Carotid intima-media thickness and metabolic syndrome in a rural population: Results from the Baependi Heart Study

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Keywords: Carotid intima-media thickness (cIMT) is a strong predictor of cardiovascular events and associated with metabolic syndrome (MetS). MetS is a cluster of cardiovascular risk factors, but the association structure between specific factors and disease development is not well-established in rural populations. We described the association structure between MetS factors and cIMT in a sample from rural Brazil.

Methods: We studied 1937 participants from the Baependi Heart Study who underwent carotid ultrasound exam. We used ATP III-2001 for MetS definition and linear mixed-effects models, adjusting by the family structure, to assess independent associations between the cardiovascular risk factors which define MetS and cIMT.

Results: The sample's mean age was 46 ± 16y, 61% female, 73% white, mean body-mass-index 26±5 kg/m², mean cIMT 0.53 ± 0.16 mm, with 35% of the sample classified with MetS. As expected, cIMT demonstrated a linear relationship with increasing age, and cIMT higher values were observed for MetS (0.58 ± 0.16 mm) compared to non-MetS (0.49 ± 0.14 mm). Considering models for cIMT with MetS and all of its factors, we found that blood pressure, glucose and obesity were independently associated with cIMT, but not HDL or triglycerides.

Conclusions: cIMT showed a linear relationship with increasing age. Blood pressure, obesity, and glucose were independently associated with cIMT, but not HDL-cholesterol or triglycerides. In a rural population, hypertension, diabetes and obesity play a more important role than lipids in determining cIMT interindividual variability.

1. Introduction

Carotid intima-media thickness (cIMT) is a traditional marker of atherosclerosis and it is a strong predictor of future cardiovascular events, especially acute myocardial infarction and stroke [1–5]. It is measured by a non-invasive ultrasound imaging method, easy to apply in epidemiological studies [4]. In addition, cIMT has been used as an early surrogate marker for atherosclerosis [3]. Indeed, a recently published systematic review showed that cIMT was higher in subjects with cardiovascular disease (CVD) compared to individuals free of CVD [6]. However, there is still a challenge of cIMT and risk association based on a non-linear relationship with vascular events in young individuals [1]. Furthermore, it has been shown that cIMT did not improve cardiovascular risk prediction in patients with elevated blood pressure [7].

In this context of uncertainty of the role of cIMT in CVD risk prediction [7,8], there is increased awareness of the context-dependent association of cardiovascular risk factors and markers of subclinical atherosclerosis, like cIMT. Recently, it has been shown that body-mass-index (BMI), a measure of obesity, is increasing faster in rural areas than in cities, contrary to the knowledge that urbanization is the most important driver of the global obesity epidemic in adults [9]. As a result, these trends pointed to a reversal of the gap in BMI between urban
and rural areas, especially in low- and middle-income countries (LMIC) and for women [9].

Metabolic syndrome (MetS) is a cluster of cardiovascular risk factors. It is characterized by a combination of elevated blood pressure, hyperglycemia, high-density lipoprotein (HDL) cholesterol levels, elevated triglycerides, and abdominal obesity [10–12], but the association structure between its specific factors and cardiovascular disease is unclear for it varies depending of other situational variables. In fact, there is growing concern on how the combined effects of traditional cardiovascular risk factors (such as those part of MetS), which have been associated with carotid atherosclerosis in the general population [10,13], modulate cardiovascular risk in different populations.

Whether the association between the different cardiovascular risk factors part of MetS and established vascular disease is different in rural populations from LMIC is unknown. Therefore, in the presence of controversies about the specific role of the cIMT in atherosclerosis disease development, and based on the strong association between cIMT and MetS [10,13], the aim of the current study is to evaluate the shape of the relationship between cIMT and age in subjects from a rural family-based population, and also to explore the independent association between cIMT and traditional cardiovascular risk factors part of the MetS definition. Here we describe the association structure between MetS factors and cIMT in a representative sample from rural Brazil.

2. Material and Methods

2.1. Study population

The Baependi Heart Study is an epidemiological study in Baependi, a city in a rural area (752 Km², 18,307 inhabitants at the 2010 census) located in Minas Gerais State, Brazil (21.95 S, 44.88 W). The overarching goal of this cohort is to evaluate genetic and environmental influences on cardiovascular risk factors. The initial data collection phase occurred between December 2005/January 2006, and one hundred and nine districts were randomly selected (from a total of twelve) were selected for study. Second, residential addresses within each district were randomly selected (by first randomly selecting a street, second a household). Finally, eligibility criteria (any individual living in the selected household who was 18 years old or above) within each household were established. Then, in 2010 during the first follow-up visit, 2239 individuals from the same families participated in the protocol. Details on the methodology of the original study have been previously published [14,15]. This current study is a cross-sectional analysis of data collected at the second evaluation visit (from 2010 to 2015) on subjects that underwent carotid ultrasonography. Individuals that presented angina, infarct, cardiac insufficiency and revascularization were removed from our analysis. We considered only individuals with complete data for all variables used in our analysis (n = 1937 subjects).

The study protocol was approved by the ethics committee of the Hospital das Clínicas (SDHC: 3485/10/074), University of São Paulo (USP), Brazil, and each subject provided informed written consent before participation.

2.2. Covariates

Demographics and anthropometrics were assessed through questionnaires and following a standard protocol by trained technicians, respectively. Height was measured in centimeters and weight in kilograms using a calibrated digital balance and BMI was calculated as body weight (Kg) divided by height squared (m²). Waist circumference was measured at the mean point between the lowest rib margin and the iliac crest with the subject standing [14,16]. Blood pressure (BP) was measured using a digital sphygmomanometer (OMRON, Brazil) on the left arm after 5 min rest, in the sitting position. Systolic BP (SBP) and diastolic BP (DBP) were calculated as an average of three readings [16]. After calculation of blood pressure values, we adjusted for medication usage by adding 15 and 10 mmHg to SBP and DBP, respectively, for individuals reported to be taking anti-hypertensive drugs [17]. Blood samples, after 12 h fasting, were collected and analyzed for lipid and glucose profiles. For lipid profile, we assessed total cholesterol, low-density lipoprotein (LDL) cholesterol, HDL cholesterol associated and triglycerides.

Based on the fact that we would like to explore the association of the cIMT and the presence or absence of MetS, we classified our analytical sample according to the MetS definition following experts classification from the National and Cholesterol Education Program—Third Adult Treatment Panel (NCEP ATP III) [18], which is the recommended classification by the Brazilian Society Guideline [19]. The ATP III 2001 suggests any three out of five features: abdominal obesity by waist circumference (≥102 cm for male and ≥88 cm for female); triglycerides (≥150 mg/dL); HDL cholesterol (<40 mg/dl for male and <50 mg/dL for female); fasting blood glucose level (≥110 mg/dL); systolic BP (≥130 mmHg) and diastolic BP (≥85 mmHg) [18].

Smoking status was collected by a questionnaire using the following question: “Did you already smoke cigarettes?” (1) Yes, in the past, but not currently; (2) Yes, and I still smoke; (3) I do not smoke. The three choices were then characterized as (1) former, (2) current, and (3) non-smokers, respectively, as previously published [20].

2.3. cIMT ultrasound imaging

The technique used to measure and calculate cIMT was to measure a double line with the definition of the light-intima and media- adventitia interfaces of the vessel. The distance between the two acoustic interfaces was considered the cIMT measure [21,22]. Measurements with reference to the light-intima and media-adventitia interfaces of the vessel were standardized using the Philips Envisor HD7 ultrasound equipment with a linear 7.5 MHz transducer.

Three measurements of the cIMT were performed on each side, starting 1.0 cm below the upper limit of the image (1.0 cm below the carotid bifurcation), using the posterior (distal) wall of the common carotid artery, with a 5 mm spacing between them. For each side, we performed the arithmetic mean of the measurements [23,24]. cIMT was calculated by the mean of the three measurements performed on the walls of the distal carotid, using Osirix™ software. In addition, carotid bifurcation was studied at 4.0 cm for plaques. Images of common carotids acquired and documented in a 4.0 cm length starting at the carotid bifurcation. When plaques occurred that did not allow the measurement, 1.0 cm below the upper limit of the image, the measurement was performed immediately after the plaque. The atheromatous plaque was defined as a focal structure that extends at least 0.5 mm to the vessel lumen or measures more than 50% of the adjacent cIMT measurement value or a measurement greater than 1.5 mm [23,24].

2.4. Statistical analysis

Descriptive data on demographics, lipid and glucose blood profiles, anthropometric measures and blood pressure are shown as mean and standard deviation for continuous variables and percentage for categorical ones. Significant differences in descriptive data between males and females were assessed using a linear mixed-effects model (LMM), adjusting by the family structure, for continuous variables and a mixed logistic regression for categorical variables assuming a p-value < 0.05.

Initially, we explored the correlation of cIMT with the well-known cardiovascular risk factors, such as age, BMI, and smoking status, as well as the MetS components separately, each one at a time. A LMM was subsequently used to analyze the association between each risk factor and cIMT taking into account family structure in the random part of the model.
Further, to test the independent association of cIMT with cardiovascular risk factors, we developed two different LMMs, one with MetS data (as a binary variable) and a second with its individual components.

Some risk factors that define MetS are highly correlated with each other and this can inflate the variances of the regression coefficients and impair the statistical power. To address this issue, we ran an exploratory factor analysis (EFA) model and a confirmatory factor analysis (CFA) model. The latent variables (factors) resulting from these analyses and the independent factors were used to fit the multiple LMM to study the independent association with cIMT. Models were corrected for age, sex, smoking status, and statin use, all well-known confounders in the context of atherosclerosis [4, 25]. To check factors and well-known confounders for multicollinearity, we assessed the variance inflation factor (VIF) in the LMM.

Univariate analysis was conducted to test the difference in means between cIMT and groups with MetS vs without MetS. We used the kinship 2 package in R (version 3.5.2) to adjust models for family structure, dlokr for exploratory analysis, coxme for univariate and multiple linear regression, stats and psych packages for exploratory factorial analysis, lavaan to confirm factorial analysis using structural equations analysis and performance to VIP analysis. The alpha level of significance was set as <0.05.

### Results

Table 1 displays results for the full analytical sample that underwent carotid ultrasound for cIMT measurement (n = 1937). In general, it is a young sample with mean age of 46 ± 16y, most female (61%), 73% white, 66% never-smoking, 8% on statin, 6% on oral hypoglycemic, and 28% on anti-hypertensive medication. Thirty five percent of the sample was classified with MetS (Supplemental Table 1S), following the ATP-III 2001 definition [18]. Table 1 also shows sex differences. Interestingly, despite several differences in the risk profile between males and females, cIMT values were quite similar between sex groups.

Considering Person’s correlation, age was the strongest variable, among all covariates, correlated to cIMT with a coefficient of 0.57 (p < 0.001) (Supplemental Table 2S). The relationship between age and cIMT was linear as shown in Supplemental Fig. 1S. We also conducted a univariate analysis checking separately all covariates, including the MetS components, and its association with cIMT, but accounting for family structure in LMM. All covariates were significantly associated with cIMT, except for HDL-cholesterol (Supplemental Table 3S).

Since several covariates, including variables used to define MetS, are highly correlated (Supplemental Table 4S), we conducted an exploratory factorial analysis to determine how many factors explain most of the phenotypic variance associated with the tested cardiovascular risk factors. We derived four distinct factors: the first one represents waist circumference and BMI, the second total cholesterol and LDL cholesterol, the third SBP and DBP and the last one represents glucose and glycated hemoglobin. HDL cholesterol and triglycerides were not represented in any factor (Supplemental Fig. 2S). Following structural equation analysis, we observed that the factors fit as latent variables representing the cited covariates (p-value of chi-square test < 0.0001). Each of these factors, as well as HDL-cholesterol and triglycerides, was tested independently for their association with cIMT using a multiple LMM, adjusted by age, sex, smoking status, statin use and familial structure. Only factors 1, 3 and 4 were significantly associated with cIMT (Supplemental Table 5S).

### Table 2

Linear mixed model analysis testing the independent association between cIMT (outcome) and traditional cardiovascular risk factors (exposures).

| Beta coefficient | se | p-value | VIF |
|------------------|----|---------|-----|
| Intercept        | 0.29341 | 0.01005 | <0.0001 |
| Factor 1         | 0.00765 | 0.00341 | 0.0251 | 1.35 |
| Factor 2         | 0.00999 | 0.00329 | 0.0024 | 1.34 |
| Factor 3         | 0.01040 | 0.00408 | 0.0107 | 1.34 |
| Age, yo          | 0.00505 | 0.000203 | <0.0001 | 1.20 |
| Smoking status   | 0.00069 | 0.00733 | 0.9250 | 1.12 |
| Smoking status   | -0.00085 | 0.00893 | 0.9240 | 1.12 |
| Statin (factor = yes) | -0.01690 | 0.01071 | 0.1147 | 1.08 |
| Sex (factor = male) | -0.00625 | 0.00597 | 0.2954 | 1.06 |

Adjustments for traditional confounders in the context of the cIMT and cardiovascular diseases were forced in the model independent of results from the univariate analysis: age, sex (reference = female), smoking status (reference = never), and statin use. cIMT = carotid intima-media thickness, se = standard error; LDL = low-density lipoprotein; BMI = body mass index. Factor 1 composed of waist circumference and BMI; Factor 3 composed of systolic blood pressure and diastolic blood pressure; Factor 4 composed of glucose and glycated hemoglobin. VIF - Variance Inflation Factor. Values in bold highlights the results those are statistically significant.
Table 3
Multiple linear mixed model analysis testing the independent association between cIMT (outcome) and MetS.

|                      | Beta coefficient | se     | p-value | VIF |
|----------------------|------------------|--------|---------|-----|
| (Intercept)          | 0.26698          | 0.00881| <0.0001 | 1.23|
| Age, yo              | 0.00547          | 0.02164| <0.0001 | 1.11|
| MetS (factor – yes)  | 0.02164          | 0.00642| 0.0008  | 1.10|
| Smoking status       | 0.00042          | 0.00722| 0.9554  | 1.11|
| Smoking status       | –0.00279         | 0.00881| 0.7514  | 1.11|
| Statin (factor – yes)| –0.01410         | 0.01048| 0.1786  | 1.07|
| Sex (factor – male)  | –0.00187         | 0.00588| 0.7504  | 1.06|

Adjustments for traditional confounders in the context of the cIMT and cardiovascular diseases were forced in the model independent of results from the univariate analysis: age, sex (reference = female), smoking status (reference = never), and statin use. Metabolic syndrome (MetS) was defined by any three out of five features: abdominal obesity by waist circumference (>102 cm for male and >88 cm for female); triglycerides (>150 mg/dL); HDL cholesterol (<40 mg/dL for male and <50 mg/dL for female); fasting blood glucose level (>110 mg/dL); systolic blood pressure (>130 mmHg) and diastolic blood pressure (>85 mmHg). VIF - Variance Inflation Factor. Values in bold highlight the results those are statistically significant.

(Tables 2 and 3). Nonetheless, MetS classification as a binary variable (MetS present/MetS absent) showed the strongest effect size (Table 3). Then, we ran LMM to test cIMT by groups (age and MetS), due to the high correlation between cIMT and age, as well as the knowledge that MetS increases with age. Both, age and MetS, influence cIMT (p-value ≤ 0.0001 and 0.0008, respectively), and the interaction between both was almost significant (p-value = 0.051).

4. Discussion

Our findings contribute to a better understanding of MetS components and the role of each component in the association with cIMT in a rural sample from Brazil. In this family-based and relatively young population, we observed that 35% were classified with MetS and those with MetS had higher values of cIMT, independently of age. From MetS defining cardiovascular risk factors, we observed that only blood pressure, obesity and glycemia, three out of five, were independently associated with cIMT, even after adjusting for main confounders in the atherosclerosis context, such as age, sex, smoking status and statin. We also confirmed the linear relationship between age and cIMT.

Because MetS is a phenotype of clustering factors, growing in prevalence worldwide particularly among rural populations [10-13], the role of each factor should be explored separately not only for quantifying factor contribution but also for getting a more specific phenotypic characterization of disease development and targeting specific therapies. In this context of phenotypic characterization, cardiovascular risk factors, such as BMI, systolic BP, hypertension, and diabetes mellitus, have been previously associated with increased cIMT [7,26], as well as sex [27,28]. However, those associations have been controversial, since studies did not assess the same risk factors and usually did not consider the correlation between them. Mannami et al. demonstrated that men present more risk factors associated with cIMT than women in a Japanese population [29], while Loboz-Rudnicka et al. showed the opposite [28]. In contrast to what has been previously described, we didn’t find any evidence of sex differences in cIMT in our population, even though a previous study in Brazil using a higher number of individuals demonstrated that cIMT is higher in men [21].

Furthermore, HDL-cholesterol and triglycerides, two of the components used to diagnose MetS, were not independently associated with cIMT in this current study. HDL-cholesterol is a protective factor for cardiovascular disease [22,30,31]. Kim et al. showed that actually a sub-fraction of HDL, the small and dense HDL particle, is responsible for the significant and inverse association with cIMT, even after adjustment by LDL and all particles of HDL [32]. In our study, we were not able to assess HDL sub-fractions. In addition, the lipid profile is known to be highly associated with atherosclerosis [33,34]. Kawamoto et al. [35] demonstrated not only a positive association of LDL-cholesterol with cIMT but also that MetS amplifies LDL levels. Nonetheless, our data showed that factor 2, composed by total cholesterol and LDL, was not significantly associated with cIMT.

Despite the fact that the cIMT has been already established as a strong predictor of future cardiovascular events [3,5], the heterogeneity regarding its effect in different age strata and the lack of information on younger individuals remained [3,5]. Our findings contribute to this gap because we explored a relatively young sample (average age of 46 ± 16yo), as well as a population without MetS (only 35% had MetS). In addition, our results showed that age is linearly associated with cIMT, even for younger individuals. Although prospective data is essential for cardiovascular risk assessment, the linear relationship between age and cIMT in young individuals pointed out that carotid ultrasound may be a very useful tool for early subclinical atherosclerosis evaluation in young participants.

Our study has limitations. First, the cross-sectional analysis does not allow causal inference. Second, independent associations were tested, and then incomplete adjustment for unmeasured confounders may be taken into account. On the other hand, we conducted a robust statistical analysis in which we assess the contribution of cardiovascular risk factors used to define MetS in the cIMT phenotype, taking into account that many risk factors are highly correlated.

5. Conclusion

In this rural cohort, cIMT showed a linear relationship with increasing age. Higher cIMT values were revealed for those with MetS compared to those without MetS. While analyzing MetS components separately, blood pressure, glucose and obesity were independently associated with cIMT, but not HDL-cholesterol and triglycerides. It appears that hypertension, diabetes, and obesity play a major role in cIMT values compared to the lipid profile. Exploring the contribution of each cardiovascular risk factor for disease development may target specific therapies and point out better practices for disease prevention.

Author contributions

Concept and design: GRG, IPS, SKT and JEK.
Analysis and interpretation of data: GRG, IPS, SKT, LMGG, ACP, and JEK.
Concept and design: GRG, IPS, SKT and JEK.
Analysis and interpretation of data: GRG, IPS, SKT, LMGG, ACP, and JEK.
Final approval: all.

Data sharing statement

Researchers can apply for data and biomaterial by submitting a proposal to the principal investigator, ACP (alexandre.pereira@incor.usp.br).

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Declaration of competing interest

This was not an industry supported study. The authors have indicated no financial conflicts of interest.
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Appendix A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijchy.2020.100043.

Abbreviations

BMI body mass index
cIMT carotid intima-media thickness
CVD cardiovascular disease
DBP diastolic blood pressure
HbA1c glycated hemoglobin
HDL high-density lipoprotein
LDL low-density lipoprotein
MetS metabolic syndrome
SBP systolic blood pressure

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