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Assessment of matrix metalloproteinase (MMP)-2, MMP-8, MMP-9, and their inhibitors, the tissue inhibitors of metalloproteinase (TIMP)-1 and TIMP-2 in obese children and adolescents

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Abstract

Objectives: To compare the circulating levels of matrix metalloproteinase (MMP)-8, pro-MMP-2, pro-MMP-9, and total MMP-9, their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMP)-1 and TIMP-2, and the MMP-8/TIMP-1, MMP-9/TIMP-1, and MMP-2/TIMP-2 ratios in normotensive obese children and adolescents with those found in non obese children and adolescents.

Design and methods: We studied 40 obese and 40 non obese (controls) children and adolescents in this cross-sectional study. MMP and TIMP concentrations were measured in plasma samples by gelatin zymography and ELISA.

Results: Obese children and adolescents had higher circulating MMP-8 concentrations, lower plasma TIMP-1 concentrations, and higher MMP-8/TIMP-1 ratios than non obese controls ($P<0.05$). We found no differences in pro-MMP-9 or total MMP-9 levels, or in MMP-9/TIMP-1 ratios between groups ($P>0.05$). While we found no significant differences in pro-MMP-2 levels ($P>0.05$) obese subjects had higher TIMP-2 concentrations and lower pro-MMP-2/TIMP-2 ratios ($P<0.05$) than non obese controls.

Conclusions: In conclusion, we found evidence indicating higher net MMP-8 (but not MMP-9 and MMP-2) activity in childhood obesity. The increased MMP-8 levels found in obese children suggest a possibly relevant pathophysiological mechanism that may be involved in the increase of cardiovascular risk associated with childhood obesity.

Keywords: Adolescents; Children; Metalloproteinases; Obesity; TIMPs

Introduction

Obesity is reaching epidemic proportions worldwide, and is now occurring at younger ages [1,2]. It is well-established that this metabolic disorder increases the risk of cardiovascular diseases leading to premature death [3]. Indeed, the adipose tissue is an active endocrine and paracrine organ that releases a large number of bioactive mediators that influence not only body weight homeostasis but also insulin resistance, circulating lipid levels, arterial blood pressure, coagulation, and inflammatory mediators that are relevant to atherosclerosis [4].

There is clear evidence that inflammatory mechanisms play a role in atherogenesis, which is a process characterized by vascular remodeling and accumulation of lipids and fibrous

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elements in the large arteries [5]. In this context, several members of the matrix metalloproteinase (MMP) family and their endogenous inhibitors, the tissue inhibitors of MMPs (TIMPs), have been implicated as primary mediators of this remodeling, and a critical equilibrium between MMPs and TIMPs must exist in order to maintain the integrity of cardiovascular system [6]. Indeed, experimental and clinical studies have shown that altered expression or activity of MMPs and/or TIMPs is an important mechanism implicated in the pathophysiology of a variety of cardiovascular diseases [7–12].

Despite the major relevance of MMPs/TIMPs to the pathophysiology of cardiovascular diseases, little information is available so far with respect to the possible alterations in MMPs/TIMPs levels in obese children and adolescents. Although two studies [13,14] by the same group described increased circulating levels of MMP-9 and TIMP-1 in obese children with coexisting hypertension, and decreased MMP-2 levels in obese children, no previous work has studied the circulating levels of TIMP-2, and the MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios in obese children and adolescents. This is important because the MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios may be better indexes of net MMP9 and MMP-2 activity, respectively, because TIMP-1 and TIMP-2 are major inhibitors of MMP-9 and MMP-2, respectively [15]. In addition, no previous study has examined the circulating levels of MMP-8 in obese children. This is of major importance because MMP-8 levels were positively associated with subclinical and clinical atherosclerosis [11,12,16], thus suggesting that circulating MMP-8 may be a marker of atherosclerosis, a condition that begins early in life and gradually progresses through adolescence and youth at an accelerated rate in children in whom risk factors are present [17].

In the present study, we aimed at comparing the plasma concentrations of MMP-8, pro-MMP-9, total MMP-9, and pro-MMP-2, as well as the plasma concentrations of TIMP-1 and TIMP-2, and MMP-8/TIMP-1, MMP-9/TIMP-1, and pro-MMP-2/TIMP-2 rations in normotensive obese children with those found in non obese normotensive children.

Methods

Subjects

Approval for use of human subjects in this cross-sectional study was obtained from the Institutional Review Board at the Federal University of Juiz de Fora, Brazil. Parents and children were informed as to nature and purpose of the study. Parents gave their written consent and children gave their verbal consent.

The study population consisted 40 normotensive obese children and adolescents (18 boys and 22 girls, aged 9.9± 1.7 years) recruited from the Endocrinology Ambulatory of the Adolescent and Child Institute at Juiz de Fora and from the Childhood Endocrinology Ambulatory of the IMEPEN Foundation at Juiz de Fora. The control group consisted of 40 healthy children and adolescents (20 boys and 20 girls, aged 10.2±1.7) recruited from local community.

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 height in meters squared. Obesity was defined as BMI greater than the 95th percentile, matched according to age and sex [18].

Systolic (SBP) and diastolic (DBP) were measured at least 3 times and the presence of hypertension was defined as SBP and/or DPB exceeding the 95th percentile [19]. Children with hypertension were not included in the study because there is evidence that hypertension may affect MMP concentrations [20].

Laboratory analyses

Glucose concentrations and lipid parameters (total cholesterol, triglycerides, high-density lipoprotein [HDL] cholesterol) were determined in plasma and serum, respectively, with routine enzymatic methods using commercial kits (Labtest Diagnostic, SA, Lagoa Santa, Brazil). Low-density lipoprotein (LDL) concentration was calculated according to the Friedewald formula.

Enzyme immunoassays of MMP-8, MMP-9, TIMP-1, and TIMP-2

Venous blood samples were collected into tubes containing EDTA in the morning before breakfast after an overnight (8–12 h) fast, centrifuged immediately at 2000 ×*g* for 10 min at room temperature and plasma samples were stored for about 3–4 months at −70 °C until analyzed. Concentrations MMP-8, MMP-9, TIMP-1, and TIMP-2 were measured in plasma using an commercially available enzyme-linked immunosorbent (ELISA) assay kits (R&D Systems, Minneapolis, MN, USA) [21,22] according to the manufacturer's instructions.

SDS-polyacrylamide gel electrophoresis (PAGE) gelatin zymography of MMP-9 and MMP-2

Gelatin zymography of MMP-9 and MMP-2 from plasma samples was performed as previously described [23–26]. Briefly, plasma samples were subjected to electrophoresis on 7% SDS-PAGE co-polymerized with gelatin (1%) as the substrate. After electrophoresis was complete, the gel was incubated for 1 h at room temperature in a 2% Triton X-100 solution, and incubated at 37 °C for 16 h in Tris–HCl buffer, pH 7.4, containing 10 mmol/L $CaCl_2$. The gels were stained with 0.05% Coomassie Brilliant Blue G-250, and then destained with 30% methanol and 10% acetic acid. Gelatinolytic activities were detected as unstained bands against the background of Coomassie blue-stained gelatin. Enzyme activity was assayed by densitometry using a Kodak Electrophoresis Documentation and Analysis System (EDAS) 290 (Kodak, Rochester, NY). The pro form of MMP-2 and MMP-9 were identified as bands at 72 and 92 kDa, respectively, by the relation of log Mr to the relative mobility of Sigma SDS-PAGE LMW marker proteins. A representative zymogram of plasma samples is shown in Fig. 1.