS, that has been recommended for children,[19] following the approach by Eisenman.[20] The use of metabolic scores or composite metabolic definitions seems to be a better measure of cardiovascular health in children than single risk factors.[21] While the use of definitions to compute a continuous score seems to have higher sensibility and give a better insight, applying a dichotomous measure could be useful for clinical purposes and have more practical implications.

In addition, previous literature has suggested that subjects with overweight/obesity could present a metabolically healthy (MH) status,[22] a definition usually based on markers of MetS. Thus, metabolic health could be to some extent independent of obesity degree. In addition, previous findings showed that poor metabolic health was associated with inflammatory status and endothelial dysfunction already in adolescence.[23] However, there is a lack of previous literature available on this association in children.

To our knowledge, there are no prospective studies carried out in children from eight European countries assessing the longitudinal association between several metabolic risk indicators, metabolic health status and inflammation at such young age. Thus, the main aim of this study is to prospectively investigate the link between MetS and its components and metabolic health status with hsCRP as a marker for inflammation in a sample of European children.

2 | METHODS

2.1 | Study population

The IDEFICS study is a multicentre population-based prospective cohort study performed in eight European countries: Belgium,

Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden, aged 2 to 10. Design and main procedures of the IDEFICS study have been previously described.[24] IDEFICS also included an intervention component, with two study regions per country, geographically apart: intervention and control. Two surveys were included in the present study: baseline (T0, September 2007 to June 2008) and, after 2 years, follow-up (T1, September 2009 to June 2010). In T0, 16 229 children participated followed by 11 041 in T1 (follow-up rate 68%). This study was conducted according to the Declaration of Helsinki. Approvals were obtained from the local Ethics Committee in each participating country. All children were informed and provided oral consent while the parents gave their written consent.

2.2 | Study sample

Out of the total sample measured in the IDEFICS study, 9601 children provided blood samples at baseline. Inclusion criteria for the present study were having data measured at T0 and T1 for all MetS components (WC, blood pressure, TG, HDL, glucose and insulin,) along with hsCRP. Finally, 2913 participants, 1509 males and 1404 females were included in the study.

2.3 | Measurements

Waist circumference was measured using an inelastic tape (SECA 200, Germany). Blood pressure was measured twice with an electronic sphyngomanometer (Welch Allyn 4200B-E2). If first and second value differed by >5%, a third measurement was recorded.

2.4 | Definition of the metabolic syndrome (MetS)

The definition presented by Ahrens et al[18] was based on three previous definitions: the definition by Cook,[25] the International Diabetes Federation (IDF)[26] and the definition by Viner.[27] These definitions consider as components of the MetS: (a) excess adiposity,[28] (b) systolic (SBP) and/or diastolic (DBP) blood pressure,[29] (c) triglycerides and HDL[30] and (d) fasting blood glucose and/or insulin.[31] However, they partly use cut-offs for adults and weigh the single components of the MetS disproportionally. The definition of Ahrens et al[18] overcomes these limitations in that it takes into account age- and sex-specific (and height-specific for blood pressure) z-scores for each of these components and by ensuring a balanced contribution of the MetS components to the overall score. A z-score standardization to calculate a continuous MetS score was used.[18] In addition, the MetS score was dichotomized to identify those children at risk of MetS. Children were divided in two categories: at risk of MetS (≥90th percentile of MetS score) vs not at risk (<90th percentile of MetS score) as previously done.[32]

2.5 | Metabolically unhealthy status definition

Children were classified as metabolically healthy (MH) or metabolically unhealthy (MU) according to the IDF definition of metabolic syndrome for young populations.[26] The IDF definition includes the following criteria: WC, blood pressure, triglycerides, HDL-c and fasting glucose. MU status was considered when at least one of these criteria was fulfilled excluding WC, which was not included in the definition for metabolic health. The MH or MU status of each child at either baseline or follow-up was computed as a new four-category variable called prospective MH/MU: (a) children being MH at both baseline and follow-up, (b) children who were MU at baseline but became MH at follow-up, (d) children who were MH at baseline and that became MU at follow-up and (e) children being MU at both baseline and follow-up.

2.6 | Biochemical analysis

Children were asked to participate in fasting blood collection after an overnight fast. The hsCRP concentrations were measured in a central laboratory with a high-sensitivity assay using latex-enhanced nephelometry (BN2-Nephelometer, Siemens, Deerfield, Illinois), and the lower limit of detection of the assay was 0.02 mg/dL. Children with concentrations of 10 mg/dL and above were excluded because they could be undergoing an inflammatory process and our findings could be biased. Assessment of glucose, HDL and TG was performed on site by a Cholestech LDX (Cholestech, Hayward, California). Insulin was measured with a luminescence immunoassay (AUTO-GA Immulite 2000, Germany). Finally, the HOMA-IR index was calculated. A complete description of blood sampling and analytical procedures has been published previously.[33]

2.7 | Statistical analysis

The distribution of hsCRP was skewed as approximately one third of the sample had a value under the detection limit of 0.02 mg/dL at both time points. The sex-specific median was calculated for those with a value over 0.02 mg/dL. Thus, children were divided into two categories (a) participants falling under the detection limit and below the sex-specific median of the hsCRP and (b) children equal/above the sex-specific median value (high values). Children whose hsCRP levels fell under category 2 were considered as being at risk of high hsCRP. Normality of variables distributions was assessed visually, and transformations were made when needed. Student t-tests were performed to assess the mean difference for all components of the MetS score by hsCRP category and at baseline and at follow-up (T0 or T1).

Multilevel logistic regression was performed to cross-sectionally assess the odds of being in the highest hsCRP category by MetS scores at baseline (hsCRP T0 with MetS score T0) and at follow-up (hsCRP T1 with MetS score T1). Separate models were conducted for each MetS component and for the MetS score. Covariables used for the multilevel

logistic regression analysis included age, sex and parental educational attainment classified according to the International Standard Classification of Education (ISCED).[34] The levels of the multi-level logistic regressions performed in this study were control/intervention and country. The binary level (control/intervention) was used for the adjustment of potential effects of an embedded community- and setting-oriented intervention program in the IDEFICS study.[35] Two models were analysed: model 1 included all previously listed variables and model 2 included the addition of body mass index (BMI).

Separate multi-level linear regression analyses were carried out to examine the associations of the MetS score and its components at baseline with hsCRP at follow-up and changes of hsCRP between baseline and follow-up. Changes in hsCRP were computed as the difference between follow-up minus baseline (T1-T0). Analyses were adjusted for potential confounders including age at baseline, sex, ISCED level, hsCRP levels at baseline and z-BMI at baseline. Control/intervention and country were entered as random intercepts. Multi-level logistic regression analyses were conducted to investigate the association between components of the MetS and MetS score at baseline and being at risk of high hsCRP at follow-up (prevalence) and becoming at high risk of hsCRP at follow-up (incidence). Incident cases included children that had low-medium concentration hsCRP levels at baseline but had high hsCRP levels at follow-up, and, therefore, those children with high hsCRP levels at baseline (prevalent children) were excluded from these analyses. Age at baseline, sex, ISCED level, hsCRP levels at baseline and z-BMI at baseline were entered as confounders. Country and intervention allocation variables were considered as grouping variables.

Multi-level logistic regression (levels: country and control vs intervention) was performed to assess the odds of being in the highest hsCRP category when 'at risk of MetS', that is, (≥90th percentile of MetS score) at baseline and follow-up, separately, considering as reference: 'not at risk of MetS'. Additionally, Odds Ratios (ORs) for having a high inflammatory state at follow-up when being 'at risk of MetS' at baseline were calculated (reference: not at risk of MetS). Two models were created, model 1 included age sex, ISCED while model 2 was Model 1 plus z-BMI. The prospective analysis was further adjusted for baseline concentration of hsCRP.

Associations between MU status at baseline and prospective MH/MU and hsCRP at follow-up and changes in hsCRP were evaluated with linear regression analyses. Analyses were adjusted for the same variables used in previous models (age at baseline, sex, ISCED level, hsCRP levels at baseline and z-BMI at baseline) with country and intervention allocation variables entered as grouping variables. These analyses were performed for the whole sample and separately according to children's weight status. The analyses were performed using Stata/IC (version 16.1). Collinearity was assessed for all the models investigated. No collinearity among variables was found. Statistical significance was set at $P < .05$.

3 | RESULTS

Mean values of biochemical parameters under study are shown in Table S1 by categories of hsCRP at both T0 and T1. At both time points, baseline (T0) and follow-up (T1) significantly higher ($P < .05$) mean values were found for BMI, SBP, DBP, WC, TG, HOMA-IR, mean arterial pressure, insulin and the MetS score in those children with higher hsCRP when compared with those with lower concentrations of hsCRP. In addition, lower mean values were found for HDL at baseline and follow-up in those children with the highest levels of hsCRP.

The cross-sectional associations between the components of the MetS score and the categories of hsCRP are shown in Table S2. The association was significant ($P < .001$) at baseline and follow-up for WC (OR = 1.065, 95% CI: 1.034-1.097 at T0; OR = 1.045, 95% CI: 1.016-1.075 at T1) and HDL (OR = 0.980, 95% CI: 0.974-0.987 at T0; OR = 0.983, 95% CI: 0.976-0.990 at T1) at baseline and follow-up, while the associations between TG and MetS score with hsCRP categories were significant only at T1, (OR = 1.004, 95%CI: 1.001-1.007 and OR = 1.070, 95% CI: 1.018-1.125, respectively).

TABLE 1 Associations between MetS factors at T0 (baseline) and hsCRP at follow-up (T1) and changes in hsCRP between baseline and follow-up (T1-T0)

	hsCRP T1[a]			ΔhsCRP[b]		
	β	95% CI	*P*-value	*β*	95% CI	*P*-value
n = 2873						
Waist circumference T0	0.05	0.04-0.06	<.001*	0.09	−0.02-0.20	.126
Systolic blood pressure T0	0.005	0.001-0.010	.022*	0.08	0.02-0.14	.005*
Diastolic blood pressure T0	0.008	0.003-0.015	.005*	0.12	0.05-0.20	.001*
HDL cholesterol T0	0.000	−0.003-0.003	.869	−0.03	−0.06-0.01	.100
Triglycerides T0	0.002	0.000-0.004	.022*	-0.01	−0.03-0.01	.332
HOMA-IR T0	0.05	−0.000-0.09	.051	0.29	−0.27-0.85	.310
MetS score T0	0.06	0.04-0.08	<.001*	0.23	0.01-0.45	.041*

Note: *Statistically significant results: *p*-value <.05.

[a]Adjusted for sex, age at T0, hsCRP at T0, z-BMI at T0, ISCED and level variables: control/intervention area and country.

[b]Changes in MetS variables and hsCRP calculated as T1 (follow-up) minus T0 (baseline).

TABLE 2 Associations between MetS factors at baseline (T0) and the prevalence of high hsCRP at follow-up (T1) and risk of being at high hsCRP at follow-up (T1)

	Prevalence hsCRP T1[a]			Risk of being at high hsCRP (incidence, n = 2465)[b] at follow-up[a]		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Waist circumference T0	1.12	1.09-1.14	<.001*	1.05	1.01-1.08	.005*
Systolic blood pressure T0	1.01	1.00-1.02	.050	0.99	0.94-1.01	.516
Diastolic blood pressure T0	1.02	1.01-1.04	.006*	0.99	0.98-1.02	.996
HDL cholesterol T0	1.00	0.99-1.01	.993	1.01	1.01-1.02	.003*
Triglycerides T0	1.00	1.00-1.01	.030*	1.00	1.00-1.01	.706
HOMA-IR T0	1.10	0.99-1.22	.078	0.91	0.77-1.07	.263
MetS score T0	1.14	1.09-1.19	<.001*	1.06	1.00-1.13	.044*

Note: *Statistically significant results: *p*-value <.05.
[a]Adjusted for sex, age at T0, hsCRP at T0, z-BMI at T0, ISCED and level variables: control/intervention area and country.
[b]Outcome 'risk of being at high hsCRP' is defined as having high hsCRP (n = 403) at follow-up. Children with high hsCRP at baseline and that remained with high hsCRP values at follow-up were excluded from this analysis (n = 412).

Table 1 shows the associations between MetS component variables at T0 with hsCRP at T1 and changes in hsCRP levels (T1-T0). Positive associations were observed for WC (β = 0.05, 95% CI = 0.04-0.06), SBP (β = 0.005, 95% CI = 0.001-0.010), DBP (β = 0.008, 95% CI = 0.003-0.015), TG (β = 0.002, 95% CI = 0.000-0.004) and MetS score (β = 0.06, 95% CI = 0.04-0.08). MetS component variables and delta values of hs-CRP were positively associated, that is, SBP (β = 0.08, 95% CI = 0.02-0.14), DBP (β = 0.012, 95% CI = 0.05-0.20) and MetS score (β = 0.23, 95% CI = 0.01-0.45). As an example, this means that a one-unit increase in MetS score was associated with a 0.23-unit higher delta hsCRP.

The prevalence and incidence of having high values of hsCRP according to each metabolic factor and MetS score at T0 are shown in Table 2. Children with higher baseline WC (OR = 1.12, 95% CI = 1.09-1.14), SBP (OR = 1.01, 95% CI = 1.00-1.02), DBP (OR = 1.02, 95% CI = 1.01-1.04), triglycerides (OR = 1.00, 95% CI = 1.00-1.01) and MetS score (OR = 1.14, 95% CI = 1.09-1.19) were more likely to be at high risk of hsCRP at follow-up (prevalence). Children with higher baseline WC (OR = 1.05, 95% CI = 1.01-1.08), HDL-c (OR = 1.01, 95% CI = 1.01-1.02) and MetS score (OR = 1.06, 95% CI = 1.00-1.13) had higher odds of becoming at high risk of hsCRP at T1 (incidence).

Table 3 shows the multi-level logistic regression between being at risk of MetS and categories of hsCRP cross-sectionally and prospectively. Those at risk of MetS had higher odds of being in the highest level of CRP at each measurement time, T0 and T1 (*P* < .001). The odds decreased when the z-BMI was entered into the model, but associations remained significant. Regarding the prospective association between being at risk of MetS at baseline and the odds of being in the highest level of hsCRP at follow-up, both models were significant (OR = 4.95, 95% CI = 3.765-6.508, and OR = 2.47, 95% CI = 1.829-3.342) when entering z-BMI.

Finally, Table 4 shows the longitudinal associations between baseline metabolic health (MU status) and changes of metabolic health over time with hsCRP at T1 and changes of hsCRP (T1-T0). These analyses are shown for all participants and stratified by weight status, that is, normal weight and children with overweight/obesity. In all participants, we found positive and significant associations

TABLE 3 Multi-level logistic regression for hsCRP by risk of metabolic syndrome (MetS) cross-sectionally and prospectively

	Model 1		Model 2	
Cross-sectional analysis		**hsCRP at T0**		**hsCRP at T0**
T0	OR	95% CI	OR	95% CI
Not at risk of MetS	-	-	-	-
At risk of MetS	3.95*	**3.029-5.151**	2.93*	**2.190-3.926**
		hsCRP at T1		**hsCRP at T1**
T1	**OR**	**95% CI**	**OR**	**95% CI**
Not at risk of MetS	-	-	-	-
At risk of MetS	7.74*	**5.817-10.312**	3.88*	**2.842-5.306**
Prospective analysis†				
	Model 1			**Model 2**
T0		**hsCRP at T1**		**hsCRP at T1**
	OR	**95% CI**	**OR**	**95% CI**
Not at risk of MetS	-	-	-	-
At risk of MetS	4.95*	**3.765–6.508**	2.47*	**1.829–3.342**

Note: T0: Baseline; T1: Follow-up. **Model 1**: Adjusted by age (T0 or T1), sex, international standard classification for education (ISCED) and levels: control/intervention and country. **Model 2**: Adjusted by age (T0 or T1), sex, zbody mass index (T0 or T1), international standard classification for education (ISCED) and levels: control/intervention and country.
*Statistically significant results indicated with and asterisk (*P*-value <.05).
†Prospective association. Adjusted by age T0, sex, body mass index T0, CRP at T0, international standard classification for education (ISCED) and levels: control/intervention and country. At risk of MetS (≥90th percentile of MetS score) vs not at risk (<90th percentile of MetS score).

TABLE 4 Longitudinal associations between metabolic health at baseline (T0) and patterns of metabolic health from baseline (T0) to follow-up (T1) and hsCRP at follow-up (T1) and changes in hsCRP between baseline and follow-up (T1-T0)

	hsCRP T1[a]			ΔhsCRP[b]		
	β	95% CI	*P*-value	*β*	95% CI	*P*-value
All (n = 2873)						
MH T0	ref.			ref.		
MU T0	0.04	−0.05-0.14	.367	1.30	0.19-2.41	.022*
Prospective MH/MU						
MH T0 and MH T1 (n = 1830)	ref.			ref.		
MU T0 and MH T1 (n = 355)	−0.02	−0.13-0.10	.795	1.33	−0.09-2.74	.066
MH T0 and MU T1 (n = 428)	0.40	0.29-0.50	<.001*	1.21	−0.09-2.50	.068
MU T0 and MU T1 (n = 260)	0.30	0.17-0.44	<.001*	1.80	0.20-3.40	.028*
Normal weight (n = 2331)						
MH T0	ref.			ref.		
MU T0	−0.04	−0.15-0.06	.396	0.25	−0.72-1.23	.61
Prospective MH/MU						
MH T0 and MH T1 (n = 1546)	ref.			ref.		
MU T0 and MH T1 (n = 298)	−0.07	−0.19-0.06	.275	0.19	−1.01-1.39	.758
MH T0 and MU T1 (n = 312)	0.34	0.22-0.46	<.001*	0.46	−0.70-1.61	.437
MU T0 and MU T1 (n = 175)	0.15	0.00-0.31	.049*	0.56	−0.93-2.05	.459
Overweight/obesity (n = 542)						
MH T0	ref.			ref.		
MU T0	0.12	−0.08-0.32	.256	3.15	−0.41-6.71	.083
Prospective MH/MU						
MH T0 and MH T1 (n = 284)	ref.			ref.		
MU T0 and MH T1 (n = 57)	0.18	−0.12-0.48	.233	1.66	3.60-6.92	.536
MH T0 and MU T1 (n = 116)	0.25	0.02-0.47	.035*	2.56	1.42-6.54	.208
MU T0 and MU T1 (n = 85)	0.19	−0.06-0.45	.132	5.38	0.89-9.87	.019*

Note: Groups: MH T0 and MH T1: Metabolically healthy at baseline and metabolically healthy at follow-up; MU T0 and MH T1: Metabolically unhealthy at baseline and metabolically healthy at follow-up; MH T0 and MU T1: Metabolically healthy at T0 and metabolically unhealthy at follow-up; MU T0 and MU T1: Metabolically unhealthy at baseline and metabolically unhealthy at follow-up.

*Statistically significant results: *p*-value <.05.

Abbreviations: MH, metabolically healthy; MU, metabolically unhealthy.

[a]Adjusted for sex, age at T0, hsCRP at T0, ISCED and level variables: control/intervention area and country.

[b]Changes in hsCRP calculated as T1 (follow-up) minus T0 (baseline).

between those that became MU over time (MH T0 and MU T1) or were already MU at baseline and remained MU over time (MU T0 and MU T1) and hsCRP at T1 ($P < .001$). The same was found for those with MU status at baseline and follow-up and the change of hsCRP ($P = .028$). Regarding MU status in normal weight participants and hsCRP at T1, we found positive and significant associations between those whose metabolic health (MH T0 and MU T1) worsened over time or were MU at baseline and at follow-up (MU T0 and MU T1) and hsCRP at T1 ($P < .001$ and $P = .049$, respectively). Finally, we found a significant and positive association between those children with overweight and obesity that became MU over time (MH T0 and MU T1) with hsCRP at T1 ($P = .035$). Likewise, a positive and significant association was found for those with overweight/obesity that were MU at both baseline and follow-up and changes (T1-T0) of hsCRP ($P = .019$).

4 | DISCUSSION

The main findings of this multicentre study with European children were the prospective positive associations between metabolic state and hsCRP. The continuous MetS score together with being at risk of MetS at baseline was associated with high levels of hsCRP at follow-up. Also, the impairment of metabolic health over time in childhood was associated with higher concentrations of hsCRP 2 years later, independently of the obesity status. These results suggest that the

metabolic definitions evaluated may be useful when assessing the association between metabolic risk and hsCRP in paediatric populations. Finally, among the metabolic factors investigated, WC seemed to be the MetS component that exerted a major effect on current and future hsCRP already in childhood. These associations were found even when controlling for BMI suggesting the major role of abdominal fat in these associations.

In our sample, 14.1% of the children were considered at risk of MetS at T1 according to the cut-off proposed by Ahrens, when MetS score was ≥90th percentile,[18] while 56.6% of the children were not at risk for MetS at baseline or follow-up (data not shown). Noteworthy, it is difficult to estimate the prevalence of MetS in children as there are no consensus regarding its definition.[36] In a sample of Spanish children, prevalence ranged from 7.6% to 30.8% depending on the definition used.[37] This could be partly explained by the previous lack of reference values for youth. However, the MetS definition based on the data of the IDEFICS study[18] has been considered an appropriate option in children.[19]

In adults, it has been observed that those with MetS are at increased risk of cardiovascular disease[38] while cardiovascular risk factors in early life may induce alterations in arteries and development of atherosclerosis.[39] However, few studies have examined the link between paediatric MetS and future cardiovascular disease or its precursors.[12,13] A 25 years follow-up study observed that MetS in childhood, combined with changes in age-specific BMI percentile, predicted cardiovascular events later in life.[13] In addition, a study from Magnussen et al[12] found that a dichotomous definition of MetS in youth could predict adult MetS, high cIMT and T2DM in early to middle adulthood. In the present study, we have found that baseline values of MetS score were associated with higher hsCRP after 2 years and that those who increased the MetS score were more likely to become at high risk of hsCRP 2 years later. The continuous score is considered useful as it takes into account the cumulative impact of the risk as it gradually increases across individuals, whereas dichotomous definitions can lead to misclassification or underestimation of the extent of variation.[40] In the present study, the MetS score was associated with the future prevalence and incidence of hsCRP, which supports its clinical use. Also, using the dichotomized MetS, we found longitudinal associations with hsCRP, suggesting that those at risk of MetS in early childhood have higher odds of high hsCRP later on. The probabilities decreased when adding z-BMI in the analysis suggesting an association with BMI,[13] but they were still high and significant.

Furthermore, in our study, we found associations between the individual markers of MetS and hsCRP. Similar results have been observed previously in adolescents.[41] In a previous study carried out in children, where cIMT was measured, hsCRP was associated with subclinical atherosclerosis in children with MetS.[42] This highlights the need for screening metabolic markers during childhood to prevent the early stages of atherosclerosis. In our study, among the components of MetS, WC and HDL cholesterol were those that increased the probabilities of hsCRP at both measurement times. Accumulation of fat mass in the abdominal area increases the risk of having elevated hsCRP and other metabolic complications, including atherosclerosis.[43] A proposed mechanism of the adverse effect of MetS on health seems to be mainly triggered by an excess of visceral fat.[44] The adipose tissue mass leads to an increased turnover of free fatty acids (FFAs) and to changes in the secretion of pro-inflammatory adipokines.[45] This secretion of hormones and adipocytokines, such as leptin, adiponectin, resistin, MCP-1, among others, as well as a variety of interleukins together with TNF-α, enhances the development and/or the progression of chronic diseases, including chronic inflammation.[46] In the longitudinal study from Ferreira et al, a relationship between central fat accumulation and future carotid atherosclerosis was also found in adolescents.[47] This highlights the importance of screening WC already in childhood to prevent metabolic risk and future CVD.

Finally, the deterioration of metabolic health over-time during childhood was associated with higher concentrations of hsCRP independently of the obesity degree and without considering WC. Thus, being at risk for just one metabolic biomarker was associated with high concentrations of hsCRP even for children with normal weight. In adults, poor metabolic health status has been associated with greater adverse changes in cardiac structure and function than obesity alone[48] and prospective changes toward metabolically unhealthy or obese states resulted in increased CVD risk.[49] Previous findings in adolescents showed that poor metabolic health was related to an unfavourable inflammatory status and endothelial dysfunction.[23] In addition, it has been observed among European adolescents that metabolic health is associated with high concentrations of hsCRP.[50] However, there are no previous studies assessing the association between metabolic status over time and inflammation during childhood. Also, it should be mentioned that the definition of MH does not consider WC, the metabolic syndrome marker that was shown to be more consistently associated with inflammation. In spite of this, we have still found associations with unhealthy metabolic status and inflammation during childhood. This suggests that both metabolic health impairment and high WC are independently associated with hsCRP already in children. Therefore, results of the present study highlight the importance of screening metabolic health already in childhood to prevent cardiovascular risk and pro-inflammatory status.

One limitation of this study is the low stability of the definitions of MetS over time in this age group. In addition, hsCRP was the only inflammatory marker measured in the present study; and one-point time analysis could not reflect chronic inflammatory state. Further studies might need to consider other inflammatory biomarkers such as pro-inflammatory adipokines, interleukins or tumour necrosis factor-α. However, it should be noted that hsCRP is the most common indicator to assess inflammatory status in epidemiological studies. Also, pubertal stage was not considered although all analyses were controlled by age. In our analyses, we considered MetS as the independent variable and hsCRP as the dependent one, but we aware of the fact that this association could have been investigated in the opposite direction. However, this study also presents some strengths. Firstly, the use of standardized and harmonized information from eight European countries. Secondly, the multi-level design, which takes into account differences by country and control/intervention areas, should be also taken into account. The prospective design of

the analysis is a further strength as it gives a better insight of long-term associations. Also, the use of the definition of MetS by Ahrens, which has been recommended for widespread use, and considers age- and sex-specific reference values.

To our knowledge, this is the first study that assesses the association between MetS and metabolic health status with hsCRP cross-sectionally and over-time in a relatively large sample of European children. Both MetS and metabolic health definitions were associated with high concentrations of hsCRP which suggests that common mechanisms are involved in the onset of each of these conditions already at early ages. Among all the individual metabolic biomarkers included in this study, WC was the factor more consistently associated with future prevalence and incidence of being at risk of high hsCRP probably due to the major role of abdominal fat tissue in the development of inflammatory status. Overall, either impairment of metabolic health over time or high waist circumference was independently associated with inflammation. These results also highlight the importance of preventing excess weight gain from early ages and effectively treating obesity early in life in order to reduce the risk of transition to a metabolically unhealthy state and incidence of high hsCRP concentrations. Consequently, screening of metabolic factors and routine measurement of WC are needed to prevent inflammatory status and related chronic diseases in children.

ACKNOWLEDGEMENTS

This work was done as part of the IDEFICS study (www.idefics.eu). We gratefully acknowledge the financial support of the European Community within the Sixth RTD Framework Programme Contract No. 016181 (FOOD).

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Silvia Bel-Serrat carried out the statistical analysis and drafted the manuscript along with Esther M. González-Gil. Luis A. Moreno, Wolfgang Ahrens, Paola Russo, Stefaan De Henauw, Toomas Veidebaum, Denes Molnar, Michael Tornaritis and Arno Fraterman designed the study, got the funding and supervised the national data collection. Annunziata Nappo, Javier Santabárbara, Maike Wolters, Monica Hunsberger, Luis A. Moreno, Licia Iacoviello and Wolfgang Ahrens revised the manuscript and interpreted the data. All the authors read and critically reviewed the manuscript and gave final approval of the version submitted.

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SUPPORTING INFORMATION

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How to cite this article: González-Gil EM, Moreno LA, Nappo A, et al. Impaired metabolic health over-time and high abdominal fat are prospectively associated with high-sensitivity C-reactive protein in children: The IDEFICS study. *Pediatric Obesity*. 2021;16(11):e12817. https://doi.org/10.1111/ijpo.12817