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Original article

Composite acute phase glycoproteins with coronary artery calcification depends on metabolic syndrome presence – The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)

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ABSTRACT

Background: Inflammation has been weakly associated with coronary artery calcium (CAC) in the overall population. However, it is currently unknown whether this varies according to the cardio-metabolic profile. We evaluated the association between GlycA, a unique composite biomarker of pro-inflammatory acute phase glycoproteins, high sensitivity C-reactive protein (hsCRP), uric acid, and their composite values (composite inflammation) in the overall population and strata according to cardiovascular risk.
Methods: This is a cross-sectional study of 3753 Sao Paulo site participants of the ELSA-Brasil cohort that were free of cardiovascular/chronic inflammatory disease and not taking statins or allopurinol. We measured GlycA by nuclear magnetic resonance spectroscopy. For each biomarker quartile (Qs), we ran adjusted logistic and linear regression for CAC > 0 and CAC score.
Results: In the overall analysis, the 4th vs. 1st GlycA Q odds ratio (OR) for CAC > 0 was 1.53 (95% CI: 1.18, 1.98, *p* trend < 0.001) adjusted for demographics and lifestyle, but null after adding metabolic syndrome (MS) components, OR 1.14 (95% CI: 0.86, 1.51, *p* trend = 0.140). Likewise, for continuous CAC values there was no difference across GlycA Qs in the fully adjusted analysis. Similarly, hsCRP, uric acid, and composite inflammation were not associated with CAC > 0 or CAC score. In stratified analysis, GlycA was associated with CAC > 0 in No-MS individuals, standardized (SD) OR 1.23 (95% CI: 1.08, 1.40); but not in MS individuals, SD OR 1.01 (95% CI: 0.89, 1.15) (*p* interaction 0.037). We found similar interaction in stratified analysis for continuous CAC on composite inflammation.
Conclusions: GlycA and composite inflammation are associated with CAC among low cardiovascular risk individuals (No-MS), but not otherwise. GlycA and composite biomarkers may better represent sources of inflammation apart from visceral obesity and traditional cardiovascular risk factors, which may have relevant effect on CAC accumulation in low cardiovascular risk individuals.

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Introduction

Inflammation is fundamental to the initiation, progression, and complications of coronary atherosclerotic disease (CAD) [1]. Coronary artery calcification (CAC) is the radiologic hallmark of subclinical CAD that is formed by calcium phosphate deposition potentiated by inflammation [2]. Despite solid histopathologic evidence supporting the role of inflammation in CAD progression [1], its association with CAC in population studies has been weak or null [3–6]. However, inflammation and CAC are entangled amidst traditional CAD risk factors, embodied by the metabolic syndrome definition [7]. It is uncertain whether inflammation is associated with CAC independently from metabolic syndrome risk factors or is just an inoffensive bystander.

The pathophysiological impact of inflammation is complex, multidimensional, and poorly captured by a single biomarker such as high sensitivity C-reactive protein (hsCRP). By contrast, composite biomarkers represent broader dimensions of inflammation and have stronger associations with CAC compared with single protein biomarkers [4,8]. In this regard, acute phase inflammatory glycoproteins, such as α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin, and transferrin, are the most abundant circulating glycoproteins. Moreover, these glycoproteins have enormous structural and functional diversity related to post-translational enzymatic glycosylation [9,10], which may better represent the complex inflammatory milieu and its effects. These acute phase glycoproteins have been successfully quantified by the proton magnetic resonance spectroscopy (NMR) signal of their glycan (carbohydrate) moiety composed of N-acetyl methyl groups [11], whose unique signal was named GlycA. It has been related to incident cardiovascular disease [12–16] and diabetes in population studies [17,18]. However, there is no data on the association of GlycA with CAC to the best of our knowledge.

We elected to address whether systemic inflammation measured by GlycA and hsCRP are associated with subclinical CAC. We also included uric acid in our analysis, despite not being a classical inflammatory marker, due its pro [19] and anti-inflammatory effects [20,21]. Besides that, uric acid is intertwined with metabolic syndrome, characterized by an inflammatory state. We also addressed the association of the composite aforementioned biomarkers with CAC, and ran stratified analyses according to cardiovascular risk profile.

Materials and methods

We evaluated the cross-sectional association of serum GlycA, hsCRP, uric acid, and their composite measurements with CAC presence and quantity in participants of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). ELSA-Brasil is a cohort of 15,105 civil servants aged 35–74 years living in 6 cities in Brazil. Per study goals, sampling was stratified by occupation: unskilled, technical/clerical, and faculty or professional staff to maximize a wide range of socioeconomic backgrounds. Other details of this cohort are described elsewhere [22–25]. The Institutional Review Boards approved the study protocol, which conforms to the ethical guidelines of the 1975 Declaration of Helsinki. All participants provided informed consent.

In the ELSA-Brasil baseline, 4,548 São Paulo site participants underwent CAC measurement. From this sample, sequential selection followed as shown (Fig. 1). Baseline anthropometric, demographic, life-style, and self-declared race/ethnicity data were collected on site by questionnaires and clinical examination between August 2008 and December 2010 [24]. We considered physically active individuals according to World Health Organization definition [26], those with at least 150 min of moderate-intensity or 75 min of high-intensity aerobic physical activity or

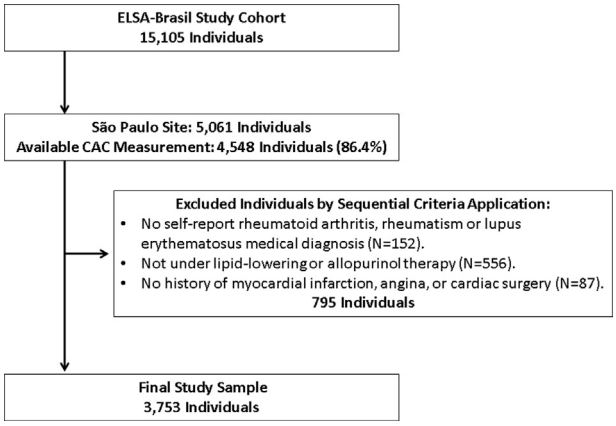

Fig. 1. Study population flow chart.

combined equivalent of both each week according to leisure time International Physical Activity Questionnaire (IPAQ). Any weekly physical activity below those levels were categorized as partly active, and the remaining sedentary. Hypertension was defined as a blood pressure above 140/90 mmHg or pharmacologic anti-hypertensive treatment. Overnight fasting blood samples were collected and analyzed at the central laboratory facility in the ELSA-Brasil Investigation Center of São Paulo at University Hospital, University of São Paulo [25]. Diabetes was defined by self-referred disease or routine use of diabetes medication, fasting plasma glucose ≥126 mg/dL, glycated hemoglobin (HbA1c) ≥6.5%, or 2-h plasma glucose during the oral glucose tolerance test ≥200 mg/dL; and dyslipidemia by low-density lipoprotein cholesterol >160 mg/dL, triglycerides >150 mg/dL, or high-density lipoprotein cholesterol (HDL-C) <50 mg/dL for women and <40 mg/dL for men.

HsCRP was measured by immunochemistry nephelometry (BN II Siemens® nephelometer, Siemens, Berlin, Germany) [27], and uric acid by the uricase method. GlycA quantified the acute phase glycoproteins by the magnetic resonance signal of their N-acetyl methyl group protons on the N-acetylglucosamine (GlcNAc) moieties located on bi-, tri-, or tetra-antennary branches [28,29]. Their signal is deconvoluted from overlapping lipoproteins (predominantly triglycerides in very-low-density lipoproteins) and their amplitude converted to micromoles per liter (μmol/L) by LipoScience (now LabCorp, Raleigh, NC, USA). GlycA intra-assay and inter-assay coefficients of variation are respectively 1.9% and 2.6%, and biological variability is low (coefficient of variation 4.3%) [11,30].

CAC was measured by a 64-detector computed tomographic scanner (Brilliance 64; Philips Healthcare, Best, The Netherlands). Following scout imaging, prospective electrocardiogram-gated images were acquired with a tube potential of 120 kV adjusted for body structure. Unidentified Images 2.5-mm thick were reconstructed by standard filtered back projection, and evaluated with semi-automatic software (Calcium Scoring, Philips Workstation) by an experienced cardiologist. CAC score was computed by Agatston score [31], and CAC >0 defined CAC presence [32].

We defined metabolic syndrome (MS) by the presence of at least three of the following: elevated fasting glucose (≥100 mg/dL or use of hypoglycemic medication), elevated triglycerides (≥150 mg/dL or use of fibrates and/or nicotinic acid), low HDL-C (<40 mg/dL for men, <50 mg/dL for women), elevated blood pressure (systolic blood pressure ≥135 mmHg and/or diastolic blood pressure ≥85 mmHg or confirmed use of antihypertensive medication) and abdominal obesity [33]. For abdominal obesity we considered the International Diabetes Foundation waist circumference criteria: in white, brown, and black, ≥94 cm for men and ≥80 cm for women; and in Asian and indigenous, ≥90 cm for men and ≥80 cm for women [34].

We described the population according to CAC score ranges: 0, 0–100, and >100 AU. We displayed continuous variables by median and interquartile range and for categorical ones by percentages. For trend analysis we applied Jonckheere–Terpstra for continuous variables and Cochran–Mantel–Haenszel for categorical. As race is a non-ordinal variable, we applied the chi square test. We ran Spearman correlations among inflammatory markers.

We conflated GlycA, hsCRP, and uric acid into one variable, named composite inflammation. For its categorical form we computed 1 score point for each biomarker value above its median distribution, therefore ranging from 0 to 3. For the continuous ones (standardized units), we computed the arithmetic mean of GlycA, hsCRP (natural logarithm), and uric acid standardized units.

We addressed the biomarkers' association with CAC >0 by logistic regression using the 1st quartile or the 0 score (composite inflammation) as the referent. For continuous CAC score, we ran analysis of covariance for CAC +1 natural logarithm. We back transformed values to Agatston units for results display. Models were incrementally adjusted for age, gender, and race (model 1), then we added family history of coronary artery disease, alcohol use, smoking, physical activity (model 2), then waist circumference (model 3), and finally diabetes, hypertension, HDL-C, and triglycerides (natural log) (model 4). We ran stratified analysis for age, gender, body mass index (BMI), waist circumference (≥ or <94 cm for men and ≥ or <80 cm for women), smoking, type 2 diabetes, low-density lipoprotein-cholesterol and metabolic syndrome. In the stratified analysis, we adjusted models by dichotomous metabolic syndrome instead of its individual components. We applied this criterion in order to avoid over adjustment and redundancy among strata and adjustment covariates, especially for metabolic syndrome stratum. We tested for interaction by including product terms. The level of significance was two tailed *p*-value below 0.05, and we used SAS version 9.3 software (SAS Institute, Cary, NC, USA).

Results

The overall study population had a median (interquartile range) age of 49 (44–55) years, 1752 (46.3%) were males, had BMI of 26.7 (23.8–29.8) kg/m^2, presented GlycA of 409 (367–453) μmol/L, hsCRP of 1.47 (0.73–3.39) mg/L, and uric acid 5.5 (4.5–6.6) mg/dL. Metabolic syndrome was present in 1429 individuals (37.8%), around three quarters had no CAC, 659 (17.4%) had CAC 0–100, and 303 (8.0%) had CAC >100. From CAC 0, to 0–100, to >100 group, there were increasing age, more men, greater proportion of white race and lower of black, increasing waist circumference, higher prevalence of current smoking, and more prevalent metabolic

Table 1
Descriptive characteristics according to CAC groups.

CAC score range	0	0–100	>100	*p* for trend
N (population study %)	2809 (74.9%)	651 (17.4%)	293 (8.0%)	
Age (years)	47 (43–52)	55 (49–60)	58 (52–64)	<0.001
Male gender *N* (%)	1111 (39.4%)	403 (61.2%)	238 (78.6%)	<0.001
Race				<0.001
White *N* (%)	1600 (57.3%)	407 (62.9%)	180 (60.2%)	
Brown *N* (%)	648 (23.2%)	135 (20.9%)	65 (21.7%)	
Black *N* (%)	422 (15.1%)	70 (10.8%)	32 (10.7%)	
Asian *N* (%)	100 (3.6%)	24 (3.7%)	21 (7%)	
Indigenous *N* (%)	24 (0.9%)	11 (1.7%)	1 (0.3%)	
Waist circumference (cm)	87.1 (79.6–96)	91.6 (83.8–99.5)	94.7 (87.2–103.4)	<0.001
Body mass index (kg/m^2)	26.4 (23.6–29.6)	27.0 (24.2–29.9)	27.3 (24.7–30.3)	<0.001
Smoking				<0.001
Never	1629 (57.8%)	303 (46.0%)	120 (39.6%)	
Former	782 (27.7%)	224 (34.0%)	115 (38%)	
Current	410 (14.5%)	132 (20.0%)	68 (22.4%)	
Alcohol consumption *N* (%)	1882 (66.7%)	476 (72.2%)	226 (74.6%)	<0.001
Physical activity				0.032
Sedentary	1830 (67.6%)	400 (62.7%)	178 (59.9%)	
Insufficiently active	249 (9.2%)	74 (11.6%)	51 (17.2%)	
Active	627 (23.2%)	164 (25.7%)	68 (22.9%)	
Family history CHD *N* (%)	100 (3.5%)	39 (5.9%)	25 (8.3%)	<0.001
Diabetes *N* (%)	381 (13.5%)	158 (24%)	100 (33%)	<0.001
Hypertension *N* (%)	627 (22.2%)	233 (35.4%)	173 (57.1%)	<0.001
Dyslipidemia *N* (%)	1308 (46.4%)	396 (60.1%)	182 (60.1%)	<0.001
Metabolic syndrome *N* (%)	940 (33.3%)	314 (47.7%)	175 (57.8%)	<0.001
CKD EPI	86 (75–96)	80 (70–90)	80 (66–93)	<0.001
Glucose (mg/dL)	103 (97–110)	108 (101–116)	110 (103–123)	<0.001
HbA1c (%)	5.2 (4.9–5.7)	5.4 (5.0–5.8)	5.4 (5.0–5.8)	<0.001
Total cholesterol (mg/dL)	208 (184–234)	222 (196–246)	224 (197–251)	<0.001
HDL cholesterol (mg/dL)	54 (46–65)	52 (45–63)	52 (44–60)	<0.001
LDL cholesterol (mg/dL)	128 (107–149)	139 (117–160)	137 (118–165)	<0.001
Triglycerides (mg/dL)	106 (76–153)	132 (92–183)	132 (101–189)	<0.001
Lipoprotein(a) (mg/dL)	12.2 (8.8–18.6)	12.4 (9.1–19.4)	16.1 (10.7–27.6)	0.062
GlycA (μmol/L)	407 (366–452)	417 (373–460)	403 (365–446)	0.092
hsCRP (mg/L)	1.45 (0.71–3.35)	1.60 (0.81–3.71)	1.40 (0.79–2.98)	0.220
Uric acid (mg/dL)	5.3 (6.3, 6.3)	5.9 (6.6, 6.9)	6.3 (6.6, 7.4)	<0.001

Categorical variables displayed by number (percentage), *p*-values for trend by Mantel–Haenszel chi-square, except for race/ethnicity where chi-square *p*-value is signaled by *. Continuous variables displayed by median (interquartile range), *p*-values for trend by Jonckheere–Terpstra. Physical activity measured by leisure time. Sedentary characterized by no activity and active by at least 150 min of moderate-intensity activity per week or 75 min of vigorous-intensity aerobic physical activity per week or an equivalent combination of moderate- and vigorous-intensity activity. Insufficiently active characterized by any level below the physically active threshold.
Abbreviations: CAC (coronary artery calcium); *p* (statistical *p* value), CKD EPI (estimated glomerular filtration rate by Chronic Kidney Disease Epidemiology Collaboration equation), HbA1c (glycated hemoglobin); HDL (high-density lipoprotein), LDL (low-density lipoprotein), hsCRP (high sensitivity C reactive protein), cm (centimeters), kg (kilograms), m (meters), mg (milligrams), μmol (micromol), dL (deciliter) and L (liter).

Table 2
GlycA, hsCRP, uric acid and composite inflammation associations with CAC presence (CAC > 0).

GlycA (μmol/L)	Q1 (<368)	Q2 (368–408)	Q3 (409–452)	Q4 (>452)	*p* for trend
Model 1	1.00 (ref.)	1.16 (0.91, 1.48)	1.59 (1.25, 2.03)	1.63 (1.27, 2.09)	<0.001
Model 2	1.00 (ref.)	1.12 (0.87, 1.45)	1.57 (1.22, 2.01)	1.53 (1.18, 1.98)	<0.001
Model 3	1.00 (ref.)	1.05 (0.81, 1.36)	1.43 (1.11, 1.85)	1.32 (1.01, 1.72)	0.008
Model 4	1.00 (ref.)	1.00 (0.77, 1.30)	1.31 (1.01, 1.70)	1.14 (0.86, 1.51)	0.140
hsCRP (mg/L)	Q1 (<0.74)	Q2 (0.74–1.46)	Q3 (1.47–3.38)	Q4 (>3.38)	*p* for trend
Model 1	1.00 (ref.)	1.27 (1.00, 1.63)	1.31 (1.02, 1.67)	1.66 (1.29, 2.14)	<0.001
Model 2	1.00 (ref.)	1.34 (1.04, 1.72)	1.33 (1.03, 1.72)	1.71 (1.32, 2.22)	<0.001
Model 3	1.00 (ref.)	1.23 (0.95, 1.59)	1.16 (0.89, 1.51)	1.38 (1.04, 1.83)	0.061
Model 4	1.00 (ref.)	1.17 (0.91, 1.52)	1.08 (0.82, 1.41)	1.28 (0.96, 1.70)	0.159
Uric acid (mg/dL)	Q1 (<4.6)	Q2 (4.6–5.4)	Q3 (5.5–6.5)	Q4 (>6.5)	*p* for trend
Model 1	1.00 (ref.)	1.20 (0.91, 1.57)	1.22 (0.93, 1.61)	1.56 (1.18, 2.06)	0.002
Model 2	1.00 (ref.)	1.29 (0.98, 1.71)	1.25 (0.94, 1.65)	1.62 (1.21, 2.16)	0.002
Model 3	1.00 (ref.)	1.20 (0.91, 1.59)	1.10 (0.82, 1.46)	1.31 (0.96, 1.77)	0.123
Model 4	1.00 (ref.)	1.17 (0.88, 1.56)	1.04 (0.78, 1.39)	1.14 (0.84, 1.56)	0.571
Inflammation score (*N* – %)	0 (765–20.4%)	1 (1083–28.9%)	2 (1182–31.5%)	3 (723–19.3%)	*p* for trend
Model 1	1.00 (ref.)	1.09 (0.84, 1.43)	1.26 (0.97, 1.64)	1.81 (1.37, 2.40)	<0.001
Model 2	1.00 (ref.)	1.12 (0.85, 1.48)	1.29 (0.98, 1.69)	1.77 (1.32, 2.37)	<0.001
Model 3	1.00 (ref.)	1.01 (0.76, 1.34)	1.11 (0.83, 1.47)	1.40 (1.02, 1.92)	0.018
Model 4	1.00 (ref.)	0.96 (0.72, 1.27)	0.97 (0.73, 1.30)	1.20 (0.87, 1.66)	0.217

Adjusted odds ratio (95% CI) by logistic regression using the first quartile as reference. Model 1: Age, gender, and race/ethnicity. Model 2: M1 + family history of coronary artery disease, alcohol use, smoking, and physical activity. Model 3: M2 + waist circumference. Model 4: M3 + diabetes, hypertension, HDL-c, and triglycerides (natural log). Inflammation score according to number of variables (GlycA, hsCRP, or uric acid) with values above median, therefore ranging from 0 to 3.
Abbreviations: hsCRP (high sensitivity C reactive protein), CAC (coronary artery calcium), Q (quartiles), *p* (statistical *p* value), μmol (micro mol), mg (milligrams), dL (deciliter) and L (liter).

syndrome criteria (Table 1). Regarding the inflammatory biomarkers, there were no increasing levels of GlycA or hsCRP across higher CAC groups, in contrast to uric acid (CAC 0: 5.3 mg/dL [95% CI: 6.3, 6.3] vs. CAC 100: 6.3 [6.6, 7,6], *p* for trend <0.001). GlycA had a moderate correlation with hsCRP (ρ 0.60, $p < 0.001$) and weak correlation with uric acid (ρ 0.13, $p < 0.001$). The correlation between hsCRP and uric acid was also weak (ρ 0.14, $p < 0.001$). These correlations did not differ according to metabolic syndrome presence or absence (data not shown).

In the model adjusted for age, gender, and race (Model 1), the 4th GlycA Q odds ratio (OR) (95% CI) vs. the 1st Q for the presence of CAC >0 was 1.63 (1.27, 2.09) (*p* for trend <0.001) (Table 2). Further adjusting for family history of coronary heart disease and life-style habits (Model 2), the 4th vs. 1st GlycA Q OR was mildly attenuated but remained significant 1.53 (1.18, 1.98) (*p* for trend <0.001). Waist circumference inclusion further attenuated the magnitude association between GlycA and CAC presence, 4th vs. 1st GlycA Q OR 1.32 (1.01, 1.72) (*p* for trend = 0.008). After adjustment for all metabolic syndrome characteristics, GlycA was no longer associated with CAC presence, OR 1.14 (0.86, 1.51) (*p* for trend = 0.140). For hsCRP, uric acid, and composite inflammation analysis, the magnitude of association with CAC presence and confounding by covariates were generally similar to GlycA (Table 2). As a reflection of the composite inflammation biomarkers, increasing inflammation score was associated with CAC presence in model 2, score 3 OR vs. 0 was 1.77 (1.32, 2.37) (*p* for trend <0.001). However, this association was nullified after metabolic syndrome components adjustment, score 3 OR vs. 0 was 1.20 (0.87, 1.66) (*p* for trend = 0.217).

In the analyses for CAC score (Agatston units), the adjusted CAC values across inflammatory markers quartiles and inflammation score were overall low due to the large number of study participants with a CAC score of 0 (Table 3). For GlycA, from the 1st to 4th Q there was increasing CAC score (95% CI), respectively 1.14 Agatston (0.84, 1.48) and 1.68 Agatston (1.29, 2.13) (*p* for trend = 0.004). After adjusting for family history of coronary artery disease and lifestyle the CAC score difference across GlycA Qs was attenuated, 1st Q 0.91 (0.75, 1.07) and 4th Q 1.09 (0.93, 1.26) (*p* for trend = 0.012). However, there was no difference across GlycA quartiles after including waist circumference and metabolic syndrome characteristics in the adjustment model. For hsCRP, uric acid, and composite inflammation analyses, the magnitude association with CAC score and attenuation by metabolic syndrome characteristics adjustment were overall identical to GlycA. Increasing composite inflammation was associated with CAC score, in model 2 the inflammation score 0 had 1.22 Agatston (0.89, 1.62) and the inflammation score 3, 1.76 Agatston (1.34, 2.27) (*p* for trend = 0.008). However, this association was nullified after metabolic syndrome adjustment, inflammation score 0 had 1.54 Agatston (1.14, 2.00) and inflammation score 3 had 1.41 Agatston (1.03, 1.86) (*p* for trend = 0.585).

In the stratified analysis for CAC > 0, there was valid interaction for GlycA, uric acid, and composite inflammation in the fully adjusted analysis (Online Table I). More specifically, uric acid presented effect modification for sex, smoking, and diabetes. From current, to former, to never smokers uric acid had increasing SD OR, respectively 0.96 (0.78, 1.18), 1.04 (0.88, 1.22), and 1.19 (1.04, 1.37) (*p* for interaction = 0.036). In diabetes-free individuals, uric acid was associated with CAC presence, 1.16 (1.03, 1.31), by contrast to diabetic ones, 0.85 (0.70, 1.03) (*p* for interaction = 0.036). GlycA association with CAC presented interaction for waist circumference categories, low SD OR 1.24 (1.07, 1.44) versus high 1.03 (0.92, 1.16) (*p* for interaction = 0.047). Finally, GlycA was associated with CAC presence in metabolic syndrome-free individuals, SD OR 1.23 (1.08, 1.40), by contrast to those with metabolic syndrome, 1.04 (0.85, 1.26) (*p* for interaction = 0.037) (Fig. 2). Similarly, composite inflammation score is associated with CAC presence in metabolic syndrome-free individuals SD OR 1.38 (1.15, 1.66), in contrast to individuals with metabolic syndrome (*p* for interaction = 0.037) (Fig. 2).

In the stratified analysis for CAC score (Agatston units) there were statistically valid interactions for GlycA, uric acid, and composite inflammation (Online Table II). In spite of that, there were no significant β values within the aforementioned uric acid and composite inflammation strata. By contrast, GlycA was associated with CAC score in those with lower waist circumference, SD β 0.11 (0.01, 0.21); and no metabolic syndrome, SD β 0.08 (0.01, 0.16) in contrast to their respective counterparts (Fig. 3).

Table 3
Adjusted CAC score for GlycA, hsCRP, uric acid, and composite inflammation quartiles.

GlycA (μmol/L)	Q1 (<368)	Q2 (368–408)	Q3 (409–452)	Q4 (>452)	*p* for trend
Model 1	1.14 (0.84, 1.48)	1.36 (1.02, 1.75)	1.58 (1.21, 2.00)	1.68 (1.29, 2.13)	0.004
Model 2	0.91 (0.75, 1.07)	0.99 (0.82, 1.15)	1.07 (0.91, 1.23)	1.09 (0.93, 1.26)	0.012
Model 3	1.68 (1.28, 2.16)	1.78 (1.36, 2.27)	1.94 (1.50, 2.46)	1.88 (1.43, 2.40)	0.328
Model 4	1.78 (1.36, 2.28)	1.79 (1.37, 2.28)	1.87 (1.44, 2.38)	1.74 (1.32, 2.25)	0.942
hsCRP (mg/L)	Q1 (<0.74)	Q2 (0.74–1.46)	Q3 (1.47–3.38)	Q4 (>3.38)	*p* for trend
Model 1	0.73 (0.58, 0.88)	0.89 (0.73, 1.04)	0.86 (0.71, 1.02)	0.99 (0.84, 1.15)	0.003
Model 2	0.85 (0.69, 1.01)	1.03 (0.86, 1.19)	0.99 (0.83, 1.16)	1.13 (0.96, 1.29)	0.003
Model 3	1.56 (1.17, 2.02)	1.89 (1.46, 2.41)	1.69 (1.29, 2.17)	1.93 (1.48, 2.47)	0.252
Model 4	1.63 (1.23, 2.10)	1.91 (1.47, 2.42)	1.64 (1.24, 2.11)	1.83 (1.39, 2.34)	0.655
Uric acid (mg/dL)	Q1 (<4.6)	Q2 (4.6–5.4)	Q3 (5.5–6.5)	Q4 (>6.5)	*p* for trend
Model 1	1.33 (0.99, 1.74)	1.24 (0.92, 1.61)	1.26 (0.94, 1.64)	1.87 (1.45, 2.37)	0.012
Model 2	1.64 (1.23, 2.12)	1.60 (1.21, 2.06)	1.57 (1.18, 2.03)	2.28 (1.76, 2.90)	0.012
Model 3	1.90 (1.44, 2.45)	1.72 (1.31, 2.20)	1.58 (1.19, 2.04)	2.12 (1.62, 2.70)	0.405
Model 4	1.99 (1.52, 2.56)	1.76 (1.35, 2.25)	1.57 (1.19, 2.03)	1.91 (1.45, 2.46)	0.796
Inflammation score	0	1	2	3	*p* for trend
Model 1	1.19 (0.87, 1.58)	1.30 (0.99, 1.66)	1.47 (1.13, 1.85)	1.82 (1.39, 2.33)	0.002
Model 2	1.22 (0.89, 1.62)	1.36 (1.03, 1.73)	1.52 (1.17, 1.92)	1.76 (1.34, 2.27)	0.008
Model 3	1.41 (1.08, 1.80)	1.48 (1.14, 1.88)	1.57 (1.17, 2.05)	1.43 (1.05, 1.88)	0.495
Model 4	1.54 (1.14, 2.00)	1.44 (1.10, 1.82)	1.40 (1.07, 1.78)	1.41 (1.03, 1.86)	0.585

Adjusted least square means (95% CI) run with natural logarithm (CAC + 1), then back transformed to Agatston units for results display. Adjusted Model 1: Age, gender, and race/ethnicity. Model 2: M1 + family history of coronary artery disease, alcohol use, smoking, and physical activity. Model 3: M2 + waist circumference. Model 4: M3 + diabetes, hypertension, HDL-c, triglycerides (natural log). Inflammation score according to number of variables (GlycA, hsCRP, or uric acid) with values above median, therefore ranging from 0 to 3.
Abbreviations: hsCRP (high sensitivity C reactive protein), CAC (coronary artery calcium), Q (quartiles), *p* (statistical *p* value), μmol (micromol), mg (milligrams), dL (deciliter) and L (liter).

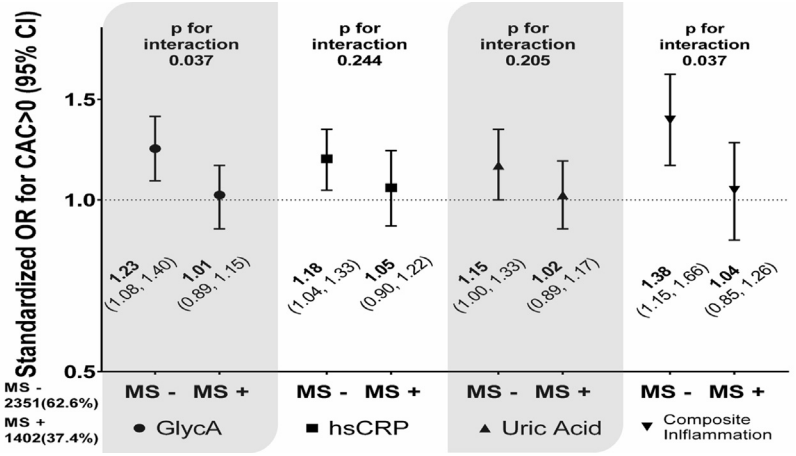

Fig. 2. Inflammation biomarkers association with CAC presence according to metabolic syndrome presence. GlycA, hsCRP, uric acid, and composite inflammation standardized (SD) odds ratio (95% CI) for CAC presence (>0) stratified by metabolic syndrome (MS) presence (logistic regression). Composite inflammation was calculated by the arithmetic mean of GlycA, hsCRP, and uric acid. Adjustment model: Age, gender, race/ethnicity, family history of coronary artery disease, alcohol use, smoking, and physical activity. CAC, coronary artery calcium; hsCRP, high sensitivity C-reactive protein.

Discussion

In individuals with no metabolic syndrome or few cardiometabolic risk factors, a novel inflammatory biomarker of composite circulating acute phase glycoproteins named GlycA and composite biomarkers of inflammation are associated with CAC. By contrast, in those with metabolic syndrome or higher cardiometabolic risk there is no such association. The association of circulating markers of inflammation with CAC according to individual cardiometabolic profile may explain the previous conflicting results in the overall population. Further studies should explore the specific drivers of circulating inflammation in individuals with low cardiometabolic risk and its effects on CAC.

This is the first study to address the association of GlycA with CAC presence and burden to the best of our knowledge. Prior data have found no association between another inflammation marker, hsCRP, and CAC in a population-based study of 3373 individuals and in 914 subjects with a family history of premature CAD [6,35]. In the MESA and other population studies, hsCRP was not associated with CAC presence and quantity after adjusting for diabetes, systolic blood pressure, BMI, and dyslipidemia [4–6]. Regarding uric acid, previous studies on apparently healthy individuals did not find any association with CAC after adjustment for metabolic syndrome [36,37]. In our study as well, the association of GlycA, hsCRP, uric acid, and composite inflammation with CAC presence and quantity was not significant after adjusting for metabolic syndrome components. If anything, in the MESA study, interleukin-6 and fibrinogen were at most weakly associated with CAC presence, but not CAC quantity [4]. In light of these data, the association of low-grade inflammation with CAC in the overall

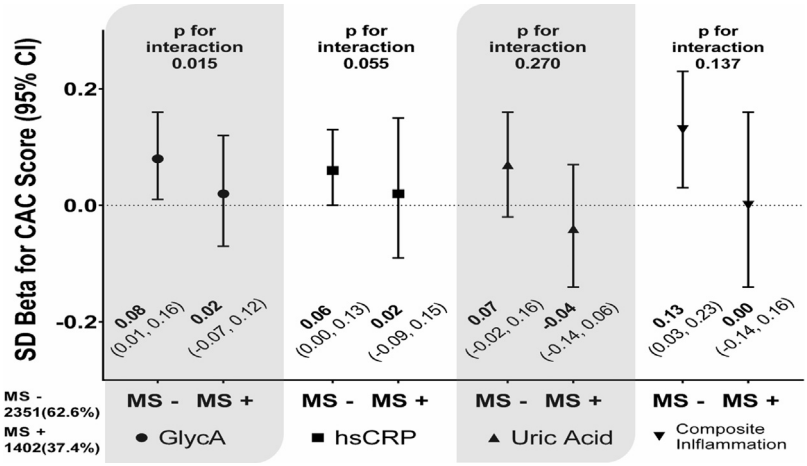

Fig. 3. Inflammation biomarkers association with CAC score according to metabolic syndrome presence. GlycA, hsCRP, uric acid, and composite inflammation standardized (SD) beta coefficients (95% CI) for CAC score (Agatston) stratified by metabolic syndrome (MS) presence (linear regression). Composite inflammation was calculated by the arithmetic mean of GlycA, hsCRP, and uric acid. Adjustment model: age, gender, race/ethnicity, family history of coronary artery disease, alcohol use, smoking, and physical activity. CAC, coronary artery calcium; hsCRP, high sensitivity C-reactive protein.

population seems considerably confounded by those with metabolic syndrome. Previous data have shown metabolic syndrome is independently associated with hsCRP and CAC [7], therefore a potential confounder on the association studied herein.

Inflammation is intimately associated with atherosclerosis initiation, progression, and complications [1]. However, in our study and previous ones, low-grade inflammation is at most weakly associated with CAC [3,4]. In contrast, hsCRP and other inflammation markers are associated with incident coronary heart disease events independently from metabolic syndrome components [38,39]. Therefore, our results seem paradoxical when compared with CAD events studies. Statin trials can be enlightening in this regard due to their concomitant effects on circulating inflammation, coronary artery plaque composition, and CAD events. On the one hand, statins decrease CAD lipid core, have anti-inflammatory effects, and reduce CAD events [40,41]. At the same time, they promote plaque "healing" and CAC deposition as documented by intravascular ultrasound studies [42,43]. Given CAD is a life span disease, longitudinal clinical studies are usually short term under this perspective. Low-grade inflammation seems rather associated with "late" clinical manifestation related to coronary plaque rupture and thrombosis than with CAC. Besides that, inflammation may wax and wane along life and its cumulative effects would be insufficiently reflected by one time measurement. Therefore, cross-sectional and longitudinal studies of circulating inflammation, may not capture an association that seems considerably complex and non linear across time.

Uric acid is not an inflammatory cytokine; however, it has been shown to have pro- [19] and anti-inflammatory effects [20,21]. It is uncertain whether uric acid is an inducer of inflammation or an anti-inflammatory counter response to it. In contrast to our findings, in white, non-diabetic men under health check-up, Santos et al. found uric acid association with CAC in those with metabolic syndrome, but not otherwise [44]. In an analysis restricted to our male sample, we did not find any interaction for metabolic syndrome (data not shown). The reasons for such conflicting results are uncertain, but diverse population profile and model adjustment could in part contribute to it. In our study, we believe there is no residual confounding by renal dysfunction as our sample has a predominantly normal glomerular filtration rate and adjusting for it did not change the results. Besides that, uric acid might have a context-dependent anti- and pro-inflammatory role poorly captured by our cross-sectional design. We also cannot rule out the play of chance for the sex interaction on uric acid association with categorical CAC; and on composite inflammation with continuous CAC.

The association of inflammation (i.e. GlycA and composite inflammation) with CAC in low cardiometabolic risk individuals, by contrast to MS, is an intriguing finding. Low-grade chronic inflammation is majorly influenced by insulin resistant intra-abdominal visceral adipose tissue [45], the underlying mechanism of MS. It is estimated that intra-abdominal visceral adipose tissue produces approximately one third of circulating interleukin-6, an upstream cytokine in the inflammatory chain [46]. Besides that, lifestyle behaviors are partly associated with circulating inflammation. Notwithstanding, diet, smoking, and physical activity have been directly associated with circulating hsCRP in normal weight individuals, but not in overweight and obese [47]. In our study as well, GlycA and overall inflammation were associated with CAC in no-MS individuals, but not otherwise. We also had similar patterns for those with lower waist circumference and diabetes-free individuals. One hypothesis is that circulating inflammation derived from intra-abdominal adiposity overshadows those driven by other sources. One source is genetically derived inflammation. In genome-wide association studies, interleukin-6 polymorphisms are associated with life span lower cardiovascular risk [48,49]. There data support some arguments for our findings. First, genetically derived inflammation may have stable behavior along life and one time measurement represents its life span level. Second, genetically driven inflammation would be more evident among low cardiometabolic risk individuals (no-MS), less "contaminated" by other inflammation sources. Therefore, genetically driven inflammation may have higher relative contribution on CAC in lower cardiometabolic risk individuals.

Another interesting fact is the GlycA association with CAC, in contrast to the null of hsCRP with CAC. This seems quite paradoxical given the robust association of hsCRP with cardiovascular and coronary artery events in epidemiological data. However, specific stimulation or blockage of hsCRP in human experiments have shown that hsCRP has no causal role on the overall inflammation cascade [50,51]. Moreover, genome-wide association studies have refuted any causal role of hsCRP on cardiovascular risk [52]. In fact, interleukin-6 has more upstream effect on the inflammatory cascade, including its influence over hsCRP and possible causal role on cardiovascular risk [48,49]. Despite the moderate correlation between GlycA and hsCRP, they capture different dimensions of inflammation. Due to the multidimensional composite nature of GlycA, it may better represent the broader effects of upstream regulators of inflammation, as interleukin-6, on CAC.

Understanding inflammation and CAC dynamics in low-risk individuals may apparently seem irrelevant. However, amidst individuals with manifest cardiovascular disease, 52.6% of women and 62.6% of women with manifest coronary heart disease have just one or none traditional cardiovascular risk factor [53]. Despite the lower individual risk, low-risk individuals are highly prevalent in the overall population. In a representative sample from the USA, 72.6% of the population presented with a Framingham risk score below 10% [54]. Because of that, low-risk individuals have major attributable cardiovascular risk in the overall population. In those with few cardiovascular risk factors, our findings may have minimal individual and clinical impact, but very relevant at the population level.

Our study must be read within its context and design. It is reasonably generalizable as we included men and women of diverse and admixed racial background. In addition, our large sample size makes a type II error improbable. Despite this, interpretation should be cautious due to the cross-sectional nature of our study. Another point is the broad structural and functional diversity of glycans, which may have implications on CAD and CAC [10]. Hitherto, glycan laboratorial profiling does not differentiate that.

Composite circulating acute phase glycoproteins measured by GlycA and composite inflammatory markers are associated with CAC only in low cardiovascular risk individuals, free from metabolic syndrome. GlycA and composite biomarkers may better represent inflammation unrelated to visceral obesity and traditional cardiovascular risk factors, which may have relevant effect on CAC accumulation in individuals with low cardiovascular risk.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jjcc.2018.09.006.

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