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Markers of subclinical atherosclerosis in school-children with obesity and metabolic syndrome

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Summary

BACKGROUND: Although increased carotid intima-media thickness (cIMT), soluble adhesion molecules and proinflammatory biomarkers are strongly implicated in the development of atherosclerotic lesions, the role of obesity and metabolic syndrome (MetS) in atherogenicity and inflammation among schoolchildren is not well investigated.

AIM: To determine the levels of cIMT, endothelial dysfunction and inflammatory biomarkers in a group of schoolchildren with obesity and MetS.

METHODS: Eighty-seven schoolchildren (age 10–15 years) were categorised into three groups: normal bodyweight group, obese group and severely obese with MetS group (17 boys and 12 girls in each group). Levels of cIMT were measured with high-resolution B-mode ultrasound. Serum proinflammatory cytokines interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), and soluble adhesion molecules E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1(ICAM-1) were measured.

RESULTS: Mean cIMT levels were significantly higher ($p \leq 0.05$) among severely obese schoolchildren with MetS (0.49 ± 0.02 mm) compared with both the obese (0.43 ± 0.03 mm) and the normal bodyweight counterparts (0.36 ± 0.03 mm). Serum levels of IL-6, TNF-α, IL-1β, E-selectin, VCAM-1 and ICAM-1 were significantly higher ($p \leq 0.05$) in severely obese with MetS and obese children compared with the normal bodyweight group. However, no significant differences ($p > 0.05$) were found between the severely obese schoolchildren with MetS and the obese without MetS.

CONCLUSIONS: Severely obese schoolchildren having MetS exhibited higher cIMT levels than obese and normal bodyweight counterparts. Biomarkers of inflammation and endothelial dysfunction were higher in obese schoolchildren, but biomarkers were not increased any further by the degree of obesity nor the MetS cluster.

Key words: atherosclerosis; childhood obesity; endothelial dysfunction; inflammation; metabolic syndrome

Introduction

Childhood obesity is strongly associated with increased risk of cardiovascular diseases [1, 2], which is mediated by obesity-related pathophysiological mechanisms including impaired glucose metabolism, dyslipidaemia and elevated blood pressure. The cluster of these alterations is defined as metabolic syndrome (MetS) [3]. MetS is a state associated with increased incidence of several noncommunicable diseases such as cardiovascular disease and type 2 diabetes mellitus [4–6]. Although the definition and the cut-off points for children remain controversial [5, 7], MetS during childhood has been associated with a higher incidence of cardiovascular disease and all-cause mortality during adulthood [8].

Pathological evidence revealed that the atherosclerotic process not only starts in childhood [9], but also develops more rapidly among children with obesity and obesity associated with MetS manifestations [8, 10, 11]. In this regard, measurement of

the carotid intima-media thickness (cIMT) of the common artery by means of high-resolution B-mode ultrasound is a noninvasive tool for subclinical atherosclerosis detection in the young population [12]. Increased cIMT is positively associated with cardiovascular risk factors among children and adolescences with obesity, and obesity related MetS [1, 10].

Furthermore, soluble adhesion molecules such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) are molecular markers for endothelial dysfunction, an early marker of atherosclerosis [13, 14]. Elevated circulating adhesion molecules are predictors of atherosclerosis events associated with cardiovascular risk factors. Additionally, soluble adhesion molecules are associated positively with obesity and may play more essential role in atherosclerosis disease than traditional risk factors [11, 13].

Obesity is a state of subclinical chronic inflammation [15], characterised by increased proinflammatory biomarkers such as interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α) and interleukin-beta (IL-1β) [6, 15]. These inflammatory biomarkers are strongly implicated in the initiation, acceleration and progression of atherosclerotic lesions [11]. It is worth noting that the inflammatory state of obesity leads to clinical and biochemical manifestations of MetS [6]. Moreover, the interaction between the clinical phenotype and the biological phenotype (e.g., dyslipidaemia) of MetS contribute to the development of a proinflammatory state and chronic, subclinical vascular inflammation, which modulates and results in atherogenicity and arthrosclerosis [16, 17]. The identification and prevention of obesity risk factors at an early age could prevent the rampant rise in the prevalence of noncommunicable disease, but the association of MetS with atherogenicity and an inflammatory state among schoolchildren is not yet well investigated; no data on this topic are available for Jordanian schoolchildren with obesity and MetS. Hence, the objective of this study was to determine the levels of cIMT, endothelial dysfunction and inflammatory biomarkers in obese schoolchildren with or without MetS. These findings might be then used to address the need of suitable public health programme planning and dietary interventions.

Materials and methods

Study design

This study included schoolchildren aged 10–15 years, 29 extremely obese with MetS, 29 obese and 29 with normal bodyweight. Inclusion criteria were for apparently healthy schoolchildren, with normal bodyweight or obesity according to the World Health Organization (WHO) body mass index (BMI) z score adjusted for age and gender [18]. Exclusion criteria were chronic illnesses (e.g., renal, cardiac or hepatic diseases), reported chronic use of medications, any type of disability other than the state of obesity, or a history of first-hand smoking.

Recruitment for this study was between March 2015 and June 2015, from four public schools that enrol pupils from the fifth to the ninth grades, taken from a list of 20 public schools located in four districts in Amman provided by the Jordanian Ministry of Education. A total of 178 schoolchildren were recruited; 18 pupils were excluded because they did not meet the inclusion criteria. Of the 160 (81 boys and 79 girls) included schoolchildren, 62 (33 boys and 29 girls) were classified as normal bodyweight and 98 (59 boys and 48girls) were classified as obese. Of the 98 obese schoolchildren, 29 (17 boys and 12 girls) were extremely obese according to the Centers for Disease Control and Prevention (CDC) classification, which was for participants who were on or above the 99th percentile of BMI adjusted for age and gender [19], and diagnosed with MetS according to the definition of the International Diabetes Federation (IDF) [3] as follows: (1) obese and having >90th percentile waist circumference according to the values reported by the National Health and Nutrition Examination Survey (NHANES) for children and the adult population [20], (2) triglycerides levels >1.7 mmol/l, (3) high density lipoprotein (HDL) levels <1.03 mmol/l, (4) blood pressure >130 mm Hg systolic or >85 mm Hg diastolic and (5) fasting plasma glucose (FPG) >5.6 mmol/l. Twenty-nine normal bodyweight children (17 boys and 12 girls) and 29 who were obese without MetS (17 boys and 12 girls) were then matched by age and gender with the severely obese-MetS group. All parents of the participating schoolchildren gave written consent before inclusion and were given a brief guidance to explain the objective and the procedures of the study to their children; verbal assent was obtained from the children after the study objective and procedures for the examination had been explained to them.

Weight, height, waist circumference, hip circumference and systolic blood pressure (SBP) and diastolic blood pressure (DBP) of the children were measured with standard procedures. BMI, and waist-to-hip ratio (WHR) were calculated.
The protocol of the study was approved by the Deanship of Academic Research at the University of Jordan, the Jordanian Ministry of Education and Abdul Hameed Shoman Foundation Research Committee, Jordan.

Carotid intima-media thickness measurement

Intima-media thickness of the common carotid artery was measured by means of B-mode ultrasound with a linear probe at 7.5 MHz frequency. Measurements were performed by one sonographer, who was not aware of each participant's study group, at the King Hussein Medical City, Jordan. Single image of the right common carotid artery were taken as a longitudinal ultrasound scan, 1 cm below the carotid bulb with participants lying in supine position, head turned by 45° to 50° rotation of the neck to the contralateral side, images were taken at the lateral angle. Measurements were taken in the far wall, and participants remained in the supine position for at least 5 minutes before the examination [12].

Biochemical analysis

Blood samples were drawn after an overnight fast (10–12 hours). Blood samples were separated, and serum aliquots were stored at –20°C until analysed. Total cholesterol , HDL, low density lipoprotein cholesterol (LDL), triglycerides and FBG concentrations were analysed with a calorimetric enzymatic method and use of commercial kits (Biolabo SAS, Maizy, France). IL-6, TNF-α, IL-1β, sVCAM-1, sICAM-1 and E-selectin levels were measured with high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (RayBiotech-Inc, Norcross, CA, USA), using a plate reader (ELX808, BioTek Instruments, Winooski, USA) at 450 nm.

Statistical analysis

Statistical analysis was performed with SPSS software (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.). Data are expressed as mean ± standard error of the mean (SEM) for continuous variables. Mean differences of continuous variables were examined using one-way analysis of variance. Means were compared with a *post hoc* Tukey HSD (honest significant difference) test. A p-value less than or equal 0.05 was considered statistically significant.

Results

Table 1 presents the general anthropometric and clinical characteristics of the participants.

Table 1: Anthropometric and clinical characteristics of the study sample.

Variable	Normal body-weight (n = 29)	Obese (n = 29)	Severely obese + MetS (n = 29)	p-value*
Males/females (n)	17/12	17/12	17/12	
Age (years)	12.6 ± 0.2[a]	12.4 ± 0.2[a]	12.9 ± 0.2[a]	0.304
Weight (kg)	44.4 ± 1.9[a]	62.2 ± 2.7[b]	79.4 ± 3.1[c]	**0.001**
Height (m)	1.6 ± 0.02[a]	1.6 ± 0.02[a]	1.6 ± 0.02[a]	0.087
BMI (kg/m^2)	18.3 ± 0. 5[a]	24.8 ± 0.6[b]	30. 9 ± 0.8[c]	**0.001**
WC (cm)	65.7 ± 1.2[a]	80.1 ± 2.3[b]	95.5 ± 2.4[c]	**0.001**
HC (cm)	82.7 ± 1.7[a]	96.9 ± 1.7[b]	107.3 ± 1.5[c]	**0.001**
WHR	0.8 ± 0.01[a]	0.8 ± 0.02[a]	0.9 ± 0.02[b]	**0.002**
SBP (mm Hg)	112.2 ± 2.5[a]	119.7 ± 2.7[b]	133.5 ± 2.0[c]	**0.001**
DBP (mm Hg)	63.2 ± 1.9[a]	72.6 ± 2.8[b]	80.1 ± 1.8[c]	**0.001**

BMI = body mass index; DBP = diastolic blood pressure; HC = hip circumference; MetS = metabolic syndrome; SBP = systolic blood pressure; WC = waist circumference; WHR = waist-hip ratio
Data are given as mean ± standard error of the mean.
* The p-value represents the difference between the three groups. Different superscript letters within the same row (a,b,c) indicate statically significant differences (p ≤0.05).

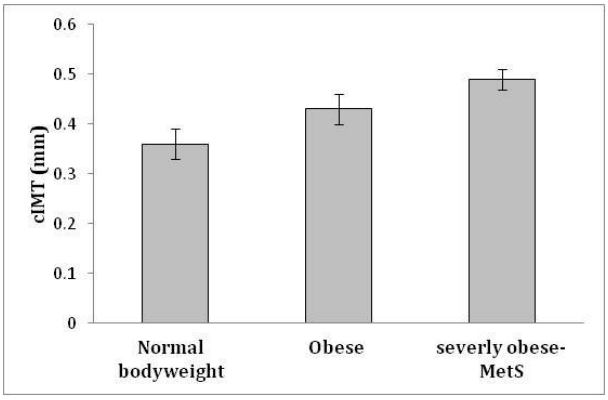

Figure 1: Mean and SEM of carotid intima-media thickness (cIMT) levels in the groups with normal bodyweight, obese without metabolic syndrome (MetS) and severely obese with MetS.

No significant differences ($p > 0.05$) were found between the study groups in age (years) and height (m). No statistical difference ($p > 0.05$) was found between the obese and normal bodyweight groups regarding to waist-to-hip ratio. Additionally, no significant differences were observed between boys and girls, nor between children (10–11 years) and young adolescents (12–15 years) regarding all examined variables in the same group and therefore data were combined (data not shown).

Figure 1 shows that mean cIMT levels were significantly higher in the severely obese-MetS group (0.49 ± 0.02 mm) as compared with the obese-without MetS (0.43 ± 0.03 mm) and normal bodyweight (0.36 ± 0.03 mm) groups ($p = 0.03$).

Table 2 shows that all tested proinflammatory biomarkers and soluble adhesion molecules were significantly ($p \leq 0.05$) higher in the severely obese-MetS and the obese groups as compared with the normal bodyweight group. Nonetheless, no statistical difference ($p > 0.05$) was found between the severely obese-MetS and the obese groups regarding the tested proinflammatory biomarkers and soluble adhesion molecules.

Table 2: Biochemical indicators (fasting) of the study population.

Variable	Normal bodyweight (n = 29)	Obese (n= 29)	Severely obese+MetS (n=29)	p-value*
FBG (mmol/l)	4.54 ± 0.13[a]	4.61 ± 0.12[a]	6.13 ± 0.21[b]	**0.001**
TC (mmol/l)	3.26 ± 0.17 [a]	4.39 ± 0.22[b]	4.60 ± 0.18[c]	**0.001**
TG (mmol/l)	1.23 ± 0.06[a]	1.49 ± 0.06[b]	2.00 ± 0.09[c]	**0.001**
HDL (mmol/l)	1.37 ± 0.09[b]	1.34 ± 0.07[b]	1.00 ± 0.03[a]	**0.031**
LDL (mmol/l)	1.95 ± 0.09[a]	2.35 ± 0.17[b]	2.29 ± 0.10[c]	**0.035**
IL-6 (pg/ml)	1.4 ± 0.1[a]	2.4 ± 0.1[b]	2.2 ± 0.3[b]	**0.006**
TNF-α (pg/ml)	2.8 ± 0.2[a]	3.5 ± 0.3[b]	3.5 ± 0.3[b]	**0.042**
IL-1β (pg/ml)	2.6 ± 0.4[a]	3.4 ± 0.3[b]	3.7 ± 0.8[b]	**0.047**
VCAM-1 (ng/ml)	219.9 ± 11.2[a]	277.3 ± 18.1[b]	238.7 ± 10.7[b]	**0.015**
ICAM-1 (ng/ml)	147.6 ± 7.9[a]	176.0 ± 7.5[b]	167.7 ± 11.4[b]	**0.05**
E-selectin (ng/ml)	23.7 ± 1.5[a]	33.3 ± 3.7[b]	35.1 ± 3.9[b]	**0.03**

FBG = fasting blood glucose; HDL = high-density lipoprotein cholesterol; ICAM-1 = intercellular adhesion molecule-1; IL-1β = interleukin-1-beta; IL-6 = interleukin 6; LDL = low-density lipoprotein cholesterol; MetS = metabolic syndrome; TC = total cholesterol; TG = triglyceride; TNF-α = tumour necrosis factor-alpha; VCAM-1 = vascular cell adhesion molecule-1

Data are given as mean ± standard error of the mean.

† The p-value represents the difference between the three groups. Different superscript letters within the same row (a,b,c) indicate statically significant differences ($p \leq 0.05$).

Discussion

Metabolic syndrome and its individual components are predictors of atherosclerosis and other cardiovascular events [5]. This study aimed at determining cIMT, inflammatory biomarkers, and soluble adhesion molecules levels in 10–15-year-old obese schoolchildren with or without MetS.

The findings of the current study show that mean cIMT levels were significantly ($p \leq 0.05$) higher among obese schoolchildren than that among their normal bodyweight counterparts. These findings are consistent with the findings of other reports [1, 2], which indicate that atherosclerosis could start during childhood [9]. Additionally, increased cIMT levels in obese boys and girls regardless of MetS state suggests that obesity and its manifestations represent a powerful determinant of the manifestations of early atherosclerosis, which may affect mechanical and structural properties of the major vessels [2, 4]. Therefore, our findings support the supposition that the onset of atherosclerosis could start at an early age among obese children and young adolescents [9].

The findings of the study demonstrate that associations in the thickness of the carotid artery were not only observed among young adolescents, but also in obese and normal bodyweight children. In other words, significant differences ($p \leq 0.05$) were found in both children and young adolescents between the three groups. This finding is not in agreement with the findings of 13 out of the 19 studies conducted mostly on adolescent populations included by Park et al. [21] in their systematic review, which included 22 cross-sectional studies and a total of 7366 children and adolescents from Western Europe and the US. The majority of the reviewed reports suggested that cIMT was not affected among children under the age of 12 years having atherosclerosis risk factors when compared with their normal counterparts. This could be attributed to genetic variations [22] and lean body mass [23], neither of which were investigated in our study.

We found a significant increase ($p \leq 0.05$) in cIMT among the severely obese schoolchildren with MetS as compared with the obese without MetS group. This is in line with the findings of Tzou et al. [24], who demonstrated that MetS increased nearly by 2.5- to 3.4-fold in the highest composite cIMT quintile (based on two different definitions of MetS). Additionally, the current findings are in line with the findings of Civilibal and colleagues [10], who reported that MetS was associated with increased cIMT when compared with normal weight counterparts (mean age 12.9 ± 3.5 years); they failed to examine the role of obesity without MetS. However, the current result could be attributed to the more severe stage of obesity and not MetS *per se*, which requires further investigation.

Our findings show an increase in the proinflammatory cytokines in the obese schoolchildren compared with normal bodyweight counterparts, which is consistent with other findings among both obese children and adolescents [11, 15, 25] and obese adults [6]. Findings suggested that proinflammatory cytokines are associated with insulin resistance and obesity associated atherogenicity [6, 26]. Interleukin-6 (IL-6), TNF-α, and IL-1β are suggested as predictors for future cardiovascular events among healthy individuals or those with coronary artery disease [27, 28].

Elevated serum levels of markers of chronic subclinical inflammation have been associated with cardiovascular disease and MetS [4, 29]. Ritchie and Connell [30] reported, in a review discussing the role of adipose tissue in secreting inflammatory biomarkers among adults, that TNF-αand IL-6 modulate lipid and glucose metabolism, inflammation and blood pressure, all of which are known as risk factors for MetS and cardiovascular disease. However, no increase was observed in our study for any of the studied inflammatory markers in the severely obese schoolchildren with MetS compared their obese counterparts not having MetS. Although MetS may be associated with a degree of systemic low-grade inflammation [6], our findings suggested that MetS *per se* may not play a role in increasing proinflammatory cytokines in younger populations, although this could be attributed to the small sample size.

Soluble adhesion molecules levels were significantly higher ($p \leq 0.05$) in obese than in normal bodyweight schoolchildren in the current study, regardless of the presence of MetS or the degree of obesity. Adhesion molecules are predictors for vascular change and have been reported to be elevated among obese children and adolescents [13, 29]. Soluble concentrations of E-selectin, ICAM-1 and VCAM-1 were correlated with the development and expansion of atherosclerotic lesions, which were suggested as markers reflecting the extent of the lesions [14, 31]. Clinical investigations using cIMT as a tool for assessing subclinical atherosclerosis have expressed these correlations [14]. Data regarding increased adhesion molecules among obese or obese-with-MetS schoolchildren remain controversial; for

instance, Kapiotis and colleges [32] reported an increase in E-selectin among obese children compared with normal bodyweight children, without a significant difference in either ICAM-1 or VCAM-1. Olza and colleges [33] reported an increase in ICAM-1 and E-selectin among prepubertal children with MetS assessed with continuous MetS score.

Severely obese schoolchildren with MetS did not show a significant increase in all studied biomarkers of inflammation and soluble adhesion molecules levels. This may be partially because obesity precedes the clustering of MetS components; therefore, obesity becomes a more sensitive marker for predicting inflammation and endothelial dysfunction in the paediatric population [34]. However, the findings of the present study are consistent with other reports [2, 15, 33, 34], which indicated that obesity among children and adolescents is associated with inflammation, endothelial dysfunction and other cardiovascular risk factors, which can compromise their health by leading to an early stage of atherosclerosis.

This study highlights the need for further investigation of the effect of metabolic syndrome on subclinical atherosclerosis in longitudinal studies of these biomarkers, in conjunction with the consistency of metabolic syndrome diagnosis during childhood and adolescence, in order to enhance our understanding of the true values of biomarkers of inflammation and endothelial dysfunction in the prognosis of later atherosclerosis.

The strength of the study lies in the number of variables measured compared with other investigations. The study participants were young, nonsmokers and did not consume medications, which strengthens our results. Also, this study may enable assessment of the level of association separately for obese children and severely obese children with metabolic syndrome. A few limitations deserve mention. Although MetS is widely used, it has several shortcomings: the absence underlying mechanisms and the use of different predictive cut-off values according to the definition used for children have been questioned [7, 35]. Other limitations were the sample size, which may reduce the statistical power, and the nature of the study design, which makes it impossible to draw conclusions on causality.

Conclusion

We demonstrated that obese schoolchildren exhibited significantly higher levels of cIMT, and biomarkers of inflammation and endothelial dysfunction; levels of biomarkers were not worsened any further by the clinical cluster “metabolic syndrome”, suggesting that it is obesity *per se* rather than metabolic syndrome that is linked to increased circulating levels of inflammation and endothelial dysfunction in children and adolescents.

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Competing interests
The authors declare no conflict of interest.

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