MMP-9 Levels and IMT of Carotid Arteries are Elevated in Obese Children and Adolescents Compared to Non-Obese
Claudio Andrade, Adriana Bosco, Valeria Sandrim, Francisco Silva
Santa Casa de Misericórdia de Belo Horizonte – Núcleo de Pós-Graduação e Pesquisa, Belo Horizonte, MG – Brazil

Abstract

Background: Childhood obesity is associated with increased risk of atherosclerosis and cardiovascular disease in adulthood. Increased intima-media thickness (IMT) of the carotid artery is linked to the initiation and progression of the chronic inflammatory processes implicated in cardiovascular disease. Matrix metalloproteinase-9 (MMP-9) plays an important role in the degradation of the extracellular matrix and, consequently, in the development, morphogenesis, repair and remodeling of connective tissues.

Objectives: (i) to determine and compare the concentrations of MMP-9, tissue inhibitor of metalloproteinase -1 (TIMP-1), and MMP-9/TIMP-1 ratio in obese and non-obese children and adolescents; (ii) to investigate the association of these markers with common and internal IMT of carotid arteries.

Methods: Cross-sectional study involving 32 obese and 32 non-obese (control) individuals between 8 - 18 years of age.

Results: Significantly (p < 0.05) higher values of MMP-9 concentration, as well as a higher MMP-9/TIMP-1 ratio were detected in the obese group compared to control counterparts. Common and internal carotid IMT values were significantly higher (p < 0.001) in the obese group compared to the control group. Positive correlations were observed between the common carotid IMT values and MMP-9 concentrations as well as MMP-9/TIMP-1 ratio.

Conclusions: Our data demonstrate that obese children and adolescents present higher mean IMT values, plasma MMP-9 and MMP-9/TIMP-1 ratio compared to the non-obese. Thus, these findings indicate that this group presents a risk profile for early atherosclerosis. (Arq Bras Cardiol. 2017; 108(3):198-203)

Keywords: Pediatric Obesity; Biomarkers; Atherosclerosis; Tissue Inhibitor of Metalloproteinase

Introduction

Childhood obesity is a major health problem because of its association with an increased risk of atherosclerosis and cardiovascular disease in adulthood. Obesity is correlated to an increased intima-media thickness (IMT) of the carotid artery, which, in turn, is linked to the initiation and progression of chronic inflammatory processes implicated in cardiovascular disease. The increase in carotid IMT starts during childhood, and nearly all children present fat deposits in these arteries by the age of three. A study by Dawson et al., with 635 adolescents and young adults, has shown that carotid IMT is significantly correlated to coronary artery risk scores; therefore, early assessment of this parameter through non-invasive methods may assist in the identification of individuals most at risk of cardiovascular disease.

Matrix metalloproteinase-9 (MMP-9) plays an important role in the degradation of the extracellular matrix and, consequently, in the development, morphogenesis, repair and remodeling of connective tissues. Since MMP-9 activity is regulated primarily by tissue inhibitor of metalloproteinase-1 (TIMP-1), an imbalance between MMP-9 and TIMP-1 could lead to the uncontrolled degradation of extracellular matrix as seen in various pathological disorders, including cardiovascular diseases. Thus, some studies in adults have correlated IMT values and circulating MMP-9/TIMP-1 concentrations; however, to our knowledge, no study has evaluated these correlations in children and adolescents. Also, increased IMT values of the carotid artery are linked to chronic inflammatory processes in cardiovascular disease, and this process involves the activation of MMP-9.

Therefore, we hypothesized that obese children and adolescents present higher concentrations of plasmatic MMP-9 and MMP-9/TIMP-1 ratio compared to the non-obese group, and that these concentrations are positively correlated to IMT values of common and internal carotid arteries. Thus, the aim of this study was to compare plasma MMP-9 and TIMP-1 levels and correlate these concentrations to IMT values of common and internal carotid arteries in obese and non-obese children and adolescents.
Methods

Study population and experimental design
Details of the cross-sectional study were presented to and approved by the Ethics Committee of the Hospital Santa Casa de Misericórdia in the city of Belo Horizonte (Belo Horizonte, MG, Brazil). Written informed consent was obtained from all participants and/or their legal guardians prior to the investigation.

Potential participants were recruited in the outpatient clinic of the Division of Endocrinology and Metabolism of Santa Casa de Misericórdia in the city of Belo Horizonte and included males and females between 8 and 18 years of age. Individuals presenting hypertension, metabolic, endocrine, autoimmune, neoplastic and infectious diseases were excluded from the study. Participants were assessed as obese (n = 32) or non-obese (n = 32; control group) according to their body mass index (BMI) referenced against the 2000 Centers for Disease Control and Prevention (CDC) sex-adjusted BMI-for-age growth charts with the cut-off point for obesity taken as ≥ 95th percentile.17 Hypertension was defined by the “VI Diretrizes de Hipertensão Arterial da Sociedade Brasileira de Cardiologia” (VI Arterial Hypertension Guidelines from the Brazilian Society of Cardiology) and for children and adolescents, it was based on percentiles. Obese and non-obese groups were not on any medication. A minimum sample size of 23 individuals per group was calculated considering an alpha error of 0.05% and a test power of 90%. Data were collected between March 2010 and March 2012.

Anthropometrical, clinical and biochemical evaluations
Anthropometrical (weight, height and BMI), clinical (carotid IMT) and biochemical (TSH, MMP-9, TIMP-1, MMP-9/TIMP-1 ratio) parameters were collected for all selected individuals. Anthropometric measurements were performed with participants barefoot and with light clothes. Body weight was measured using portable digital scales (capacity 180 kg; sensitivity 100 g), while height was determined by portable stadiometer (non-extendable 2 m measuring tape graduated in 0.1 cm divisions) with the subject in the orthostatic position. Systolic (SBP) and diastolic (DBP) blood pressures were measured at least three times after 15 min of rest and hypertension was defined as SBP and/or DBP exceeding the 95th percentile. Hypertension was defined as SBP and/or DBP exceeding the 95th percentile.19

Serum TSH was estimated with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Quibasa Química Básica, Belo Horizonte, MG, Brazil). Plasma was collected in tubes containing EDTA as anticoagulant. MMP-9 and TIMP-1 tests were performed using human MMP-9/TIMP-1 complex DuoSet kit (R&D Systems, Minneapolis, MN, USA).

IMT measurements
Common carotid artery: average measurement of the thickness on both sides, longitudinal projection, exactly 1 cm before the bifurcation. Internal carotid artery: average measurement of the thickness on both sides, longitudinal projection at the origin.

Results
Clinical and biochemical characteristics of subjects enrolled in study are shown in Table 1. Although both groups exhibited serum TSH values within the normal range, the mean value of this parameter in the obese group was significantly higher (p < 0.05) than that recorded in the non-obese group (2.7 ± 0.8 vs 2.0 ± 0.8 µIU/mL, p < 0.05).

Plasma MMP-9 concentrations were significantly higher in the obese group compared to the non-obese group (p < 0.05), while plasma TIMP-1 concentrations were similar (p > 0.05) in both groups. Mean MMP-9/TIMP-1 ratio was significantly higher (p < 0.05) in the obese group in comparison to the non-obese. Mean IMT values of the common and internal carotid arteries of obese individuals were significantly greater (p < 0.001) than those of their control counterparts.

There was a direct and statistically significant correlation among plasma MMP-9, MMP-9/TIMP-1 ratio, and IMT values of the common carotid artery (p = 0.02 and p = 0.04, respectively: Figure 1A and E). In contrast, there was no significant correlation between plasma TIMP-1 and IMTs of common and internal carotid arteries (Figure 1C and D) or MMP-9 and IMT of internal carotid arteries (Figure 1B).

Discussion
To our knowledge, this is the first study to correlate plasma MMP-9 and TIMP-1 levels to common and internal IMT in obese and non-obese children and adolescents. Following an evaluation of matrix metalloproteinases in obese and non-obese children and adolescents, Glowińska-Olszewska et al.12 reported high concentrations of the atherosclerosis marker MMP-9 in the obese group and even higher concentrations in hypertensive obese individuals. These authors argued that the abnormally high concentrations of MMP-9 could indicate modifications in the metabolism of the extracellular matrix of blood vessels and heart muscle, and that such alterations could speed up the atherosclerotic process. Additionally, the same research team described...
Table 1 – Demographic, anatomical and biochemical characteristics of obese and non-obese children and adolescents recruited in the outpatient clinic of the Division of Endocrinology and Metabolism of Santa Casa de Misericórdia de Belo Horizonte (Belo Horizonte, MG, Brazil).

| Variable                | Obese group [n = 32] | Non-obese group [n = 32] |
|-------------------------|-----------------------|--------------------------|
|                         | Minimum | Maximum | Mean/% | SD | Minimum | Maximum | Mean/% | SD |
| Age [years]             | 8       | 17      | 13     | 2  | 12      | 18      | 15*    | 2  |
| Height [m]              | 1.28    | 1.79    | 1.57   | 0.13 | 1.52    | 1.84    | 1.63*   | 0.08 |
| Weight [kg]             | 47      | 120     | 73     | 17 | 35      | 71      | 56*    | 9  |
| BMI [kg/m²]             | 26      | 40      | 29     | 5  | 15      | 23      | 22*    | 2  |
| SBP (mmHg)              | 90      | 120     | 103    | 6  | 90      | 110     | 103    | 6  |
| DBP (mmHg)              | 50      | 70      | 60     | 7  | 50      | 80      | 63     | 7  |
| Gender (% Female)       | -       | -       | 59     | -  | -       | -       | 47     | -  |
| TSH [μIU/mL]            | 1.5     | 4.6     | 2.7    | 0.8 | 0.7     | 4.2     | 2.0*   | 0.8 |
| Common carotid IMT [mm] | 0.38    | 0.58    | 0.45   | 0.04 | 0.38    | 0.45    | 0.42*   | 0.02 |
| Internal carotid IMT [mm]| 0.36    | 0.46    | 0.42   | 0.03 | 0.37    | 0.44    | 0.40*   | 0.02 |
| MMP-9 [ng/mL]           | 127     | 1208    | 343    | 249 | 92      | 925     | 246*   | 151 |
| TIMP-1 [ng/mL]          | 322     | 1165    | 677    | 214 | 207     | 1522    | 709    | 284 |
| MMP-9/TIMP-1 ratio      | 0.15    | 1.47    | 0.48   | 0.25 | 0.11    | 1.59    | 0.41*   | 0.31 |

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TSH: thyroid-stimulating hormone; IMT: intima-media thickness; MMP-9: metalloproteinase-9; TIMP-1: tissue inhibitor of metalloproteinase-1; SD: standard deviation. * Significant differences p < 0.05 compared to obese group.

that MMP-9 and TIMP-1 concentrations were elevated in obese children and adolescents, and that the values of these parameters increased even further when obesity was accompanied by hypertension. Moreover, Belo et al. reported that genotypes and haplotypes of MMP-9 gene modulate circulating MMP-9 levels in obese children and adolescents. In the present study, plasma MMP-9 and the ratio MMP-9/TIMP-1 were significantly higher in obese individuals compared to their control counterparts, but the two groups presented no statistical difference in plasma TIMP-1. Although weak, it was possible to demonstrate a direct relationship between the concentrations of MMP-9 and MMP-9/TIMP-1 ratio, but not those of TIMP-1 and IMT values of common carotid arteries, suggesting a potential participation of this gelatinase in artery remodeling. Furthermore, no such relationship could be established with internal carotid IMT. This difference of correlations could be explained by the magnitude of the IMT of the internal carotid that is lower than that of the common carotid; therefore, the difference of magnitude may have interfered in the correlation. It is important to note that plasma MMP-9 concentrations reflect the systemic MMP-9 production and not only the vascular production, which may reduce the magnitude of correlations between this biomarker and IMT.

In the present study, mean IMT values of the common and internal carotid arteries of the obese group (0.47 and 0.43 mm, respectively) were significantly increased (p < 0.001) compared to those of the control group (0.42 and 0.40 mm, respectively); a result that is in agreement with previous reports. Thus, in a case-control study carried out in Belgium by Beauloye et al., involving healthy subjects between 8 and 18 years of age, the mean value of carotid IMT of the obese group (0.470 mm) was significantly greater than that of the non-obese control group (0.438 mm), even though the mean age of the two groups did not differ significantly. Furthermore, these authors were able to demonstrate a significant positive correlation between carotid IMT and relative BMI. Moreover, studying Brazilian adolescents, Silva et al. demonstrated, in 35 obese and 18 non-obese subjects between 10-16 years old, that cIMT, triglycerides, HOMA-IR, insulin, and CRP values were higher, while high-density lipoprotein cholesterol (HDL-c), adiponectin, and VO₂max values were lower in the obese group than in the non-obese group.

Based on mean IMT values of the common carotid artery determined in the obese and control groups in the present study, a cut-off point of 0.44 mm was established. A sonographic evaluation of common carotid and femoral arteries of 247 healthy subjects between 10 and 20 years of age revealed that mean IMT values increased almost linearly from 0.38 to 0.40 mm with increasing age. Since the adopted cut-off point was considerably higher than the value previously ascribed to healthy individuals in the age range 18 to 20 years of age, it is possible to state that children and adolescents comprising the obese group in the present study exhibited abnormally increased carotid IMT values. Moreover, it was possible to estimate from the data obtained that the risk of the obese group exhibiting elevated common carotid IMT was 2 to 5 times higher than that of the control group, while the risk of increased internal carotid IMT was 1.5 to 4 times greater.

Non-invasive techniques are reliable tools for identifying adults with increased risk of atherosclerosis and cardiovascular risk, but for children and adolescents, such techniques have been reserved mainly for research purposes. Ultrasound imaging appears to be a reliable technique to estimate IMT values of human arteries in vivo, since Pignoli et al. were able to confirm that there were no significant differences between B
mode-determined IMTs of the common carotid arteries evaluated in pathogenic examination and those evaluated in vivo in young subjects. Moreover, while the analysis of IMT has often been used in cross-sectional studies, only a few clinical trials with children have employed this parameter. The Cardiovascular Risk in Young Finns study, which comprised a 21 year follow-up longitudinal investigation, suggested that obesity indices, such as BMI, skinfold, serum lipoproteins, insulin, glucose and blood pressure, measured in youth, are significantly associated to increased IMT and decreased elasticity of the carotid artery in adulthood. These findings emphasize the importance of weight control from youth to adulthood in reducing cardiovascular risk.

Although mean TSH value of the obese group was higher than that of the control group (2.85 versus 1.98 µIU/mL), no cases of hypothyroidism were diagnosed in obese participants. Conventionally, a serum TSH concentration of 4 to 5 µIU/mL is considered elevated; however, recent data from large population studies have indicated that a lower TSH cut-off point in the region of 2 to 2.5 µIU/mL would be more appropriate. Likewise, the National Academy of Clinical Biochemistry has recommended an upper limit of 2.5 µIU/mL for serum TSH, a value that is below the mean concentration of the obese group determined in the present study. However, it is not possible to state with certainty that cases of subclinical hypothyroidism were absent within the obese group of the present study.

Figure 1 – Correlations among biomarkers [MMP-9 (A,B), TIMP-1(C,D) and MMP-9/TIMP-1 ratio (E,F)] and common (A,C,E) and internal (B,D,F) carotid IMT. The correlations among plasma biomarkers and common and internal carotid IMT were analyzed using Spearman’s correlation.
In addition, numerous studies have revealed a positive association between measures of obesity and serum thyroid-stimulating hormone (TSH) concentrations, although the mechanisms responsible for this association require further elucidation, it is proposed that variations in thyroid hormone could affect lipoproteins and oxidation steps contributing to vascular remodeling and endothelial function. Interestingly, a significant correlation has also been demonstrated between carotid IMT and TSH values within normal reference values, suggesting an increased cardiovascular risk in subjects with low normal thyroid function. Yap and Jasul found a positive correlation between serum TSH and BMI, and inferred that an increase in TSH concentration, even within the generally accepted limits, could contribute to weight problems. The present study demonstrated that the group of obese children and adolescents exhibited increased TSH concentrations, although the concentrations were within the normal range, similarly to findings previously reported by Aypak et al. However, this problem clearly requires further investigation since hypothyroidism may be associated with markers of atherosclerosis and, consequently, with increased carotid IMT. A limitation of our study is the small number of subjects enrolled.

Conclusion
Our data demonstrate that obese children and adolescents present higher mean IMT values, plasma TSH, plasma MMP-9 and MMP-9/TIMP-1 ratio compared to the non-obese. Thus, these findings indicate that this group presents a risk profile for early atherosclerosis.

Acknowledgements
The study was financed by IEP – Santa Casa de Misericórdia de Belo Horizonte, MG, Brazil and Fundação de Amparo à Pesquisa do Estado de Minas Gerais.

Author contributions
Conception and design of the research: Andrade C; Acquisition of data: Bosco A, Sandrim V; Analysis and interpretation of the data: Andrade C, Bosco A, Sandrim V, Silva F; Statistical analysis: Bosco A, Sandrim V; Writing of the manuscript: Andrade C, Bosco A, Sandrim V; Critical revision of the manuscript for intellectual content: Silva F.

Potential Conflict of Interest
No potential conflict of interest relevant to this article was reported.

Sources of Funding
This study was Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Study Association
This article is part of the thesis of master submitted by Claudio Andrade, from Santa Casa de Misericórdia de Belo Horizonte.

References
1. Oren A, Vos LE, Uiterwaal CS, Gorissen WH, Grobbee DE, Bots ML. Change in body mass index from adolescence to young adulthood and increased carotid intima-media thickness at 28 years of age: the Atherosclerosis Risk in Young Adults study. Int J Obes Relat Metab Disord. 2003;27(11):1383-90. 2. Iannuzzi A, Licenziai MR, Acampora C, Renis M, Agrusta M, Romano L, et al. Carotid artery stiffness in obese children with the metabolic syndrome. Am J Cardiol. 2006;97(4):528-31. 3. Heiss G, Sharrett AR, Barnes R, Chambless LE, Szklo M, Alzola C. Carotid atherosclerosis measured by B-mode ultrasound in populations: associations with cardiovascular risk factors in the ARIC study. Am J Epidemiol 1991;134(3):250-6. 4. Davis PH, Dawson JD, Riley WA, Lauer RM. Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: the Muscatine Study. Circulation. 2001;104(23):2815-9. 5. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation. 2007;115(4):459-67. 6. Davis PH, Dawson JD, Riley WA, Lauer RM. Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: the Muscatine Study. Circulation. 2001;104(23):2815-9. 7. Bosco A, Sandrim V, Silva F, Andrade C. Childhood markers of inflammation and cardiovascular disease: a systematic review and meta-analysis. Arq Bras Cardiol. 2017;108(3):198-203.
16. Tan C, Liu Y, Li W, Deng F, Liu X, Wang X, et al. Associations of matrix metalloproteinase-9 and monocyte chemoattractant protein-1 concentrations with carotid atherosclerosis, based on measurements of plaque and intima-media thickness. Atherosclerosis. 2014;232(1):199-3.

17. Daniels SR, Khoury PR, Morrison JA. The utility of body mass index as a measure of body fatness in children and adolescents: differences by race and gender. Pediatrics. 1997;99(6):804-07.

18. Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999-2000. JAMA. 2002;288(14):1728-32.

19. Update on the 1987 task force report on high blood pressure in children and adolescents: a working group report from the National High Blood Pressure Education Program. National high blood pressure education program working group on hypertension control in children and adolescents. Pediatrics. 1996;98(4 Pt 1):649-58.

20. Urbina EM, Williams RV, Alpert BS, Collins SR, Hayman L, et al; American Heart Association Atherosclerosis, Hypertension, and Obesity in Youth Committee of the Council on Cardiovascular Disease in the Young. Noninvasive assessment of subclinical atherosclerosis in children and adolescents: recommendations for standard assessment for clinical research: a scientific statement from the American Heart Association. Hypertension. 2009;54(5):919-50. Erratum in: Hypertension. 2010;56(3):e36.

21. Belo VA, Souza-Costa DC, Luizão MR, Lanna CM, Carneiro PC, Izidoro-Toledo TC, et al. Matrix metalloproteinase-9 genetic variations affect MMP-9 levels in obese children. Int J Obes (Lond). 2012;36(1):69-75.

22. Iannuzzi A, Licenziati MR, Acampora C, Salvatore V, Auriemma L, Romano ML, et al. Increased carotid intima-media thickness and stiffness in obese children. Diabetes Care. 2004;27(10):2506-8.

23. Beauloye V, Zech F, Tran HT, Clapuyt P, Maes M, Brichard SM. Determinants of early atherosclerosis in obese children and adolescents. J Clin Endocrinol Metab. 2007;92(8):3025-32.

24. Silva LR, Cavagliera C, Lopes WA, Pizzi J, Coelho-e-Silva MJ, Leite N. Endothelial wall thickness, cardiorespiratory fitness and inflammatory markers in obese and non-obese adolescents. Braz J Phys Ther. 2014;18(1):47-55.

25. Jourdan C, Wühl E, Litwin M, Fahr K, Trelewiecz J, Jobs K, et al. Normative values for intima-media thickness and distensibility of large arteries in healthy adolescents. J Hypertens. 2005;23(9):1707-15.

26. Pigoli P, Tornelli E, Poli A, Oreto P, Padletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation. 1986;74(6):1399-406.

27. Raitakari OT, Juonala M, Viikari JS. Obesity in childhood and vascular changes in adulthood: insights into the Cardiovascular Risk in Young Finns study. Int J Obes (Lond). 2005;29 Suppl 2:S1-4.

28. Brabant G, Beck-Peccoz P, Jazalb B, Lauberg P, Orgiazzi J, Szabolcs I, et al. Is there a need to redefine the standard upper limit of TSH? Eur J Endocrinol. 2006;154(5):633-7.

29. Zöphel K, Wunderlich G, Kotzerke J. Should we really determine a reference population for the definition of thyroid stimulating hormone reference interval Clin Chem. 2006;52(2):329-30.

30. de Moura Souza A, Sichieri R. Association between serum TSH concentration within the normal range and adiposity. Eur J Endocrinol. 2011;165(1):11-5.

31. Takamura N, Akishimino A, Hayashida N, Kadoya K, Yamasaki H, Usu T, et al. Thyroid function is associated with carotid intima-media thickness in euthyroid subjects. Atherosclerosis. 2009;204(2):e77-81.

32. Yap SE, Jaul G. Correlation of thyroid-stimulating hormone concentrations with body mass index among adult patients seen at the weight management center in a tertiary hospital. Philippine J Internal Med. 2011;50(2):1-6.

33. Aypak C, Türedi O, Yüce A, Görgülenolgu S. Thyroid-stimulating hormone (TSH) concentration in nutritionally obese children and metabolic co-morbidity. J Pediatr Endocrinol Metab. 2013;26(7-8):703-8.

34. Valentina VN, Marijan B, Chedo D, Branka K. Subclinical hypothyroidism and risk to carotid atherosclerosis. Arq Bras Endocrinol Metabol. 2011;55(7):475-80.

35. Gunduz M, Gunduz E, Kircelli F, Okur N, Ozkaya M. Role of surrogate markers of atherosclerosis in clinical and subclinical thyroidism. Int J Endocrinol. 2012;2012:109797.