ORIGINAL RESEARCH

Association Between Smoking and Serum GlycA and High-Sensitivity C-Reactive Protein Levels: The Multi-Ethnic Study of Atherosclerosis (MESA) and Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)

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***Background*—**Inflammation is suggested to be a central feature of atherosclerosis, particularly among smokers. We studied whether inflammatory biomarkers GlycA and high-sensitivity C-reactive protein are associated with cigarette smoking.

***Methods and Results*—**A total of 11 509 participants, 6774 from the MESA (Multi-Ethnic Study of Atherosclerosis) and 4735 from ELSA-Brasil (The Brazilian Longitudinal Study of Adult Health) were included. We evaluated the cross-sectional association between multiple measures of smoking behavior and the inflammatory biomarkers, GlycA and high-sensitivity C-reactive protein, using regression models adjusted for demographic, anthropometric, and clinical characteristics. Participants were 57.7±11.1 years old and 46.4% were men. Never, former, and current smokers comprised 51.7%, 34.0%, and 14.3% of the population, respectively. Multivariable-adjusted mean absolute difference in GlycA levels (μmol/L) with 95% confidence interval (CI) were higher for former (4.1, 95% CI, 1.7–6.6 μmol/L) and current smokers (19.9, 95% CI, 16.6–23.2 μmol/L), compared with never smokers. Each 5-unit increase in pack-years of smoking was associated with higher GlycA levels among former (0.7, 95% CI, 0.3–1.1 μmol/L) and current smokers (1.6, 95% CI, 0.8–2.4 μmol/L). Among former smokers, each 5-year increase in time since quitting smoking was associated with lower GlycA levels (−1.6, 95% CI, −2.4 to −0.8 μmol/L) and each 10-unit increase in number of cigarettes/day was associated with higher GlycA among current smokers (2.8, 95% CI, 0.5–5.2 μmol/L). There were similar significant associations between all measures of smoking behavior, and both log-transformed GlycA and high-sensitivity C-reactive protein.

***Conclusions*—**Acute and chronic exposure to tobacco smoking is associated with inflammation, as quantified by both GlycA and high-sensitivity C-reactive protein. These biomarkers may have utility for the study and regulation of novel and traditional tobacco products. **(*J Am Heart Assoc*. 2017;6:e006545. DOI: 10.1161/JAHA.117.006545.)**

Key Words: atherosclerosis • inflammation • prevention • risk assessment • smoking

Tobacco smoking is one of the major preventable causes of death and cardiovascular disease (CVD) in the world.[1] The high prevalence of old and new tobacco products among both smokers and nonsmokers poses a potentially significant population-wide health hazard around the world.[2,3] Therefore, from the perspective of the US Food and Drug Administration, it is important to identify sensitive markers of subclinical cardiovascular injury that might be useful for toxicological study and subsequent regulation of both existing and new tobacco products.[4]

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Accompanying Tables S1 through S7 are available at http://jaha.ahajournals.org/content/6/8/e006545/DC1/embed/inline-supplementary-material-1.pdf

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Received May 3, 2017; accepted July 12, 2017.

Clinical Perspective

What Is New?

- We found similar significant associations between different measures of smoking behavior and higher GlycA and high-sensitivity C-reactive protein levels.

What Are the Clinical Implications?

- The US Food and Drug Administration New Tobacco Deeming Rule mandates evaluation of the safety of traditional and novel tobacco products.
- GlycA and high-sensitivity C-reactive protein appear to be among the most sensitive markers of cardiovascular injury associated with tobacco exposure.
- GlycA and high-sensitivity C-reactive protein can be used for the further study and regulation of all tobacco products.

Overt CVD is usually preceded by detectable subclinical cardiovascular injury, with 1 suggested mechanism being increased systemic inflammation.[5] Previous studies have shown that higher levels of some inflammatory markers are reflective of smoking exposure, and hence, may be useful for the study and regulation of tobacco products. Several measures of smoking behavior are associated with higher levels of C-reactive protein (CRP), IL-6, and fibrinogen.[5–9]

GlycA, as measured by nuclear magnetic resonance spectroscopy, is a composite biomarker that shows the integrated concentrations of glycosylated states of circulating acute phase reactants, which are modulated by multiple cytokines secreted from activated neutrophils. These glycosylated acute phase reactants predominantly include α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin, and transferrin.[10] Glycosylation involves protein folding and stabilization, cellular adhesion, antigen recognition, and cell signaling. Therefore, GlycA shows established inflammatory states, as it shows both the concentration and molecular alterations of acute phase reactants.

Analytic imprecision and intraindividual variability of GlycA are lower than CRP as measured by high-sensitivity techniques (hsCRP), making GlycA a potentially more reliable biomarker for quantitative assessment of systemic inflammation.[10] Previous prospective studies have demonstrated that GlycA is positively associated with CVD, total cancer, and all-cause mortality after adjustment for hsCRP, IL-6, and D-dimer.[11,12]

No studies have evaluated the association between smoking and GlycA. In this cross-sectional study, we investigated whether smoking is associated with systemic inflammation as measured by GlycA levels. We also sought to compare the strength of association of smoking and GlycA versus the association of smoking and hsCRP, which is shown to be a sensitive candidate inflammatory biomarker of smoking exposure.[13]

The MESA (Multi-Ethnic Study of Atherosclerosis) and the ELSA-Brasil (Brazilian Longitudinal Study of Adult Health) have included modern cohorts, and share very similar study design and methodology for measuring serum biomarkers. For example, GlycA was measured for both MESA and ELSA-Brasil in the same site by the same staff. Therefore, we combined these 2 cohorts with different racial/ethnic distributions to increase the generalizability of results related to the association between smoking and inflammatory biomarkers.

Materials and Methods

Study Population

The study design and participant recruitment for MESA and ELSA-Brasil have been previously described.[14,15] Briefly, MESA and ELSA-Brasil are ethnically diverse, community-based, multisite, prospective cohort studies. Between July 2000 and August 2002, MESA recruited 6814 participants, aged 45 to 84 years at baseline, who were white, Chinese-American, black, and Hispanic enrolled from 6 centers in the United States: Forsyth County, North Carolina; New York City, New York; Baltimore, Maryland; St. Paul, Minnesota; Chicago, Illinois; and Los Angeles, California. Full details of the study methods are available at the MESA website (http://www.mesa-nhlbi.org).

ELSA-Brasil participants were 15 105 active or retired employees, aged 35 to 74 years at baseline, who were white, brown, black, Asian, and indigenous enrolled from 6 cities of Brazil (Belo Horizonte, Porto Alegre, Rio de Janeiro, Salvador, São Paulo, and Vitória) between 2008 and 2010. Brown race was referred to as racially/ethnically mixture of white, black, and to a lesser extent indigenous ancestry. The study design, methods, and recruitment of participants have been described previously.[15]

All MESA and ELSA-Brasil participants gave written informed consent, and both studies were approved by their corresponding Institutional Review Boards from all field centers. At the baseline visit, participants completed self-administered questionnaires, standardized interviews, and in-person examinations of medical history, anthropometric measurements, and laboratory data. All MESA participants and only ELSA-Brasil participants from the São Paulo site (N=5061) underwent laboratory testing for inflammatory biomarkers. MESA participants were free of clinical CVD at baseline. We excluded 316 participants in ELSA-Brasil who had prevalent CVD including self-reported diagnosis of myocardial infarction, congestive heart failure, stroke, and coronary revascularization. We excluded participants with

missing data regarding GlycA and hsCRP (N=28) or measures of smoking behavior (N=22). A final study population of 11 509 MESA-ELSA participants was used for this study. (Figure 1)

Ascertainment of Smoking Exposure

In both cohorts, smoking exposures were ascertained through self-report including past and current cigarette-smoking habits. The primary smoking variables were smoking status (never, former, current), smoking burden (among former and current smokers), time since quitting (among former smokers), and smoking intensity (among current smokers). Never-smoking status was defined as lifetime consumption of <100 cigarettes. Former smokers had a past history of smoking and had not smoked cigarettes within the past 30 days. Ever smokers included both former and current smokers. Smoking burden was defined as pack-years of smoking calculated by the average number of packs of cigarettes smoked per day multiplied by the duration of smoking in years. Time since quitting smoking was calculated by the difference between current age and age at which former smokers stopped smoking. Smoking intensity was defined as number of cigarettes smoked per day. Second-hand smoking was defined as self-reported smoking exposure at home, transportation, or work.

Measuring GlycA and hsCRP

In MESA and ELSA-Brasil, blood samples were collected at baseline after a 12-hour overnight fast and participants were recommended to abstain from smoking for 12 hours. Bloodworks were performed at all centers by staff, who had no knowledge of participants' characteristics.

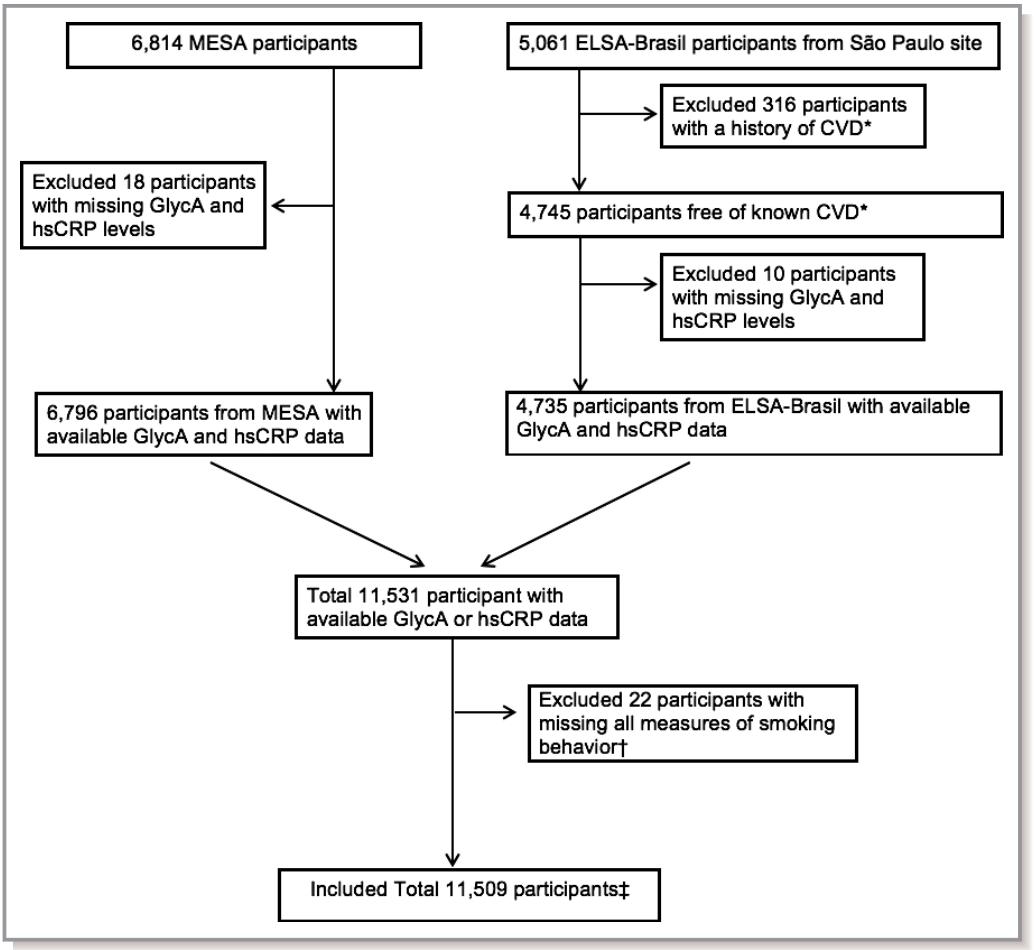

Figure 1. Flow diagram of enrollment of participants with available GlycA or hsCRP levels. *Including self-reported medical diagnosis of myocardial infarction, congestive heart failure, stroke, and coronary revascularization (coronary artery bypass graft or percutaneous coronary intervention). †All participants were from MESA. ‡Including 6774 MESA and 4735 ELSA-Brasil participants. CVD indicates cardiovascular disease; ELSA-Brasil, Brazilian Longitudinal Study of Adult Health; hsCRP, high-sensitivity C-reactive protein; MESA, Multi-Ethnic Study of Atherosclerosis.

In MESA and ELSA-Brasil, GlycA (μmol/L) was measured using nuclear magnetic resonance spectra acquired from ethylenediaminetetraacetic acid plasma samples used by LabCorp clinical laboratory in Raleigh, NC. The nuclear magnetic resonance Profiler platform consisted of a 9.4-T (400-MHz 1 H frequency) spectrometer with an integrated fluidic sample delivery system. Proprietary deconvolution software was used to quantify the GlycA signal.[16] The intra-assay and interassay coefficients of variability for GlycA measurement were 1.9% and 2.6%, respectively.[10]

In MESA, serum levels of hsCRP (mg/L) were measured at baseline using the BNII nephelometer (Dade Behring, Deerfield, IL) at the Core Lab at the University of Vermont, Burlington, Vermont. In ELSA-Brasil, hsCRP was measured using immunochemistry (nephelometry; Siemens) in São Paulo. Analytical intra-assay coefficients of variation of hsCRP ranged from 2.3% to 4.4% and interassay coefficients of variation ranged from 2.1% to 5.7%.

Additional Study Covariates

Sociodemographic characteristics such as age, sex, race/ethnicity, educational history, and other health and medical history, such as alcohol consumption, family history of myocardial infarction, and medication use, were ascertained through self-report. The anthropometric parameters, such as weight, height, and body mass index, were measured using standard equipment and techniques. Resting blood pressure and heart rate were measured 3 times with 1-minute intervals with subjects in the seated position after a 5-minute rest; an average of the second and third measurements was recorded for the analyses. Estimated glomerular filtration rate was calculated using the Cockcroft–Gault equation from obtained blood samples. Total cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured in the baseline blood samples following a 12-hour overnight fast. Low-density lipoprotein cholesterol was estimated using the Friedewald equation when the triglyceride level was lower than 400 mg/dL. Low-density lipoprotein cholesterol was measured directly if triglyceride levels were >400 mg/dL.

Statistical Analyses

We used plots and Kolmogorov–Smirnov test of normality to examine for normal distribution of data. For comparison of discrete variables across the smoking status groups, χ^2 test was performed. To compare normally distributed continuous variables, mean±SD was calculated and the ANOVA test was performed. For skewed variables, medians and interquartile ranges were reported and Kruskal–Wallis tests were performed.

The associations of smoking variables with GlycA were performed using multivariable linear regression models with the output of β-coefficients indicating difference in mean GlycA between former and current versus never smokers and with the unit increase in pack-years, years since quit smoking, and number of cigarettes smoked per day. Two models were used: First, a limited model with demographic characteristics including age, sex, race, and education. Second, a multivariable adjusted model including variables in model 1 plus alcohol use, studied cohort, body mass index, systolic blood pressure, estimated glomerular filtration rate, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, family history of heart disease, history of diabetes mellitus, and use of antihypertensive, hypoglycemic, statin, and aspirin medications.

Consistent with prior reports,[5] for smoking burden, we evaluated the difference in GlycA levels for every 5-year increase in pack-years of smoking among ever, former, and current smokers. We assessed the difference in GlycA levels for every 10-year increase in time since quitting smoking among former smokers and 10-unit increase in number of cigarettes per day among current smokers adjusting for duration of smoking.

Pearson correlation coefficients were calculated to evaluate the relationship between GlycA and hsCRP levels. To compare the results for strength of associations of smoking with GlycA and hsCRP, we sought to create a similar scale for the biomarkers by ln-transforming both variables and comparing per SD change for each with measures of smoking behavior. To calculate T-statistics, we divided β-coefficients by the SE for each measure of smoking behavior. T-statistics reveal the size of difference in GlycA and hsCRP levels relative to their corresponding variability in the sample size, thus correlating with percent of the variability in the marker explained.[17] Restricted cubic splines were used in multivariable adjusted linear regression models to graph the associations between log-transformed inflammatory biomarkers and smoking burden and intensity.

We secondarily evaluated the association between secondhand smoking (yes versus no) and GlycA with and without adjusting for smoking burden stratified by smoking status. Interaction terms were evaluated between measures of smoking behavior and age, sex, education, and studied cohorts in their associations with GlycA and hsCRP. We evaluated whether results are consistent after further adjustment for waist circumference and prevalent inflammatory disease. Finally, we assessed whether hsCRP mediated the association between smoking and GlycA by adding hsCRP to the models.

All analyses were conducted using Stata 14. A P value of <0.05 was considered statistically significant (2-sided).

Table 1. Baseline Characteristics of the Study Population Including MESA and ELSA Brasil Participants Free of Prevalent CVD (N=11 509) by Categories of Smoking Status

	Smoking Status				
Variable	Total Population (N=11 509)	Never Smokers (n=5947)	Former Smokers (n=3913)	Current Smokers (n=1649)	*P* Value
Age, y	57.7±11.1	57.2±11.6	59.7±10.7	54.8±9.3	<0.001
Male, %	46.4	38.9	56.1	50.0	<0.001
Race,* %					<0.001
White	47.1	45.2	51.8	42.9	
Brown (mixed)	8.7	8.9	7.2	11.6	
Black	21.9	19.5	22.9	28.6	
Hispanic	13.1	12.8	12.5	12.4	
Asian	8.8	12.5	5.2	3.7	
Indigenous	0.5	0.30	0.5	0.9	
Cigarettes/d	15.6±15.4	0±0	16.5±14.3	13.2±13.8	<0.001
Duration of smoking, y	26.3±13.9	0±0	21.9±13.0	36.7±10.1	<0.001
Pack-y of smoking	10.0±18.9	0±0	19.7±23.3	23.7±21.3	<0.001
Years since quitting*	…	…	20.1±12.8	…	…
Current alcohol use, %	68.4	66.1	68.2	75.6	<0.001
Body mass index, kg/m^2	27.9±5.3	27.8±5.3	28.4±5.2	27.2±5.0	<0.001
Systolic blood pressure, mm Hg	123.6±19.8	123.4±19.9	124.8±19.4	121.6±20.3	<0.001
Diastolic blood pressure, mm Hg	73.2±10.5	72.9±10.4	73.6±10.5	73.6±11.3	<0.001
eGFR, mL/min per 1.73 m^2	88.5±26.8	87.2±26.9	88.5±26.8	99.9±26.7	<0.001
Antihypertensive medication use, %	28.8	29.0	31.4	22.2	<0.001
Statin use, %	13.2	12.7	15.3	10.1	<0.001
Steroid use, %	2.1	2.1	2.4	1.5	<0.001
NSAID use, %	10.7	9.5	12.3	10.7	<0.001
Total cholesterol, mg/dL	202.2±39.3	202.4±37.8	200.7±39.7	204.6±43.3	<0.001
Triglycerides, mg/dL	112 (79, 161)	109 (78, 157)	114 (78, 162)	122 (86, 179)	<0.001
LDL-C, mg/dL	122.8±33.3	123.2±32.7	121.3±32.7	124.6±36.5	0.002
HDL-C, mg/dL	53.2±14.9	53.9±14.7	52.8±15.2	51.2±14.5	<0.001
Diabetes mellitus, %	11.3	11.1	11.4	12.1	0.552
Family history of MI*, %	30.2	27.9	33.2	31.0	<0.001

Continuous variables presented as means (SDs) while categorical variables are presented as percentages. *P* value for continuous variables was calculated using 1-way ANOVA and for categorical variables using the χ^2 test among never, former, and current smokers. Numbers may not add up to total because of missing data and may not round up to 100% because of rounding. CVD indicates cardiovascular disease; eGFR, estimated GFR; ELSA-Brasil, Brazilian Longitudinal Study of Adult Health; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MESA, Multi-Ethnic Study of Atherosclerosis; MI, myocardial infarction; NSAID, nonsteroidal anti-inflammatory drug.

Results

A total of 11 509 MESA-ELSA participants aged 57.7±11.1, with 46.4% men, were included in this study. Of this total, 5947 were never smokers (51.7%), 3913 former smokers (34.0%), and 1649 current smokers (14.3%). Former smokers were older (59.7±10.7) than never (57.2±11.6) and current smokers (54.8±9.3) (*P*<0.001) and were more likely to be men (56.1%) (*P*<0.001). Most former smokers were white (51.8%). Blacks comprised 28.6% of current smokers, but 19.5% and 22.9% of never and former smokers, respectively (*P*<0.001). Number of cigarettes per day was higher among former (16.5±14.3) than current smokers (13.2±13.8) (*P*<0.001). However, duration and pack-years of smoking were higher among current smokers,

compared with former smokers ($P<0.001$). More participants among former (68.2%) and current smokers (75.6%) consumed alcohol at the time of examination compared with never smokers. Former smokers had higher body mass index (28.4±5.2 kg/m^2) compared with never (27.8±5.3 kg/m^2) and current smokers (27.2±5.0 kg/m^2, $P<0.001$). However, triglyceride and low-density lipoprotein cholesterol levels were higher among current smokers than former and never smokers. Other anthropometric and clinical characteristics of participants are summarized in Table 1. Characteristics of participants in MESA and ELSA-Brasil are also summarized in Tables S1 and S2, respectively.

Smoking and GlycA

GlycA levels were normally distributed. Mean GlycA levels for never, former, and currents smokers were 391.4±65.2 μmol/L, 392.7±66.5 μmol/L, and 414.3±70.6 μmol/L, respectively. Compared with never smokers, former and current smokers had significantly higher adjusted means of GlycA levels in the multivariable models (4.1 [95% CI, 1.7–6.6] μmol/L and 19.9 [95% CI, 16.6–23.2] μmol/L, respectively). Each 5-unit increase in pack-years of smoking was associated with higher GlycA levels among former (0.7 [95% CI, 0.3–1.1] μmol/L) and current smokers (1.6 [95% CI, 0.8–2.4] μmol/L). There was a negative association between GlycA and time since quitting (−1.6 [95% CI, −2.4 to −0.8] μmol/L). Also, after controlling for duration of smoking, each 10-unit increase in number of cigarettes smoked per day was associated with higher GlycA levels among current smokers (3.3 [95% CI, 0.8–5.8] μmol/L, Table 2).

Comparing the Associations of Smoking With ln-GlycA and ln-hsCRP

There was a significant moderate correlation between GlycA and hsCRP ($r=0.43$; $P<0.001$). Results were significant for the association between all measures of smoking behavior and ln-GlycA and ln-hsCRP. Compared with ln-hsCRP, absolute values of T-statistics were nominally higher for ln-GlycA with regard to former and current smoking status, pack-years of smoking among ever and former smokers, and time since quitting (Table 3). T-statistics were similar for pack-years of smoking and number of cigarettes smoked per day among current smokers. Multivariable-adjusted restricted cubic splines showed similar trends for ln-GlycA and ln-hsCRP levels with increasing pack-years of smoking and number of cigarettes smoked per day (Figure 2).

Secondary and Sensitivity Analyses

There was an interaction between secondhand smoking and smoking status in their association with GlycA levels. Further analyses demonstrated that GlycA levels were higher for those with secondhand exposure among the whole cohort (3.00 [95% CI, 0.62–5.40] μmol/L) and former smokers (4.88 [95% CI, 0.98–8.78] μmol/L) in the multivariable-adjusted model, as compared with those without secondhand exposure

Table 2. Multivariable Adjusted Baseline Mean Absolute Difference With 95% CIs in GlycA Levels (μmol/L) Among Former and Current Smokers by Different Modes of Smoking Exposure in a Cohort Including MESA and ELSA-Brasil Participants

	Former Smokers			Current Smokers	
Exposure	β (95% CI)		*P* Value	β (95% CI)	*P* Value
Smoking status (compared with never smokers)	Model 1*	4.2 (1.6–6.8)	0.002	19.4 (15.9–22.9)	<0.001
	Model 2†	4.1 (1.7–6.6)	0.001	19.9 (16.6–23.2)	<0.001
Pack-y of smoking (per 5-unit increase)	Model 1	1.0 (0.5–1.4)	<0.001	1.5 (0.6–2.3)	0.001
	Model 2	0.7 (0.3–1.1)	0.002	1.6 (0.8–2.4)	<0.001
Time since quitting (per 5-y increase)‡	Model 1	−2.6 (−3.5 to −1.8)	<0.001		…
	Model 2	−1.6 (−2.4 to −0.8)	<0.001	…	…
Number of cigarettes/d (per 10-unit increase)	Model 1	…	…	3.3 (0.8–5.8)	0.011
	Model 2	…	…	2.8 (0.5–5.2)	0.020

CIs indicates confidence intervals; ELSA-Brasil, Brazilian Longitudinal Study of Adult Health; MESA, Multi-Ethnic Study of Atherosclerosis.
*Model 1 was adjusted for age, sex, race, and education.
†Model 2 was adjusted for model 1 variables studied cohort, body mass index, estimated glomerular filtration rate, systolic blood pressure, low-density lipoprotein cholesterols, high-density lipoprotein cholesterols, triglycerides, family history of heart disease, history of diabetes mellitus, and use of antihypertensive, hypoglycemic, statin, and nonsteroidal anti-inflammatory drugs, and steroids.
‡Also adjusted for durations of smoking for both Model 1 and Model 2.

Table 3. Multivariable-Adjusted Baseline Mean Absolute Difference With 95% CIs for Naturally Log-Transformed GlycA (ln-GlycA) vs ln-hsCRP Levels by Different Modes of Smoking Exposure in a Cohort Including MESA and ELSA-Brasil Participants

	Ln-GlycA			Ln-hsCRP		
Exposure	β-Coeff (95% CI)	T Statistics	*P* Value	β-Coeff (95% CI)	T Statistics	*P* Value
Smoking status						
Never	REF	…	…	REF	…	…
Former	1.009 (1.003–1.015)	2.91	0.004	1.050 (1.006–1.097)	2.23	0.026
Current	1.050 (1.042–1.059)	11.86	<0.001	1.269 (1.198–1.345)	8.03	<0.001
Smoking burden*						
Ever smokers†	1.003 (1.002–1.004)	6.46	<0.001	1.018 (1.012–1.025)	5.45	<0.001
Former smokers	1.002 (1.000–1.003)	3.43	0.001	1.009 (1.002–1.017)	2.49	0.013
Current smokers	1.004 (1.002–1.006)	3.91	<0.001	1.029 (1.015–1.043)	4.15	<0.001
Years since quitting smoking†						
Per 5-y increase	0.996 (0.994–0.998)	−4.30	<0.001	0.976 (0.963–0.991)	−3.27	0.001
Smoking intensity‡						
Per 10-cigarettes/d increase	1.007 (0.001–0.012)	2.47	0.014	1.050 (1.011–1.092)	2.53	0.011

CIs indicates confidence intervals; ELSA-Brasil, Brazilian Longitudinal Study of Adult Health; ln-hsCRP, naturally log-transformed high-sensitivity C-reactive protein; MESA, Multi-Ethnic Study of Atherosclerosis.

Models were adjusted for age, sex, race, education, studied cohort, body mass index, estimated glomerular filtration rate, systolic blood pressure, low-density lipoprotein cholesterols, low-density lipoprotein cholesterols, triglycerides, family history of heart disease, history of diabetes mellitus, and use of antihypertensive, hypoglycemic, statin, and nonsteroidal anti-inflammatory drugs, and steroids.

*For every 5-unit increase in pack-y of smoking among current and former smokers.

†For every 5-y increase in time since quitting smoking among former smokers.

‡For every 10-unit increase in number of cigarettes/d among current smokers. Also adjusted for duration of smoking.

(Table 4). For GlycA, among current smokers there were significant interactions between sex and smoking burden ($P_{Interaction}$=0.036), and sex and smoking intensity ($P_{Interaction}$=0.011), such that with the increase in pack-years of smoking and number of cigarettes per day, female smokers had higher GlycA levels than male smokers. For hsCRP, there was an interaction between age and smoking status ($P_{Interaction}$<0.001), such that among former smokers, hsCRP levels were higher for those older than 55, but not younger participants. Among current smokers, male smokers had higher hsCRP levels, as compared with females ($P_{Interaction}$=0.001). However, there was a stronger association between smoking intensity and hsCRP among females than males ($P_{Interaction}$=0.027). No interaction was found between different measures of smoking behavior and race/ethnicity in their association with GlycA and hsCRP (Tables S3 and S4). There was also no interaction between any measures of smoking behaviors and studied cohorts in these associations. Consistent with this, stratified analyses showed similar results for MESA (Table S5) and ELSA-Brasil (Table S6) cohorts. Results remained significant after further adjustment for waist circumference and prevalent inflammatory disease. Including ln-hsCRP in multivariable models attenuated associations between measures of smoking behavior and GlycA, but results remained significant (Table S7).

Discussion

In this study of asymptomatic individuals from clinical CVD in a combined MESA and ELSA-Brasil cohort, we demonstrated significant associations between all measures of smoking behavior, including smoking status, intensity, burden, and secondhand smoking, and 2 biomarkers of subclinical inflammation, GlycA and hsCRP. Our results are the first to suggest a strong relationship between smoking and the emerging inflammatory biomarker GlycA.

Our study has several strengths. We increased the generalizability of our findings by combining 2 multiethnic populations including about 11 500 participants from 2 cohort studies that shared similar design and methods. Smoking behaviors were well characterized. Our complete data allowed head-to-head comparisons of unitless log-transformed biomarker levels, demonstrating that GlycA may be a useful biomarker in addition to hsCRP for quantifying subclinical inflammation following tobacco exposure.

Atherothrombotic CVD is described as a chronic inflammatory condition, which involves processes mediated by cytokines and acute phase reactants.[18] Indeed, higher CVD risk in chronic inflammatory conditions has been shown to be associated with higher GlycA levels.[19–21] In addition, GlycA is

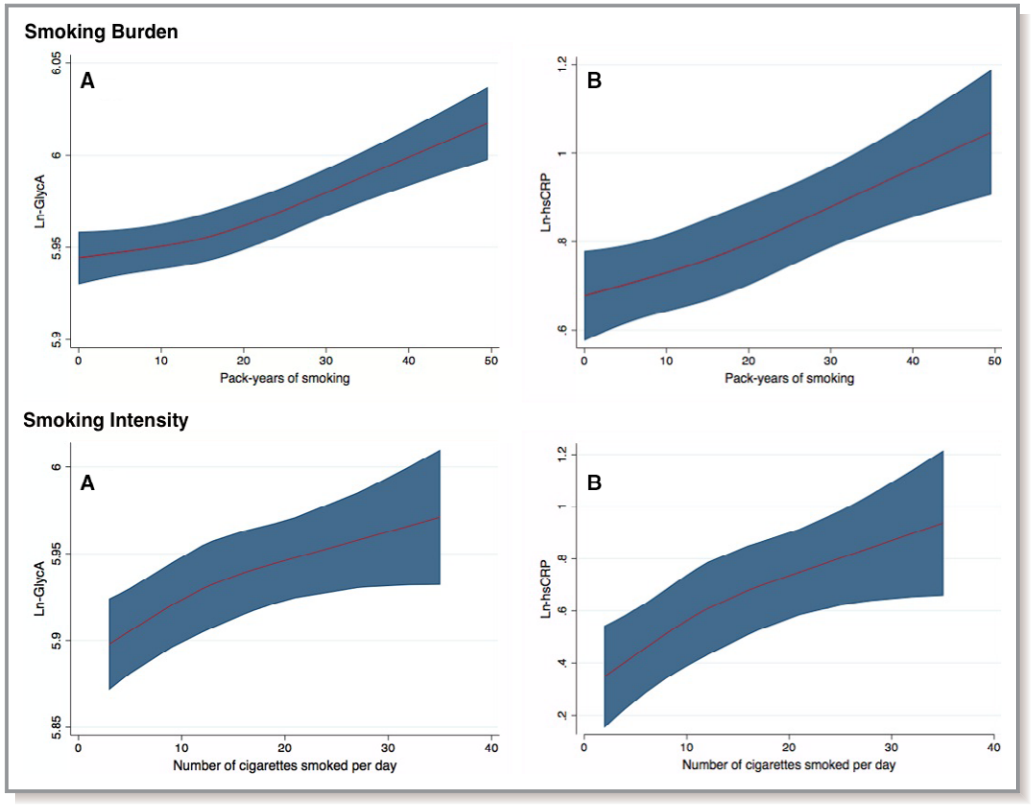

Figure 2. Restricted cubic spline graphs showing the association between smoking burden (as measured by pack-years of smoking) and smoking intensity (as measured by number of cigarettes smoked per day), and (A) log-transformed GlycA and (B) log-transformed hsCRP. Results were adjusted for age, sex, race, education, studied cohort, body mass index, systolic blood pressure, estimated glomerular filtration rate, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, family history of heart attack, blood pressure medications, statins, steroids, nonsteroidal anti-inflammatory drugs, and history of diabetes mellitus. Intensity models were further adjusted for duration of smoking. Blue shadows indicate 95% confidence intervals. hsCRP indicates high-sensitivity C-reactive protein

shown to be associated with other measures of subclinical CVD. A recent study including 3783 asymptomatic participants from ELSA-Brasil demonstrated that GlycA is associated with coronary artery calcium independent of hsCRP.[22] In our study, smokers free of clinical CVD had higher GlycA levels that reinforce the underlying subclinical CVD related to tobacco consumption. These findings support the role of GlycA as a sensitive biomarker for gauging the detrimental cardiovascular effects of tobacco, even for remote exposure.

We found a moderate positive correlation between GlycA and hsCRP. Previous studies have shown that there is a progressive increase in the CVD risk with the increase in both GlycA and hsCRP beyond traditional CVD risk factors and independent of each other.[11,12,23,24] A recent study by

Table 4. Baseline Mean Absolute Difference With 95% CIs in Levels of GlycA (μmol/L) by Secondhand Smoking Status Among MESA and ELSA-Brasil Participants

Secondhand Smoking	All		Former Smokers		Current Smokers		Never Smokers	
	β-Coeff (95% CI)	*P* Value	β-Coeff (95% CI)	*P* Value	β-Coeff (95% CI)	*P* Value	β-Coeff (95% CI)	*P* Value
Model 1	0.27 (−2.27 to 2.81)	0.834	0.58 (−3.57 to 4.73)	0.783	−6.66 (−15.68 to 2.37)	0.148	−0.61 (−4.03 to 2.82)	0.729
Model 2	3.00 (0.62 to 5.40)	0.014	4.88 (0.98 to 8.78)	0.014	−2.82 (−11.44 to 5.79)	0.520	0.31 (−2.96 to 3.56)	0.854

CIs indicates confidence intervals; ELSA-Brasil, Brazilian Longitudinal Study of Adult Health; MESA, Multi-Ethnic Study of Atherosclerosis.
Model 1 is adjusted for age, sex, race, and education. Model 2 is adjusted for Model 1 plus alcohol use, studied cohort, body mass index, systolic blood pressure, estimated glomerular filtration rate, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, family history of heart disease, history of diabetes mellitus, and use of antihypertensive, hypoglycemic, statin, and nonsteroidal anti-inflammatory drugs, and steroids.

McGarrah et al showed that GlycA could independently predict coronary artery disease, all-cause death, CVD death, and non-CVD death in models adjusted for 10 CVD risk factors.[24] Another study showed that GlycA is associated with similar CVD and non-CVD outcomes after adjusting for several inflammatory markers including hsCRP, IL-6, and D-dimer.[11] Similarly, in our study, we found persistent associations between smoking and GlycA when hsCRP was added to the models. Therefore, despite some overlap between hsCRP and GlycA, they seemingly reflect different and complementary dimensions of the complex inflammatory cascade. On the other hand, CRP is a downstream inflammatory marker produced by liver and cells in atherosclerotic plaques in response to IL-6 production, and may be found when inflammation is well established.[25] One advantage of GlycA over other inflammatory markers, such as hsCRP, is that GlycA has higher reliability. Otvos et al showed that intraindividual variability of GlycA (4.3%) was lower than that of hsCRP (29.2%), following weekly measurements in a 35-day study.[10]

The recent US Food and Drug Administration deeming rule mandates evaluation of the safety of traditional and novel tobacco products such as electronic nicotine delivery systems, whose prevalence is considerably increasing and potentially pose a considerable health concern worldwide. This study supports that GlycA and hsCRP are robust candidate markers of the potential CVD toxicity of tobacco exposure. Future studies should seek to demonstrate whether novel tobacco products, such as electronic nicotine delivery systems, have negative health effects by similar mechanisms. Therefore, the sensitive inflammatory biomarkers identified in this report may be used for the study and regulation of novel tobacco products such as electronic cigarettes, before results from prospective clinical event data become available.

One limitation of this study is the potential for residual confounding. However, we adjusted for many CVD risk factors, anthropometric measures, and medications that are most likely to produce confounded associations. Also, this cross-sectional study was not able to show the causal relationship between smoking and inflammation. We could not capture non-tobacco-related environmental exposures and the change in tobacco habits over time, which may have potentially impacted the studied inflammatory markers. In addition, although participants were told to abstain from smoking for at least 12 hours before examination, their adherence to smoking abstinence and time since last smoke was not accurately known. Finally, cotinine levels were not measured in ELSA-Brasil, although a previous MESA indicated that there is a high correlation between self-report of smoking and urine cotinine levels, with only 1.2% of current smokers being misclassified as never smokers. Therefore, the self-report method of data collection regarding smoking behavior is shown to be acceptable in cohort studies.[26]

Conclusions

Both GlycA and hsCRP appear to be reliable and sensitive biomarkers of subclinical cardiovascular injury associated with exposure to combustible tobacco cigarettes. Further study is needed to confirm whether these biomarkers remain robust for the measurement of potential cardiovascular toxicity of novel tobacco products, such as e-cigs, which are becoming increasingly more prevalent worldwide.

Acknowledgments

We thank the other investigators, staff, and participants of the MESA and ELSA-Brasil for their valuable contributions.

Sources of Funding

This study was supported by funding from the American Heart Association Tobacco Regulation and Addiction Center (A-TRAC, NIH 1 P50 HL120163-01), a member of the FDA Tobacco Centers of Regulatory Science for Research Relevant to the Family Smoking Prevention and Tobacco Control Act (P50). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the FDA. The MESA baseline study was supported by contracts N01-HC-95 159, N01- HC-95 160, N01-HC-95 161, N01-HC-95 162, N01-HC-95 163, N01- HC-95 164, N01-HC-95 165, N01-HC-95 166, N01-HC-95 167, N01- HC-95 168, and N01-HC-95 169 from the National Heart, Lung, and Blood Institute and by grants UL1-TR-000 040 and UL1-TR-001 079 from NCRR. The ELSA-Brasil baseline study was supported by the Brazilian Ministry of Health (Science and Technology Department) and the Brazilian Ministry of Science and Technology and National Counsel of Technological and Scientific Development (CNPq)—National Research Council (grants no. 01 06 0010.00 RS, 01 06 0212.00 BA, 01 06 0300.00 ES, 01 06 0278.00 MG, 01 06 0115.00 SP, and 01 06 0071.00 RJ).

Disclosures

Dr Lotufo receives honoraria (modest) from AbbVie Brazil. The remaining authors have no disclosures to report.

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SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics of the study population including The Multi-Ethnic Study of Atherosclerosis (MESA) participants free of prevalent cardiovascular disease (N=6,774) by categories of smoking status

	Smoking status				
Variable	**Total population (N=6,774)**	**Never smokers (n=3,409)**	**Former smokers (n=2,484)**	**Current smokers (n=881)**	**P-value**
Age, years	62.2 ± 10.2	62.2 ± 10.5	63.5 ± 9.8	58.2 ± 9.1	<0.001
Male, %	47.2	38.0	57.9	52.8	<0.001
Race*, %					<0.001
White (Caucasian)	38.5	33.9	46.5	33.9	
Brown (mixed)	---	---	---	---	
Black (African American)	27.6	24.7	27.8	38.0	
Hispanic	22.1	23.7	19.6	22.9	
Asian	11.8	17.7	6.2	5.1	
Indigenous	---	---	---	---	
College level education or higher, %	63.8	61.5	68.0	60.6	<0.001
Cigarettes/day	15.6 ± 15.4	0 ± 0	16.5 ± 14.3	13.2 ± 13.8	<0.001
Duration of smoking, years	27.5 ± 14.9	0 ± 0	23.1 ± 13.8	39.6 ± 10.5	<0.001
Pack-years of smoking	11.3 ± 20.9	0 ± 0	21.7 ± 25.1	26.7 ± 23.9	<0.001
Years since quitting*	---	---	23.1 ± 13.7	---	---
Current alcohol use, %	68.5	68.5	66.6	74.4	<0.001
Body mass index, kg/m^2	28.3 ± 5.5	28.1 ± 5.5	28.8 ± 5.5	28.0 ± 5.3	<0.001
Systolic blood pressure, mmHg	126.6 ± 21.5	126.9 ± 21.8	127.1 ± 20.9	123.7 ± 21.7	<0.001
Diastolic blood pressure, mm Hg	71.9 ± 10.3	71.5 ± 10.1	72.3 ± 10.2	72.4 ± 11.0	<0.001
eGFR	85.8 ± 28.5	83.4 ± 27.5	88.5 ± 29.6	93.0 ± 28.4	<0.001
Antihypertensive medication use, %	33.2	34.2	34.0	26.7	<0.001
Statin use, %	14.9	14.6	16.6	11.0	<0.001
Steroid use, %	1.6	1.4	1.8	1.4	<0.001
NSAID use, %	17.5	16.1	18.9	18.8	<0.001
Total cholesterol, mg/dL	194.1 ± 35.7	195.8 ± 35.3	192.5 ± 34.9	192.4 ± 39.1	<0.001

Triglycerides, mg/dL	111 (78, 161)	111 (79, 161)	109 (76, 157)	118.5 (84, 172)	<0.001
LDL-C, mg/dL	117.2 ± 31.4	118.1 ± 31.3	116.4 ± 30.1	115.7 ± 33.4	0.002
HDL-C, mg/dL	50.9 ± 14.8	51.7 ± 14.7	50.8 ± 15.1	48.3 ± 14.3	<0.001
Diabetes mellitus, %	5.5	5.7	5.1	5.9	0.552
Family history of MI* (%)	42.74	40.3	45.3	45.0	<0.001

Abbreviations: BMI, body mass index; eGFR, estimated GFR; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MI, myocardial infarction; and NSAID, non-steroidal anti-inflammatory drug.

Continuous variables presented as means (standard deviations) while categorical variables are presented as percentages.

P-value for continuous variables was calculated using one-way ANOVA and for categorical variables using the chi-square test among never, former, and current smokers.

Numbers may not add up to total due to missing and may not round up to 100% due to rounding.

Table S2. Baseline characteristics of the study population including The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) participants free of prevalent cardiovascular disease (N=4,735) by categories of smoking status

	Smoking status				
Variable	**Total population (N=4,735)**	**Never smokers (n=2,538)**	**Former smokers (n=1,429)**	**Current smokers (n=768)**	**P-value**
Age, years	51.2 ± 8.9	50.4 ± 9.3	53.0 ± 8.6	50.9 ± 7.7	<0.001
Male, %	45.2	40.2	53.0	53.1	<0.001
Race*, %					<0.001
White (Caucasian)	59.6	60.6	61.0	53.4	
Brown (mixed)	21.3	20.9	19.9	25.0	
Black (African American)	13.8	12.4	14.3	17.5	
Hispanic	---	---	---	---	
Asian	4.3	5.4	3.5	2.1	
Indigenous	1.1	0.7	1.4	2.0	
College level education or higher, %	45.3	51.1	43.1	29.8	<0.001
Cigarettes/day	14.7 ± 12.0	0 ± 0	15.8 ± 13.1	12.7 ± 9.1	<0.001
Duration of smoking, years	24.6 ± 12.2	0 ± 0	19.9 ± 11.2	33.3 ± 8.6	<0.001
Pack-years of smoking	8.1 ± 15.4	0 ± 0	16.3 ± 19.2	20.2 ± 17.3	<0.001
Years since quitting*	---	---	16.0 ± 10.7	---	---
Current alcohol use, %	68.2	63.9	70.9	77.0	<0.001
Body mass index, kg/m^2	27.3 ±4.9	27.3 ± 5.0	27.9 ± 4.7	26.3 ± 4.5	<0.001
Systolic blood pressure, mmHg	119.4 ± 16.3	118.7 ± 15.8	120.8 ± 15.9	119.1 ± 18.2	<0.001
Diastolic blood pressure, mm Hg	75.2 ± 11.1	73.5 ± 12.1	74.1 ± 11.1	74.2 ± 11.3	<0.001
eGFR	92.3 ± 25.0	92.4 ± 27.3	91.9 ± 24.6	92.8 ± 34.2	0.706
Antihypertensive medication use, %	22.6	22.0	26.8	17.1	<0.001
Statin use, %	10.9	10.2	13.0	9.0	0.006
Steroid use, %	2.9	2.9	3.4	1.7	0.078
NSAID use, %	0.9	0.8	0.8	1.3	0.402
Total cholesterol, mg/dL	213.6 ± 41.4	211.3 ± 39.3	215.0 ± 43.3	218.6 ± 43.8	<0.001

Triglycerides, mg/dL	113 (80, 162)	106 (76, 151)	122 (84, 176)	126 (88.5, 185)	<0.001
LDL-C, mg/dL	130.6 ± 34.3	129.8 ± 33.3	129.9 ± 34.1	134.6 ± 37.5	0.002
HDL-C, mg/dL	56.3 ± 14.3	56.9 ± 14.2	56.1 ± 14.8	54.6 ± 13.9	<0.001
Diabetes mellitus, %	19.6	18.3	22.3	19.1	0.009
Family history of MI* (%)	13.1	12.2	13.3	15.6	0.05

Abbreviations: BMI, body mass index; eGFR, estimated GFR; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; MI, myocardial infarction; and NSAID, non-steroidal anti-inflammatory drug.

Continuous variables presented as means (standard deviations) while categorical variables are presented as percentages.

P-value for continuous variables was calculated using one-way ANOVA and for categorical variables using the chi-square test among never, former, and current smokers.

Numbers may not add up to total due to missing and may not round up to 100% due to rounding.

Table S3. Stratified analyses by age, sex, race and education for GlycA where interaction P-values were < 0.05 by smoking status, burden, years since quitting and intensity

	Overall	**P-value**	**Males**	**P-value**	**Females**	**P-value**
Never	REF		REF		REF	
Former	4.17 (1.72 – 6.62)	**0.001**	3.64 (0.15 – 7.14)	0.041	4.50 (1.03 – 7.97)	0.011
Current	19.6 (16.26 – 22.86)	**<0.001**	21.61 (16.83 – 26.37)	**<0.001**	16.01 (11.43 – 20.59)	**<0.001**
Pack-years of smoking*	1.14 (0.77 – 1.51)	**<0.001**	1.39 (0.94 – 1.84)	**<0.001**	0.84 (0.22 – 1.46)	**0.008**
Years since quit†	-1.63 (-2.43 – -0.83)	**<0.001**	-2.48 (-3.48 – -1.48)	**<0.001**	-0.37 (-1.67 – 0.93)	**0.572**
Smoking intensity‡	3.33 (0.98 – 5.68)	**0.006**	2.53 (-0.14 – 5.19)	**0.063**	9.38 (3.94 – 14.82)	**0.001**

	Overall	**P-value**	**Age<55**	**P-value**	**Age≥55**	**P-value**
Never	REF		REF		REF	
Former	4.17 (1.72 – 6.62)	**0.001**	5.73 (1.88 – 9.58)	0.004	3.38 (0.17 – 6.58)	0.039
Current	19.6 (16.26 – 22.86)	**<0.001**	16.86 (12.31 – 21.42)	**<0.001**	23.37 (18.52 – 28.26)	**<0.001**
Pack-years of smoking*	1.14 (0.77 – 1.51)	**<0.001**	1.44 (0.68 – 2.21)	**<0.001**	1.08 (0.66 – 1.50)	**<0.001**
Years since quit†	-1.63 (-2.43 – -0.83)	**<0.001**	-1.21 (-2.94 – 0.51)	**0.168**	-1.77 (-2.66 – -0.87)	**<0.001**
Smoking intensity†	3.33 (0.98 – 5.68)	**0.006**	2.49 (-0.18 – 5.16)	**0.068**	5.57 (0.73 – 10.41)	**0.024**

	Overall	**P-value**	**High school or less**	**P-value**	**More than high school**	**P-value**
Never	REF		REF		REF	
Former	4.17 (1.72 – 6.62)	**0.001**	6.02 (1.97 – 10.07)	0.004	2.21 (-0.85 – 5.28)	0.50
Current	19.6 (16.26 – 22.86)	**<0.001**	19.18 (14.27 – 24.08)	**<0.001**	19.34 (14.81 – 23.87)	**<0.001**
Pack-years of smoking*	1.14 (0.77 – 1.51)	**<0.001**	1.54 (0.93 – 2.16)	**<0.001**	0.88 (0.43 – 1.35)	**<0.001**
Years since quit†	-1.63 (-2.43 – -0.83)	**<0.001**	-2.47 (-3.77 – -1.16)	**<0.001**	-1.23 (-2.24 – -0.22)	**0.017**
Smoking intensity‡	3.33 (0.98 – 5.68)	**0.006**	7.33 (1.89 – 12.76)	**0.008**	2.21 (-0.27 – 4.68)	**0.080**

	Overall	P-value	Whites	P-value	Non-Whites	P-value
Never	REF		REF		REF	
Former	4.17 (1.72 – 6.62)	**0.001**	5.25 (1.89 – 8.60)	**0.002**	2.89 (-0.70 – 6.48)	0.24
Current	19.6 (16.26 – 22.86)	**<0.001**	19.78 (14.98 – 24.58)	**<0.001**	18.97 (14.41 – 23.54)	**<0.001**
Pack-years of smoking*	1.14 (0.77 – 1.51)	**<0.001**	1.17 (0.70 – 1.64)	**<0.001**	1.11 (0.51 – 1.71)	**<0.001**
Years since quit†	-1.63 (-2.43 – -0.83)	**<0.001**	-1.39 (-2.50 – -0.27)	**0.015**	-1.82 (-2.98 – -0.66)	**0.002**
Smoking intensity‡	3.33 (0.98 – 5.68)	**0.006**	2.81 (0.21 – 5.41)	**0.034**	6.06 (0.67 – 11.46)	**0.028**

	Overall	P-value	Browns	P-value	Non-Browns	P-value
Never	REF		REF		REF	
Former	4.17 (1.72 – 6.62)	**0.001**	2.98 (-6.27 – 12.34)	0.527	4.29 (1.75 – 6.84)	0.001
Current	19.6 (16.26 – 22.86)	**<0.001**	18.84 (8.13 – 29.55)	**0.001**	19.79 (16.31 – 23.28)	**<0.001**
Pack-years of smoking*	1.14 (0.77 – 1.51)	**<0.001**	0.96 (-0.86 – 2.79)	**0.300**	1.16 (0.79 – 1.54)	**<0.001**
Years since quit†	-1.63 (-2.43 – -0.83)	**<0.001**	-3.88 (-7.68 – -0.08)	**0.046**	-1.54 (-2.36 – -0.73)	**<0.001**
Smoking intensity‡	3.33 (0.98 – 5.68)	**0.006**	12.23 (-1.02 – 25.48)	**0.070**	3.05 (0.68 – 5.43)	**0.012**

	Overall	P-value	Blacks	P-value	Non-Blacks	P-value
Never	REF		REF		REF	
Former	4.17 (1.72 – 6.62)	**0.001**	4.49 (-1.16 – 10.14)	0.119	4.14 (1.42 – 6.86)	**0.003**
Current	19.6 (16.26 – 22.86)	**<0.001**	26.59 (19.52 – 33.66)	**<0.001**	17.10 (13.35 – 20.85)	**<0.001**
Pack-years of smoking*	1.14 (0.77 – 1.51)	**<0.001**	1.13 (0.27 – 1.98)	0.010	1.16 (0.76 – 1.57)	**<0.001**
Years since quit†	-1.63 (-2.43 – -0.83)	**<0.001**	-1.24 (-2.95 – 0.47)	**0.154**	-1.75 (-2.66 – -0.85)	**<0.001**
Smoking intensity‡	3.33 (0.98 – 5.68)	**0.006**	4.82 (-2.53 – 12.17)	0.199	3.20 (0.73 – 5.66)	0.011

*For every 5-unit increase among current and former smokers

†For every 5-year increase in time since quitting smoking among former smokers

‡For every 10-unit increase in number of cigarettes per day among current smokers

HsCRP indicates high-sensitivity C-reactive protein.

Bold items are statistically significant interaction terms ($P<0.05$).

Table S4. Stratified analyses by age, sex, race and education for ln hsCRP where interaction P-values were < 0.05 by smoking status, burden, years since quitting and intensity

	Overall	**P-value**	**Males**	**P-value**	**Females**	**P-value**
Never	REF		REF		REF	
Former	0.05 (0.01 – 0.09)	**0.024**	0.04 (-0.02 – 0.10)	0.165	0.04 (-0.02 – 0.10)	0.198
Current	0.24(0.18 – 0.29)	**<0.001**	0.37 (0.28 – 0.45)	**<0.001**	0.09 (0.01 – 0.17)	**0.025**
Pack-years of smoking*	0.02 (0.01 – 0.02)	**<0.001**	0.02 (0.01 – 0.03)	**<0.001**	0.01 (-0.00 – 0.02)	**0.008**
Years since quit†	-0.02 (-0.04 – -0.00)	**0.001**	-0.03 (-0.05 – -0.01)	**0.003**	-0.02 (-0.04 – 0.00)	0.125
Smoking intensity‡	0.06 (0.02 – 0.09)	**0.004**	0.04 (0.00 – 0.08)	**0.034**	0.15 (0.05 – 0.24)	**0.002**

	Overall	**P-value**	**Age<55**	**P-value**	**Age≥55**	**P-value**
Never	REF		REF		REF	
Former	0.05 (0.01 – 0.09)	**0.024**	0.00 (-0.06 – 0.07)	0.932	0.08 (0.03 – 0.14)	**0.002**
Current	0.24(0.18 – 0.29)	**<0.001**	0.15 (0.07 – 0.23)	**<0.001**	0.34 (0.26 – 0.43)	**<0.001**
Pack-years of smoking*	0.02 (0.01 – 0.02)	**<0.001**	0.02 (0.01 – 0.03)	**0.001**	0.02 (0.00 – 0.02)	**<0.001**
Years since quit†	-0.02 (-0.04 – -0.00)	**0.001**	-0.00 (-0.03 – 0.03)	**0.792**	-0.03 (-0.05 – -0.01)	**<0.001**
Smoking intensity†	0.06 (0.02 – 0.09)	**0.004**	0.04 (-0.00 – 0.09)	0.052	0.10 (0.23 – 0.18)	**0.010**

	Overall	**P-value**	**High school or less**	**P-value**	**More than high school**	**P-value**
Never	REF		REF		REF	
Former	0.05 (0.01 – 0.09)	**0.024**	0.04 (-0.03 – 0.10)	0.312	0.05 (-0.00 – 0.10)	0.100
Current	0.24(0.18 – 0.29)	**<0.001**	0.23 (0.15 – 0.32)	**<0.001**	0.21 (0.13 – 0.30)	**<0.001**
Pack-years of smoking*	0.02 (0.01 – 0.02)	**<0.001**	0.03 (0.02 – 0.04)	**<0.001**	0.01 (0.00 – 0.02)	**0.005**
Years since quit†	-0.02 (-0.04 – -0.00)	**0.001**	-0.02 (-0.05 – -0.00)	**0.033**	-0.02 (-0.04 – -0.01)	**0.009**
Smoking intensity‡	0.06 (0.02 – 0.09)	**0.004**	0.13 (0.04 – 0.21)	**0.003**	0.04 (-0.01 – 0.08)	0.093

	Overall	P-value	Whites	P-value	Non-Whites	P-value
Never	REF		REF		REF	
Former	0.05 (0.01 – 0.09)	**0.024**	0.34 (-0.02 – 0.09)	0.253	2.89 (-0.70 – 6.48)	0.24
Current	0.24(0.18 – 0.29)	**<0.001**	0.20 (0.11 – 0.28)	**<0.001**	0.06 (-0.01 – 0.12)	0.073
Pack-years of smoking*	0.02 (0.01 – 0.02)	**<0.001**	0.01 (0.01 – 0.02)	**0.001**	0.02 (0.01 – 0.03)	**<0.001**
Years since quit†	-0.02 (-0.04 – -0.00)	**0.001**	-0.02 (-0.04 – -0.00)	**0.036**	-0.03 (-0.05 – -0.00)	**0.019**
Smoking intensity‡	0.06 (0.02 – 0.09)	**0.004**	0.04 (-0.00 – 0.08)	0.073	0.13 (0.05 – 0.22)	**0.002**

	Overall	P-value	Browns	P-value	Non-Browns	P-value
Never	REF		REF		REF	
Former	0.05 (0.01 – 0.09)	**0.024**	-0.07 (-0.22 – 0.09)	0.377	0.06 (0.02 – 0.11)	**0.008**
Current	0.24(0.18 – 0.29)	**<0.001**	0.28 (0.11 – 0.46)	**0.002**	0.23 (0.17 – 0.29)	**<0.001**
Pack-years of smoking*	0.02 (0.01 – 0.02)	**<0.001**	0.03 (-0.01 – 0.06)	**0.102**	0.02 (0.01 – 0.02)	**<0.001**
Years since quit†	-0.02 (-0.04 – -0.00)	**0.001**	-0.02 (-0.09 – 0.04)	0.509	0.02 (-0.04 – -0.01)	**0.001**
Smoking intensity‡	0.06 (0.02 – 0.09)	**0.004**	0.13 (-0.06 – 0.31)	0.194	0.05 (0.01 – 0.09)	**0.007**

	Overall	P-value	Blacks	P-value	Non-Blacks	P-value
Never	REF		REF		REF	
Former	0.05 (0.01 – 0.09)	**0.024**	0.12 (0.02 – 0.22)	**0.016**	0.03 (-0.02 – 0.808)	0.214
Current	0.24(0.18 – 0.29)	**<0.001**	0.32 (0.20 – 0.44)	**<0.001**	0.21 (0.14 – 0.28)	**<0.001**
Pack-years of smoking*	0.02 (0.01 – 0.02)	**<0.001**	0.03 (0.01 – 0.04)	**0.001**	0.01 (0.01 – 0.22)	**<0.001**
Years since quit†	-0.02 (-0.04 – -0.00)	**0.001**	-0.03 (-0.06 – 0.00)	0.059	-0.02 (-0.04 – -0.00)	**0.012**
Smoking intensity‡	0.06 (0.02 – 0.09)	**0.004**	0.13 (0.00 – 0.25)	**0.046**	0.05 (0.01 – 0.09)	**0.019**

*For every 5-unit increase among current and former smokers

†For every 5-year increase in time since quitting smoking among former smokers

‡For every 10-unit increase in number of cigarettes per day among current smokers

HsCRP indicates high-sensitivity C-reactive protein.

Bold items are statistically significant interaction terms (P<0.05).

Table S5. Multivariable-adjusted baseline mean absolute difference with 95% confidence intervals for naturally log-transformed GlycA (ln-GlycA) versus naturally log-transformed hsCRP (ln-hsCRP) levels by different modes of smoking exposure among 6,774 MESA participants.

Exposure	**Ln-GlycA**			**Ln-hsCRP**		
Smoking status	**β-coeff (95% CI)**	**P-value**	**T-statistics**	**β-coeff (95% CI)**	**P-value**	**T-statistics**
Never	REF	---	---	REF	---	---
Former	1.007 (0.999 - 1.015)	0.079	1.75	1.078 (1.018 – 1.140)	0.010	2.56
Current	1.054 (1.042– 1.066)	<0.001	9.10	1.307 (1.206 - 1.418)	<0.001	6.48
Smoking burden*						
Ever smokers†	1.003 (1.002 - 1.004)	<0.001	4.99	1.019 (1.012 – 1.027)	<0.001	4.96
Former smokers	1.002 (1.000– 1.003)	0.003	2.97	1.012 (1.004– 1.021)	0.005	2.83
Current smokers	1.003 (1.000– 1.005)	0.023	2.28	1.027 (1.009– 1.044)	0.002	3.04
Years since quitting smoking†						
Per 5-year increase	0.996 (0.994 – 0.998)	0.001	-3.33	0.970 (0.955 – 0.987)	0.001	-3.46
Smoking intensity‡						
Per 10-cigarettes/day increase	1.004 (0.999 – 1.011)	0.116	1.57	1.041 (0.997 – 1.089)	0.070	1.81

*For every 5-unit increase in pack-years of smoking among current and former smokers
†For every 5-year increase in time since quitting smoking among former smokers
‡For every 10-unit increase in number of cigarettes per day among current smokers. Also adjusted for duration of smoking.

Models were adjusted for age, sex, race, education, studied cohort, body mass index, estimated glomerular filtration rate, systolic blood pressure, low-density lipoprotein cholesterols, low-density lipoprotein cholesterols, triglycerides, family history of heart disease, history of diabetes, and use of anti-hypertensive, hypoglycemic, statin, and non-steroidal anti-inflammatory drugs, and steroids.

Table S6. Multivariable-adjusted baseline mean absolute difference with 95% confidence intervals for naturally log-transformed GlycA (ln-GlycA) versus naturally log-transformed hsCRP (ln-hsCRP) levels by different modes of smoking exposure among 4,735 ELSA-Brasil participants.

Exposure	**Ln-GlycA**			**Ln-hsCRP**		
Smoking status	**β-coeff (95% CI)**	**P-value**	**T-statistics**	**β-coeff (95% CI)**	**P-value**	**T-statistics**
Never	REF	---	---	REF	---	---
Former	1.014 (1.004 - 1.023)	0.002	3.03	1.039 (0.972 – 1.111)	0.258	1.13
Current	1.049 (1.037– 1.061)	<0.001	8.12	1.263 (1.160 - 1.374)	<0.001	5.43
Smoking burden*						
Ever smokers†	1.003 (1.002 - 1.006)	<0.001	4.63	1.019 (1.006 – 1.031)	0.003	2.93
Former smokers	1.002 (1.000– 1.004)	0.040	2.06	1.005 (0.990– 1.020)	0.496	0.68
Current smokers	1.006 (1.003– 1.009)	0.001	3.48	1.032 (1.009– 1.056)	0.006	2.75
Years since quitting smoking†						
Per 5-year increase	0.996 (0.992 – 0.999)	0.024	-2.27	0.994 (0.968 – 1.022)	0.691	-0.40
Smoking intensity‡						
Per 10-cigarettes/day increase	1.016 (1.004 – 1.029)	0.008	2.65	1.107 (1.018 – 1.204)	0.018	2.37

*For every 5-unit increase in pack-years of smoking among current and former smokers
†For every 5-year increase in time since quitting smoking among former smokers
‡For every 10-unit increase in number of cigarettes per day among current smokers. Also adjusted for duration of smoking.

Models were adjusted for age, sex, race, education, studied cohort, body mass index, estimated glomerular filtration rate, systolic blood pressure, low-density lipoprotein cholesterols, low-density lipoprotein cholesterols, triglycerides, family history of heart disease, history of diabetes, and use of anti-hypertensive, hypoglycemic, statin, and non-steroidal anti-inflammatory drugs, and steroids.

Table S7. Multivariable-adjusted baseline mean absolute difference with 95% confidence intervals for ln-GlycA without and with adjusting for hsCRP levels by different modes of smoking exposure in a cohort including MESA and ELSA-Brasil participants.

Exposure	**Ln-GlycA without adjusting for hsCRP**			**Ln-GlycA adjusting for hsCRP**		
Smoking status	**β-coeff (95% CI)**	**T-statistics**	**P-value**	**β-coeff (95% CI)**	**T-statistics**	**P-value**
Never	REF	---	---	REF	---	---
Former	1.009 (1.003 - 1.015)	2.91	0.004	1.008 (1.002 – 1.013)	2.79	0.005
Current	1.050 (1.042– 1.059)	11.86	<0.001	1.043 (1.036 - 1.051)	11.21	<0.001
Smoking burden*						
Ever smokers†	1.003 (1.002 - 1.004)	6.46	<0.001	1.002 (1.000 – 1.003)	5.54	<0.001
Former smokers	1.002 (1.000– 1.003)	3.43	0.001	1.001 (1.000– 1.003)	3.20	0.001
Current smokers	1.004 (1.002– 1.006)	3.91	<0.001	1.002 (1.000– 1.004)	2.65	0.008
Years since quitting smoking†						
Per 5-year increase	0.996 (0.994 – 0.998)	-4.30	<0.001	0.996 (0.995 – 0.998)	-3.82	<0.001
Smoking intensity‡						
Per 10-cigarettes/day increase	1.007 (0.001 – 0.012)	2.47	0.014	1.005 (1.000 – 1.009)	1.92	0.056

*For every 5-unit increase in pack-years of smoking among current and former smokers
†For every 5-year increase in time since quitting smoking among former smokers
‡For every 10-unit increase in number of cigarettes per day among current smokers. Also adjusted for duration of smoking.

Models were adjusted for age, sex, race, education, study site, body mass index, estimated glomerular filtration rate, systolic blood pressure, low-density lipoprotein cholesterols, low-density lipoprotein cholesterols, triglycerides, family history of heart disease, history of diabetes, and use of anti-hypertensive, hypoglycemic, statin, and non-steroidal anti-inflammatory drugs, and steroids. Abbreviation: hsCRP, high-sensitivity C Reactive Protein