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Elevated matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 in obese children and adolescents

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Abstract

Matrix metalloproteinases (MMPs) have been implicated in the atherosclerotic process and risk factors for the disease such as hypertension, hyperlipidemia, or diabetes mellitus in adults. So far, circulating levels of MMPs and their tissue inhibitors (TIMPs) have not been assessed in children and adolescents with obesity, a known risk factor for cardiovascular disease. Plasma levels of MMP-9 and TIMP-1 were measured immunoenzymatically in 45 obese children and adolescents, aged 15 ± 1.8 years. The control group consisted of 28 healthy children, aged 14.5 ± 2.5 years. MMP-9 and TIMP-1 concentrations were higher in obese children than in the control group (MMP-9: 553.5 ± 311 vs 400.4 ± 204 ng/mL, respectively; $P = .02$; TIMP-1: 161.2 ± 32 vs 143.1 ± 20.1 ng/mL, respectively; $P = .03$). We found significantly higher levels of MMP-9 in obese children with coexisting hypertension than in obese normotensive patients (635 ± 308 vs 450 ± 289 ng/mL, respectively; $P = .04$). MMP-9 correlated with body mass index (BMI) ($r = 0.33$, $P = .005$) and fasting insulin ($r = 0.3$, $P = .013$); TIMP-1 correlated with BMI ($r = 0.35$, $P = .006$). In the group of obese hypertensive children (n = 25), MMP-9 correlated with BMI ($r = 0.41$, $P = .001$), systolic blood pressure ($r = 0.41$, $P = .002$), fasting insulin ($r = 0.37$, $P = .006$), and homeostasis model assessment index of insulin resistance ($r = 0.27$, $P = .03$). TIMP-1 correlated with BMI ($r = 0.33$, $P = .025$) and systolic ($r = 0.38$, $P = .008$) and diastolic ($r = 0.47$, $P = .001$) blood pressure. In the regression models, MMP-9 was found to be dependent on fasting insulin ($R^2 = 0.16$, $P = .04$), and TIMP-1 on BMI ($R^2 = 0.14$, $P = .04$). In the obese hypertensive group, TIMP-1 was dependent on diastolic blood pressure ($R^2 = 0.18$, $P = .04$). Obese children and adolescents have elevated plasma concentrations of MMP-9 and TIMP-1. Coexistence of hypertension may exacerbate alterations of extracellular matrix turnover in these patients. It might be hypothesized that elevated MMP and TIMP concentrations may be related to increased cardiovascular risk in obese and particularly in obese hypertensive children and adolescents.

1. Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases capable of degrading components of the extracellular matrix (ECM) and basement membranes. This ability to modify the structural integrity is essential in physiologic tissue remodeling. However, the dysregulation or activation of MMP expression is associated with numerous pathologic conditions associated with an unbalanced turnover of the ECM such as arthritis, wound healing, and tumor growth [1,2]. Lately, MMPs and their inhibitors (tissue inhibitors of metalloproteinases [TIMPs]) have been implicated in various cardiovascular diseases (CVD) that include acute coronary events, restenosis, dilated cardiomyopathy, heart failure, and myocardial infarction [2-4]. A large body of evidence asserts the importance of MMPs in atherosclerosis [1].

Atherosclerosis is a chronic inflammatory disease involving, among others, production and degradation of the ECM and the accumulation of lipids in the arterial wall. MMPs may play a central role in subendothelial vascular ECM remodeling. MMP degradation of the ECM may facilitate infiltration of leukocytes through the endothelial layer, contribute to a decrease in endothelial barrier function with increased influx of plasma lipoproteins, and facilitate migration of vascular smooth muscle cells through the internal elastic lamina into the intimal space where they proliferate and contribute to plaque formation [5-8].

Cardiovascular disease constitutes the leading cause of morbidity and mortality worldwide [9]. Atherosclerosis is the main underlying pathophysiologic process involved,

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although it remains not fully understood. MMPs and TIMPs may constitute an additional group of biomarkers in the inflammatory atherosclerotic process. Alterations in MMP expression in the arterial intima can be detected by determination of their systemic concentration. Some studies have revealed elevation in circulating levels of MMPs not only after acute coronary syndromes, but also in patients with early atherosclerosis and risk factors for the disease [10,11]. Atherosclerosis begins early in life and gradually progresses through adolescence and youth. The process is accelerated in children in whom risk factors for the disease are present. Autopsy studies have shown that the presence of fatty streaks and more advanced changes in the aorta and coronary arteries of children and young adults was related to body mass index (BMI) [12,13].

Obesity in childhood is becoming a significant health issue in developed as well as in developing countries [14,15]. Childhood obesity increases the risk of atherosclerosis and premature all-cause mortality later in life [16]. Already in the young, it is correlated with the constellation of other atherosclerosis risk factors such as hypertension, hyperlipidemia, and insulin resistance [17]. In our previous studies we found elevated levels of adhesion molecules (sICAM-1, sVCAM-1) and sE-selectin in obese children and adolescents, concluding that endothelial activation appears in these children and adhesion molecules are related to the earliest stages of atherosclerosis [18].

No information is available so far on the levels of MMPs in childhood obesity and their possible role in the process of atherosclerosis in the young. We chose to measure plasma MMP-9 and TIMP-1 because clinical data support an important role of these 2 enzymes in cardiovascular pathology and identified them as novel predictors of cardiovascular risk [19-22]. The aim of this study was to investigate the levels of MMP-9 and TIMP-1 in obese children and adolescents and to estimate the MMP-9 and TIMP-1 levels in disturbances connected with childhood obesity, such as hypertension, insulin resistance, hyperlipidemia, and positive family history of CVDs.

2. Methods

2.1. Subjects

The study population consisted of 45 obese children and adolescents (22 boys and 26 girls, aged 15 ± 1.8 years) recruited from patients of the 2nd Department of Children's Diseases of the Medical University of Białystok, Poland, and its related outpatient clinic for endocrinology. Obese children were divided and then compared according to accompanying alterations into groups: (1) hypertensive (n = 25) vs normotensive, (2) hyperlipidemic (n = 16) vs normolipidemic, (3) insulin resistant (n = 11) vs insulin sensitive, and (4) children with positive family history (n = 11) vs negative family history of CVDs. The control group consisted of 28 healthy children and adolescents (13 boys and 15 girls, aged 14.5 ± 2.5 years). The groups were matched for age and sex. None of the parents of the children from the control group reported a history of CVD. The general characteristics of the study groups are presented in Table 1.

All children underwent thorough physical examination. Height was measured to the nearest 0.1 cm by using a wall-mounted stadiometer. Body weight was measured with a digital scale to the nearest 0.1 kg. BMI was calculated as the weight in kilograms divided by the height in meters squared. Obesity was defined as BMI greater than the 95th percentile, matched according to age and sex, by using centile charts for the assessment of somatic development in children and adolescents [23]. Children with secondary obesity were not included in the study.

Before the blood pressure measurements, the children lay for 10 minutes in a quiet and comfortable room. Systolic (SBP) and diastolic blood pressure (DBP) were measured 3 times, once daily, using a standard mercury sphygmomanometer with cuff bladder width approximately 40% of the arm circumference of the child. The presence of hypertension was defined as SBP and/or DBP exceeding the 95th centile. If high arterial blood pressure was found, we performed 24-hour ambulatory blood pressure monitoring (ABPM), using the Medilog DX apparatus (Oxford, UK). Hypertension was diagnosed when high measurements recurred frequently, ie, at least 30% of the 24-hour recordings exceeded the 95th centile, matched for age and sex [24]. Mean SBP and DBP values were calculated from ABPM. For all children, an underlying cause for their hypertension, such as hormonal, renal, or cardiac was sought. Such children were not included in the study.

To recognize hyperlipidemia we adopted guidelines from the American Report of the Expert Panel on Blood Cholesterol in Children and Adolescents of the National Cholesterol Education Program [25]. The estimate of

Table 1
General and clinical characteristics of the study and control groups

	Obese group	Control group
No. of patients	45	28
Boys	22	13
Girls	26	15
Age (y)	15 ± 1.8	14.5 ± 2.5
Height (m)	1.6 ± 0.1	1.6 ± 0.1
Body mass (kg)	86 ± 17*	51 ± 14
BMI	30 ± 3.8*	19 ± 2.8
Total cholesterol (mmol/L)	4.31 ± 0.7*	3.93 ± 0.5
Triglycerides (mmol/L)	1.24 ± 0.5*	0.93 ± 0.2
LDL cholesterol (mmol/L)	2.24 ± 0.7*	1.9 ± 0.4
HDL cholesterol (mmol/L)	1.35 ± 0.2*	1.55 ± 0.2
SBP (mmHg)	133 ± 21*	115 ± 7
DBP (mmHg)	77 ± 8*	70 ± 5
Fasting glucose (mmol/L)	4.4 ± 1	4.1 ± 0.4
Fasting insulin (IU/mL)	17.5 ± 18*	9.7 ± 3.5
HOMA IR index	3.2 ± 2.4*	1.9 ± 0.7

Data are presented as mean ± SD.
* $P < .05$ compared with control group.

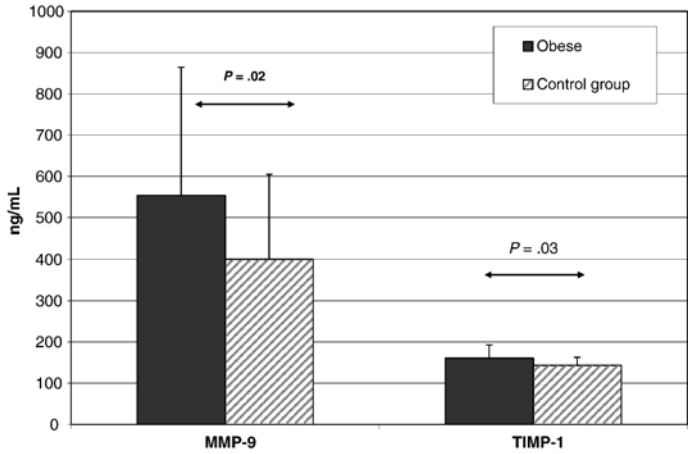

Fig. 1. MMP-9 and TIMP-1 levels in the study and control groups. Data are presented as mean ± SD.

insulin resistance by a homeostasis model assessment index (HOMA IR) was calculated with the formula: [fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L)]/22.5, as described by Matthews et al [26]. Insulin resistance was recognized when the HOMA IR index reached the upper quartile.

Information was obtained from hospital documentation and interviews with parents regarding family history of CVD (coronary heart disease, myocardial infarction, and stroke).

2.2. Laboratory analysis

For analysis, 6 mL of venous blood was taken by venipuncture in the morning before breakfast after an overnight (8-12 hours) fast. To assess MMP-9 and TIMP-1 levels, plasma samples were collected, frozen, and stored at −70°C until analysis. Serum concentrations of MMP-9 and TIMP-1 were determined by using commercially available enzyme-linked immunoassays (Quantikine human MMP-9 [total] and Quantikine human TIMP-1; R&D Systems, Minnneapolis, MN) with the use of an ELx 800 Automated Microplate Reader, Bio-Tek Instruments (Winooski, VT). The intra- and interassay precision coefficients of variation (in percent) were as follows: MMP-9 <2.9/<7.9; TIMP-1 <5.0/<4.9. The minimum detectable dose of MMP-9 is less than 0.156 ng/mL and of TIMP-1 less than 0.08 ng/mL.

Glucose concentrations and lipid parameters (total cholesterol, triglycerides, high-density lipoprotein [HDL] cholesterol) were determined by routine enzymatic methods using commercial kits. Analysis was performed in the hospital's laboratory by use of standard laboratory instruments (Hitachi 912, La Roche, Tokyo, Japan). Low-density lipoprotein (LDL) concentration was calculated according to the Friedewald formula. Plasma insulin was measured by use of immunoenzymatic chemiluminescent method (Immulite 2000, DPC).

Agreement was obtained from the Local Bioethics Committee of the Medical University of Białystok, Poland. Parents and children were informed as to the nature and purpose of the study. Parents gave their written consent; children gave their verbal consent.

2.3. Statistical analysis

Statistical analysis was performed with the use of the computer program Statistica 5.0 (StatSoft, Kraków, Poland). The Kolmogorov-Smirnov test of normality was used to test the distribution of variables. For normally distributed variables, the unpaired Student *t* test was used, and for nonnormally distributed variables, the Mann-Whitney *U* test was used to compare the differences between 2 groups. Data are presented as mean and SD. Correlations among MMP-9, TIMP-1, and other factors studied were calculated with Pearson correlation coefficient for parametric data and Spearman rank coefficient for nonparametric data. Multiple linear regression analysis was performed to verify the statistical significance of the measured parameters. In our

Table 2
Levels of MMP-9 and TIMP-1 in the study group according to hypertension, hyperlipidemia, insulin resistance, family history of CVDs, and sex

	MMP-9 (ng/mL)	*P*	TIMP-1 (ng/mL)	*P*
Hypertension				
Positive (n = 25)	635 ± 308	.04	158 ± 23	NS
Negative	450 ± 289		165 ± 43	
Hyperlipidemia				
Positive (n = 16)	545 ± 282	NS	159 ± 41	NS
Negative	560 ± 345		162 ± 20	
Insulin resistance				
Positive (n = 11)	612 ± 358	NS	156 ± 21	NS
Negative	541 ± 303		162 ± 35	
Family history of CVD				
Positive (n = 11)	525 ± 294	NS	165 ± 25	NS
Negative	557 ± 234		160 ± 35	
Sex				
Male	656 ± 335	.03	161 ± 23	NS
Female	468 ± 259		162 ± 38	

Data are presented as mean ± SD.

Table 3
Correlation analysis among MMP-9 and TIMP-1 and BMI, lipids, blood pressure, glucose, insulin, and HOMA IR score

	Study group				Obese hypertensive group (n = 25)			
	MMP-9		TIMP-1		MMP-9		TIMP-1	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
BMI	0.33	.005	0.35	.006	0.41	.001	0.33	.025
Total cholesterol	0.09	.43	0.13	.32	0.07	.58	0.15	.32
Triglycerides	0.03	.7	0.05	.67	0.03	.78	−0.24	.1
LDL cholesterol	0.1	.38	0.21	.11	0.06	.61	0.26	.07
HDL cholesterol	−0.02	.8	−0.09	.47	−0.006	.96	−0.1	.49
SBP	0.16	.17	0.11	.37	0.41	.002	0.38	.008
DBP	0.15	.18	0.22	.08	0.19	.16	0.47	.001
Fasting glycemia	−0.21	.08	0.04	.72	−0.12	.09	−0.06	.67
Fasting insulin	0.3	.013	0.02	.86	0.37	.006	0.07	.61
HOMA IR	0.15	.21	0.07	.59	0.27	.03	0.07	.62

regression model, MMP-9 and TIMP-1 were the dependent variables, and age, BMI, fasting insulin, HOMA IR, and blood pressure values were the independent variables. $P < .05$ was considered statistically significant.

3. Results

The clinical characteristics of the study groups are presented in Table 1. The obese group had higher body mass and BMI, as expected. Lipid levels, blood pressure values, fasting insulin, and HOMA IR were also found to be significantly different from that of the control group (Table 1).

MMP-9 concentration was significantly higher in obese children than in the control group (553.5 ± 311 vs 400.4 ± 204 ng/mL, respectively; $P = .02$). TIMP-1 concentration was also higher in the study group than in controls (161.2 ± 32 vs 143.1 ± 20.1 ng/mL, respectively; $P = .03$) (Fig. 1).

We found significantly higher levels of MMP-9 in obese children with accompanying hypertension than in obese normotensive patients (635 ± 308 vs 450 ± 289 ng/mL; $P = .04$). Boys had higher levels of MMP-9 than girls (656 ± 335 vs 468 ± 259 ng/mL, respectively; $P = .03$). No differences were revealed between hyperlipidemic and normolipidemic, between insulin-resistant and insulin-sensitive children, as well as between obese children with positive and negative family history of CVD. We did not find any differences in TIMP-1 levels in the groups of patients (Table 2).

Correlation analysis among MMPs and BMI, lipid levels, blood pressure values, glucose, and insulin and HOMA IR index showed significant correlations between MMP-9 and BMI ($r = 0.33$, $P = .005$), between MMP-9 and fasting insulin ($r = 0.3$, $P = .013$), and between TIMP-1 and BMI ($r = 0.35$, $P = .006$) (Table 3). Because more than half of the children studied (n = 25) had recognized hypertension, correlation analysis was performed separately for the obese hypertensive children. Statistically significant correlations were found between MMP-9 and BMI ($r = 0.41$, $P = .001$), SBP ($r = 0.41$, $P = .002$), fasting insulin ($r = 0.37$, $P = .006$), and HOMA IR index ($r = 0.27$, $P = .03$), as well as between TIMP-1 and BMI ($r = 0.33$, $P = .025$), SBP ($r = 0.38$, $P = .008$), and DBP ($r = 0.47$, $P = .001$) (Table 3; Fig. 2).

Multiple linear regression analysis was performed to verify the statistical significance of these associations. In the regression models, MMP-9 was found to be dependent on

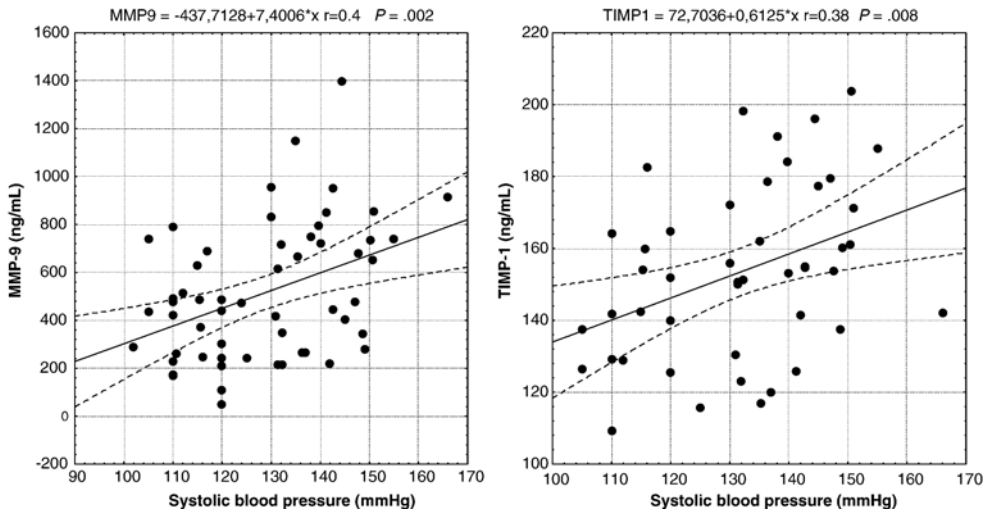

Fig. 2. Correlations between MMP-9 and TIMP-1 with SBP in the group of obese/hypertensive children.

fasting insulin (R^2 = 0.16, B = 7.2, P = .04), and TIMP-1 was dependent on BMI (R^2 = 0.14, B = 1.4, P = .04). In obese hypertensive group, TIMP-1 was revealed to be dependent on DBP (R^2 = 0.18, B = 0.9, P = .04).

4. Discussion

The main finding of this study is that obese children and adolescents, a group at increased risk of CVD, have elevated circulating levels of MMP-9 and TIMP-1. Both MMP-9 and TIMP-1 correlated positively with BMI. Data on the levels of MMPs and TIMPs in obesity in humans appear to be lacking. To our knowledge there are no data in such a young population so far.

In morbidly obese adult women (BMI, 42.5 kg/m^2) undergoing gastric banding and 1-year postsurgical treatment, a positive correlation between BMI and MMP-9 was observed, as in our study. Weight loss was associated with a pronounced decrease in the MMP-9 level [27]. Ghanim et al [28] showed elevated levels of MMP-9 messenger RNA expression in the peripheral blood mononuclear cells of obese subjects. Plasma concentration was also significantly higher, together with increased C-reactive protein, interleukin 6, and tumor necrosis factor α, known inflammatory mediators of atherosclerosis. This may suggest that MMPs might be another proinflammatory marker of the atherosclerotic process. This is especially important because mononuclear cells are known to migrate to the arterial wall to form foam cells in the atherosclerotic lesions and into adipose tissue to activate adipocytes into producing proinflammatory cytokines [28]. Recently, elevated level of MMP-9 in obese children and adolescents with type 1 diabetes mellitus was reported [29]. Experimental studies showed strongly induced messenger RNA levels for several MMPs and TIMP-1 in obese adipose tissues compared with lean tissues. MMP/TIMP balance in obesity is shifted toward increased matrix degradation [30]. It is suggested that adipose tissue expresses a large array of MMPs and TIMPs, which modulate adipocyte differentiation [31].

Another important finding of our study is that obese children with accompanying hypertension have higher MMP-9 levels compared with obese normotensive subjects. In the obese hypertensive group of patients, we showed significant correlations between MMP-9 and SBP, as well as between TIMP-1 and SBP and DBP values.

Tayebjee et al [32] found increased circulating MMP-9 and TIMP-1 levels in adult patients with untreated hypertension and postulated that it could reflect an increased deposition and retention of type I collagen at the expense of other components of the ECM within the cardiac and vascular ECM. Circulating levels of MMP-9 decreased, but TIMP-1 levels increased, after high blood pressure was treated, which was surprising [32]. Hypertension is a systemic disease with a major impact on the arterial tree, and ECM abnormalities may originate in the vascular tree rather than cardiac changes per se. Cardiac and arterial remodeling in hypertension is regarded as pathologic. Changes in tissue structure seem likely to require changes in the concentration and activity of a range of matrix enzymes. The measurement of these MMPs could be a valuable tool to assess the state of tissue composition and turnover, and this may link to clinical outcomes and prognoses.

It has been hypothesized that circulating TIMP-1 may be a marker of left ventricular fibrosis or hypertrophy, diastolic dysfunction, and heart failure, as TIMP-1 levels were found to be elevated in hypertension and correlated with left ventricle mass and indices of diastolic dysfunction. TIMP-1 is thought to increase tissue concentrations of collagen type I by preventing its breakdown by MMPs. The findings of Tayebee et al [33] add weight to a hypothesis suggesting that TIMP-1 may be a key mediator of left ventricular diastolic dysfunction.

Increasing arterial stiffness is well recognized in systolic hypertension and is associated with alterations in the ECM. Aortic stiffness is related to MMP-9 levels not only in hypertension, but also in younger, apparently healthy individuals [34]. Papadopoulos et al [35] found increased MMP-9 levels in healthy normotensive individuals with high-normal blood pressure. TIMP-1 levels were decreased. However, the role of MMPs in hypertension remains controversial because increased and decreased levels of MMP-1, MMP-2, and MMP-9 have been reported [36-38].

Although our study did not confirm differences in MMP levels between insulin-resistant and insulin-sensitive children, significant correlations were found between MMP-9 and fasting insulin in the whole group of obese children, and between MMP-9 with HOMA IR index and fasting insulin in the obese hypertensive group. This suggests that glucose/insulin metabolism alterations, known to appear in obesity and in hypertension, may influence ECM turnover.

Hyperglycemia can increase MMP-9 activity in vascular endothelial cells; however, this effect could be secondary to effects of increased insulin [39,40]. Recently, some studies in adults have reported altered levels of MMPs in disorders connected with insulin resistance. Roberts et al [41] found elevated levels of MMP-9 in men with metabolic syndrome and proved significant reductions in BMI, insulin, HOMA IR, and MMP-9 after a 3-week diet and exercise intervention. In women with polycystic ovary syndrome, the common endocrinopathy of reproductive age characterized by insulin resistance and increased prevalence of cardiovascular risk factors, elevated levels of MMP-9, MMP-2, and TIMP-1 were reported [42]. The authors concluded that elevated MMP concentrations in polycystic ovary syndrome may be related to increased cardiovascular risk in this disease.

MMP/TIMP balance in insulin resistance in obesity needs further investigation, particularly in the light of the alarming information about the dramatic increase of obesity, metabolic syndrome, and type 2 diabetes mellitus in children.

In our study we found higher levels of MMP-9 in boys than in girls. In the control group, there were no sex differences in the MMPs studied. Because all our study

subjects were in the pubertal and postpubertal age, we cannot exclude the impact of sex hormones, although if so, we should expect the differences in the control group as well. Further analysis of these differences revealed that in our study boys had higher SBP and lower HDL cholesterol levels compared with girls. We conclude that elevated MMP-9 in boys may be related to the mentioned alterations. Tayebjee et al [43] did not confirm any influence of gender on circulating levels of several MMPs in healthy adult volunteers from 4 ethnic groups. However, in the Framingham Heart Study, plasma MMP-9 was associated with echocardiographic left ventricular measures of remodeling in men but not in women [44]. Additional investigations are required to identify plausible mechanism for sex-related differences.

In conclusion, our study demonstrates elevated concentrations of MMP-9 and TIMP-1 in obese, and especially in obese/hypertensive, children and adolescents. Changes in collagen turnover of the vessels may occur early in the disease process in high-risk patients, before CVD is clinically detectable. Circulating levels of MMPs and TIMPs might in fact provide information on the existence and possibly the degree of progression of atherosclerotic disease. It is conceivable that modulation of MMP activity or the MMP/TIMP balance may be useful in the management and prevention of atherosclerosis. It may be speculated that MMPs levels underlie important and specific effects due to obesity in the network of interactions contributing to atherosclerosis. Whether the coexistence of hypertension may exacerbate this mechanism remains to be investigated.

5. Limitations

Our study has, however, certain limitations, so that conclusions should be drawn very carefully. Peripheral blood measurements of MMPs and/or TIMPs are limited by the lack of standardized analytical procedures, including the nature of tested samples (serum, heparinized plasma), the nature of the measured MMP molecule (total MMP, pro MMP), and the detection limit of commercially available assays. Ideally, correlations should be established between tissue activity and circulating MMP-9 and TIMP-1 levels. However, tissue sampling in humans, and particularly in children, is problematic. We hope that in spite of the limitations, the results of our study may contribute, although to a small extent, to the explanation of the mechanisms of early atherosclerosis in young patients with risk factors for the disease.

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