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Citation
Akinkuolie, Akintunde O., Robert J. Glynn, Latha Padmanabhan, Paul M Ridker, and Samia Mora. 2016. "Circulating N-Linked Glycoprotein Side-Chain Biomarker, Rosuvastatin Therapy, and Incident Cardiovascular Disease: An Analysis From the JUPITER Trial.” Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease 5 (7): e003822. doi:10.1161/JAHA.116.003822.

Published Version
doi:10.1161/JAHA.116.003822

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Circulating N-Linked Glycoprotein Side-Chain Biomarker, Rosuvastatin Therapy, and Incident Cardiovascular Disease: An Analysis From the JUPITER Trial

Akintunde O. Akinkuolie, MBBS; Robert J. Glynn, PhD; Latha Padmanabhan, MSc; Paul M Ridker, MD; Samia Mora, MD, MHS

Background—GlycA, a novel protein glycan biomarker of N-acetyl side chains of acute-phase proteins, was recently associated with incident cardiovascular disease (CVD) in healthy women. Whether GlycA predicts CVD events in the setting of statin therapy in men and women without CVD but with evidence of chronic inflammation is unknown.

Methods and Results—In the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial (NCT00239681), participants with low-density lipoprotein cholesterol <130 mg/dL and high-sensitivity C-reactive protein (hsCRP) ≥2 mg/L were randomized to rosuvastatin 20 mg/day or placebo. GlycA was quantified by nuclear magnetic resonance spectroscopy in 12 527 before randomization and 10 039 participants at 1 year. A total of 310 first primary CVD events occurred during maximum follow-up of 5.0 years (median, 1.9). GlycA changed minimally after 1 year on study treatment: 6.8% and 4.7% decrease in the rosuvastatin and placebo groups, respectively. Overall, baseline GlycA levels were associated with increased risk of CVD: multivariable-adjusted hazard ratio (HR) per SD increment, 1.20 (95% CI, 1.08–1.34; \( P=0.0006 \)). After additionally adjusting for hsCRP, this was slightly attenuated (HR, 1.18; 95% CI, 1.04–1.35; \( P=0.01 \)). On-treatment GlycA levels were also associated with CVD; corresponding multivariable-adjusted HRs per SD before and after additionally adjusting for hsCRP: 1.27 (95% CI, 1.13–1.42; \( P<0.0001 \)) and 1.24 (95% CI, 1.07–1.44; \( P=0.004 \)), respectively. Tests for heterogeneity by treatment arm were not significant (\( P \) for interaction, >0.20).

Conclusion—In the JUPITER trial, increased levels of GlycA were associated with an increased risk of CVD events independent of traditional risk factors and hsCRP.

Clinical Trials Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00239681. (J Am Heart Assoc. 2016;5: e003822 doi: 10.1161/JAHA.116.003822)

Key Words: cardiovascular disease • epidemiology • glycoprotein • metabolomics • statin intervention

Although statins are effective agents in preventing cardiovascular disease (CVD) in combination with optimal lifestyle behaviors, cardiovascular events remain the leading cause of morbidity and mortality worldwide and still commonly occur during statin therapy.\(^1\) Recent research has also found that specific glycan changes facilitate the course of atherosclerosis and also track with cardiometabolic risk factors.\(^2,3\) In addition, glycan attachments have been known to functionally modify cytokines and other inflammatory mediators implicated in atherosclerosis.\(^4\) Yet, the potential role of glycans in CVD prevention has not been well explored. Considerable technological advances for glycan analysis are now facilitating the investigation of glycans and their side chains as novel diagnostic and pharmacotherapeutic targets for various conditions, such as infectious diseases and cancers.\(^5\)

To this effect, we recently reported that GlycA, a novel proton nuclear magnetic resonance (NMR) biomarker that identifies a consensus sequence of glycans common to a host of acute phase glycoproteins,\(^6\) was associated with the risk of incident CVD in initially healthy middle-aged and older women in a manner similar to high-sensitivity C-reactive protein (hsCRP)\(^7\) despite the negligible contribution of hsCRP to the GlycA signal.\(^6\) Nonetheless, glycan structures change in response to the phenotypic and metabolic state of a cell, and
it is unknown whether the glycan sequence present in GlycA predicts CVD in a high-risk population recruited on the basis of elevated hsCRP. Furthermore, although GlycA was correlated with modifiable CVD risk factors, the effect of statin therapy on GlycA and its association with CVD events during treatment with high-intensity statin therapy is unknown.

Therefore, in the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER), we measured baseline and on-treatment GlycA levels in order to assess: (1) the effect of 1 year of rosuvastatin treatment on GlycA; (2) the association of baseline and on-statin GlycA with incident CVD; and (3) whether the CVD relative risk reduction attributable to rosuvastatin treatment in JUPITER was modified by GlycA levels.

Methods

Study Population

JUPITER (ClinicalTrial.gov No.: NCT00239681) was a double-blind, placebo-controlled trial that evaluated rosuvastatin 20 mg daily versus placebo in the primary prevention of first major CVD events among 17,802 apparently healthy men ≥50 years and women ≥60 years with low low-density lipoprotein cholesterol (LDL-C; <130 mg/dL), but who were at increased risk of cardiovascular events on the basis of elevated hsCRP (≥2 mg/L). Key exclusion criteria for JUPITER included previous or current use of lipid-lowering therapy, current use of postmenopausal hormonal therapy, diabetes mellitus, and inflammatory conditions, such as severe arthritis, lupus or inflammatory bowel disease, or treatment with immunosuppressant medications. The trial protocol required study participants to provide a baseline blood sample before randomization and after 1 year on study treatment. Study participants were also requested, but not required, to provide samples for additional phenotyping. For this study, we analyzed a total of 12,527 participants who provided a sufficient blood sample at baseline for NMR GlycA measurements; and of these, 10,039 participants had a sufficient blood sample at both baseline and at 1 year. The JUPITER trial protocol was approved by the local institutional review board at each participating center, and all study participants provided written informed consent.

Laboratory Methods

Standard lipids, apolipoprotein-B, and hsCRP were assayed immediately after blood collection in a core laboratory as previously described. For the current analysis, GlycA was measured for each participant from baseline and 1-year samples that were paired together and stored in liquid nitrogen until the time for assay. GlycA signals were quantified from signal amplitudes generated from the automated proton (H\(^1\)) NMR LipoProfile test at the CLIA-certified Liposcience, Inc. (now LabCorp, Raleigh, NC) clinical laboratory. The GlycA signal is centered at 2.00±0.01 ppm in H\(^1\) NMR spectra of plasma, and only N-acetylglucosamine with specific glycosidic linkage, that is, β(1→2) or β(1→6) with a preceding mannose residue, contributes to the GlycA signal. NMR signal amplitudes originating from the N-acetyl methyl group protons of the N-acetylglucosamine moieties located on the bi-, tri-, and tetra-antennary branches of specific serum proteins (mainly α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin, and transferrin) were used to calculate the concentrations of GlycA expressed in μmol/L of N-acetyl methyl groups.

Outcomes

The primary endpoint of the current study is the trial primary endpoint, defined as nonfatal myocardial infarction, nonfatal stroke, hospitalization for unstable angina, arterial revascularization, or cardiovascular death. We additionally examined the expanded endpoint of the primary endpoint and all-cause death, consistent with previous biomarker analyses in JUPITER. All components of the primary endpoint occurring through the end of the JUPITER trial (March 30, 2008) were adjudicated in a blinded fashion by an independent endpoint committee and were confirmed using standardized diagnostic criteria. Only deaths adjudicated to be clearly attributed to a cardiovascular or cerebrovascular cause were included in the primary endpoint. For the expanded outcome, all deaths were included regardless of whether data were available to confirm the cause of death.

Statistical Analyses

Statistical analyses were performed with SAS software (version 9.3; SAS Institute Inc., Cary, NC). Baseline characteristics are expressed as medians (25th–75th percentiles) or percentages. Spearman coefficients were used to compare correlations of GlycA with risk factors. Wilcoxon signed-rank tests compared baseline and 1-year GlycA levels by randomization arm. Changes in GlycA after 1 year of randomization are expressed as percentages within each arm. The effect of 1 year of rosuvastatin therapy versus placebo on GlycA levels was assessed by the Wilcoxon rank-sum tests. We performed a similar analysis for hsCRP.

Person-time of follow-up was assessed from the time of randomization to the first occurrence of a primary endpoint or the date of death, last study visit, withdrawal from study, loss to follow-up, or trial completion, whichever came first. Absolute event rates were calculated per 100 person-years. Consistent with previous published analyses examining on-treatment hsCRP and LDL-C in relation to cardiovascular
outcomes, on-treatment GlycA levels were defined as the values obtained after the first year of treatment. A cumulative incidence plot and log-rank statistics were used to compare the unadjusted primary event rates between baseline quartiles of GlycA. Hazard ratios (HRs) and 95% CIs for the association of baseline and on-treatment GlycA with cardiovascular outcomes were quantified using Cox proportional hazards regression according to quartiles of GlycA and per SD. The P value for linear trend was computed by fitting a continuous variable that assigned the median value for each quartile in regression models. We used 3 models that sequentially adjusted for demographic variables (age, sex, race, and randomization treatment assignment), traditional CVD risk factors (smoking, blood pressure, body mass index, fasting glucose, LDL-C, high-density lipoprotein cholesterol [HDL-C], triglycerides, and family history of premature coronary disease), and natural log-transformed hsCRP. We tested for treatment interaction by including a cross-product term between GlycA and randomized treatment and by stratifying the analysis according to randomization arm. We evaluated the incremental predictive value of GlycA by calculating the likelihood ratio $\chi^2$ statistic and corresponding P value comparing models with and without the addition of GlycA.

We performed additional analyses to examine whether the association of GlycA with events was modified by sex (male or female), age ($\leq$65 or $>$65 years), smoking (yes or no), family history of coronary heart disease (CHD; yes or no), metabolic syndrome (yes or no), time to event ($\leq$24 or $>$24 months), or fasting glucose ($<$100 or $\geq$100 mg/dL). Finally, we examined whether the efficacy of rosuvastatin therapy on the primary outcome was differential in subgroups defined by quartiles of baseline GlycA levels. All probability values were 2-tailed, with values <0.05 considered statistically significant.

### Results

#### Baseline Characteristics

Baseline characteristics of study participants with measurable GlycA levels were similar to that of the entire JUPITER cohort except for more white participants in the current study (Table 1). GlycA was normally distributed, with a mean (SD) of 5.7 (5.4–5.9) mg/dL.

| Characteristics                              | Current Study n=12 527 | Not in Current Study n=5275 | Overall Study n=17 802 |
|---------------------------------------------|------------------------|-----------------------------|------------------------|
| Age, y                                       | 66 (60–71)             | 66 (61–71)                  | 66 (60–71)             |
| Female                                       | 4542 (36.3)            | 2259 (42.8)                 | 6801 (38.2)            |
| Rosuvastatin                                 | 6182 (49.4)            | 2719 (51.6)                 | 8901 (50.0)            |
| Race/ethnicity                               |                        |                             |                        |
| White                                        | 10 251 (81.8)          | 2432 (46.1)                 | 12 683 (71.3)          |
| Black                                        | 886 (7.1)              | 1338 (25.4)                 | 2224 (12.5)            |
| Asian                                        | 184 (1.5)              | 99 (1.9)                    | 283 (1.6)              |
| Hispanic                                     | 1105 (8.8)             | 1156 (21.9)                 | 2261 (12.7)            |
| Other/unknown                                | 99 (0.8)               | 250 (4.7)                   | 349 (2.0)              |
| Body mass index, kg/m²                        | 28.4 (25.5–32.0)       | 28.1 (25.0–32.0)            | 28.4 (25.3–32.0)       |
| Systolic blood pressure, mmHg                 | 134 (124–146)          | 134 (125–144)               | 134 (124–145)          |
| Diastolic blood pressure, mmHg                | 80 (75–86)             | 80 (76–89)                  | 80 (75–87)             |
| Current smoker                               | 1883 (15.0)            | 937 (17.8)                  | 2820 (15.9)            |
| Family history of premature coronary disease  | 1575 (12.6)            | 470 (9.0)                   | 2045 (11.5)            |
| Metabolic syndrome                           | 4987 (40.4)            | 2329 (44.5)                 | 7316 (41.6)            |
| Fasting glucose, mg/dL                       | 95 (88–102)            | 93 (86–101)                 | 94 (88–102)            |
| Hemoglobin A1c, %                            | 5.7 (5.4–5.9)          | 5.7 (5.5–6.0)               | 5.7 (5.5–5.9)          |
| High-sensitivity C-reactive protein, mg/L     | 4.1 (2.8–6.9)          | 4.7 (3.0–7.9)               | 4.3 (2.9–7.1)          |
| LDL cholesterol, mg/dL                       | 109 (95–119)           | 107 (91–118)                | 108 (94–119)           |
| Total cholesterol, mg/dL                     | 186 (170–200)          | 183 (165–198)               | 185 (169–200)          |
| Triglycerides, mg/dL                         | 116 (84–167)           | 122 (88–175)                | 118 (85–169)           |
| HDL cholesterol, mg/dL                       | 49 (41–60)             | 47 (39–58)                  | 49 (40–60)             |

Values stated are median (25th–75th percentile) or n (%). Percentages may not add up because of rounding off. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.
411 (70) μmol/L and a median value of 404 μmol/L (25th–75th percentile: 364–449 μmol/L); corresponding median value in men was 395 μmol/L (356–439) whereas that in women was 419 μmol/L (381–466). GlycA correlated positively in increasing magnitude with apolipoprotein B, triglycerides, hemoglobin A1c, and hsCRP (Spearman, r=0.13–0.46, but not substantively with fasting glucose, LDL-C, body mass index, and apolipoprotein B (Spearman, r<0.07; Table 2).

**Effect of Rosuvastatin on GlycA**

After 1 year of study treatment, GlycA levels were minimally decreased in both placebo and rosuvastatin treatment groups to median values of 383 (345–427) and 375 μmol/L (337–419), respectively; P<0.0001 for 1-year vs baseline values in each group. The resulting median percent change (25th–75th percentile) was −4.7% (−12% to 2.7%) in the placebo group and −6.8% (−13.8% to 0.8%) in the rosuvastatin group (P comparing changes between treatment groups <0.0001; Table 3 and Figure 1). The corresponding percent changes in hsCRP were −20% (−49.2% to 20%) and −48% (−68.8% to −15.2%), respectively. Similar changes were observed in analyses stratified by age, sex, metabolic syndrome, family history of premature coronary disease, and current smoking (data not shown).

In the rosuvastatin treatment group, 1-year change in GlycA correlated positively with 1-year change in hsCRP (Spearman, r=0.44), but not substantively with 1-year changes in LDL-C, apolipoprotein B, and triglycerides (Spearman, r=0.13; Table 2). Generally, similar correlations were noted in the placebo group.

**Association of Baseline and On-Treatment GlycA With CVD**

Among 12,527 JUPITER participants with baseline GlycA levels, the study primary outcome was confirmed in 310 participants over a maximum follow-up of 5.0 years (median, 1.9). Of these, 201 (1.6%) primary events occurred in the placebo group and 109 (0.87%) in the rosuvastatin group, the proportion of these were similar to those observed in the overall JUPITER trial.9 The cumulative primary event rates diverged increasingly according to quartiles of GlycA with the

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**Table 2. Spearman Correlation Coefficients**

| Biomarker               | GlycA | hsCRP, mg/L | Total cholesterol, mg/dL | LDL cholesterol, mg/dL | Apolipoprotein B, mg/dL | HDL cholesterol, mg/dL | Apolipoprotein A-1, mg/dL | Triglycerides, mg/dL | Body mass index, kg/m² | Fasting glucose, mg/dL | Hemoglobin A1c, % |
|-------------------------|-------|-------------|--------------------------|------------------------|-------------------------|-------------------------|---------------------------|----------------------|----------------------|----------------------|------------------|
| Correlation Coefficient |       |             |                          |                        |                         |                         |                           |                      |                      |                      |                  |
| hsCRP, mg/L             | 0.46  |             |                          |                        |                         |                         |                           |                      |                      |                      |                  |
| Total cholesterol, mg/dL| 0.09  |             |                          |                        |                         |                         |                           |                      |                      |                      |                  |
| LDL cholesterol, mg/dL  | −0.02 |             |                          |                        |                         |                         |                           |                      |                      |                      |                  |
| Apolipoprotein B, mg/dL | 0.13  |             |                          |                        |                         |                         |                           |                      |                      |                      |                  |
| HDL cholesterol, mg/dL  | −0.03 |             |                          |                        |                         |                         |                           |                      |                      |                      |                  |
| Apolipoprotein A-1, mg/dL| 0.01* |             |                          |                        |                         |                         |                           |                      |                      |                      |                  |
| Triglycerides, mg/dL    | 0.20  |             |                          |                        |                         |                         |                           |                      |                      |                      |                  |
| Body mass index, kg/m²  | 0.07  |             |                          |                        |                         |                         |                           |                      |                      |                      |                  |
| Fasting glucose, mg/dL  | 0.01* |             |                          |                        |                         |                         |                           |                      |                      |                      |                  |
| Hemoglobin A1c, %       | 0.21  |             |                          |                        |                         |                         |                           |                      |                      |                      |                  |

1HDL indicates high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL-C, low density lipoprotein cholesterol.

*Nonsignificant P values for Spearman correlations; otherwise, P values are <0.01.

†Denotes change at 1 year; otherwise, biomarkers represent baseline measurements.

**Table 3. Median (25th–75th Percentile) Concentrations of GlycA and High-Sensitivity C-Reactive Protein by Randomized Treatment Group**

| Biomarker | Baseline | Year 1* | Change* | % Change |
|-----------|----------|---------|---------|----------|
| GlycA, μmol/L |          |         |         |          |
| Placebo   | 404 (363, 446) | 383 (345, 427) | −19 (−48, 10) | −4.7 (−11.7, 2.7) |
| Rosuvastatin | 404 (364, 448) | 375 (337, 419) | −27 (−57, 3) | −6.8 (−13.8, 0.8) |
| hsCRP, mg/L |          |         |         |          |
| Placebo   | 4.1 (2.8, 6.7) | 3.4 (1.9, 5.9) | −0.7 (−2.3, 0.7) | −20 (−49.2, 20) |
| Rosuvastatin | 4.1 (2.8, 6.7) | 2.1 (1.2, 4.2) | −1.7 (−3.4, −0.5) | −48 (−68.8, −15.2) |

Values obtained from individuals with both baseline and 1-year measurements (n=10,039). hsCRP indicates high-sensitivity C-reactive protein.

*P values from the Wilcoxon signed-rank test comparing baseline and year 1 values were statistically significant (P<0.0001).

†P values from the Wilcoxon rank-sum test comparing the change among the rosuvastatin group with the change among the placebo group were <0.0001.
In a multivariable model that included age, race, sex, randomized treatment assignment, smoking, blood pressure, body mass index, fasting glucose, LDL-C, HDL-C, triglycerides, and family history of premature coronary disease, HRs for the primary endpoint for quartiles 1 to 4 of baseline GlycA were 1.00, 1.05 (95% CI, 0.74–1.49), 1.23 (95% CI, 0.88–1.72), and 1.57 (95% CI, 1.12–2.18; \( P_{\text{linear trend}} = 0.004\). This association was slightly attenuated, but remained statistically significant, after adjustment for hsCRP: HR for quartile 4 versus 1 was 1.45 (95% CI, 1.01–2.10; \( P_{\text{linear trend}} = 0.03\)). Risk estimates per SD increase in baseline GlycA levels were 1.20 (95% CI, 1.08–1.34; \( P=0.0006\)) and 1.18 (95% CI, 1.04–1.35; \( P=0.01\)) in the corresponding multivariable adjusted models (Table 4). Results were similar when baseline GlycA was examined in relation to the expanded primary endpoint that included all-cause death (528 events); the HR in the fully adjusted model for quartile 4 versus 1 was 1.83 (95% CI, 1.38–2.42; \( P_{\text{linear trend}} = 0.0001\), with a corresponding HR per SD increase in baseline GlycA of 1.25 (95% CI, 1.15–1.37; \( P<0.0001\); Table 4).

Somewhat stronger magnitudes of associations were observed among the 10 039 participants with on-treatment levels of GlycA: the HRs for quartile 4 versus 1 of on-treatment GlycA with the primary endpoint (224 events) in multivariable adjusted models with and without hsCRP were 2.25 (95% CI, 1.48–3.42; \( P_{\text{linear trend}} = 0.0001\)) and 2.05 (95% CI, 1.28–3.28; \( P_{\text{linear trend}} = 0.002\); Table 5). The corresponding HR per SD increase in on-treatment GlycA levels were 1.27 (95% CI, 1.13–1.42; \( P<0.0001\)) and 1.24 (95% CI, 1.07–1.44; \( P=0.0004\), respectively. In analyses examining on-treatment GlycA levels with the expanded primary endpoint that included all-cause death (315 events), the HR in the fully adjusted model for quartile 4 versus 1 was 1.98 (95% CI, 1.33–2.94; \( P_{\text{linear trend}} = 0.0002\), with a corresponding HR per SD increase in on-treatment GlycA of 1.37 (95% CI, 1.22–1.54; \( P<0.0001\); Table 5).

We found no evidence of statistical interaction by GlycA across varied profiles of CVD risk factors when assessed at baseline (Figure 3A) or on-treatment (Figure 3B; \( P>0.05\)).

**Association of Baseline and On-Treatment GlycA With CVD in the Rosuvastatin and Placebo Groups**

Risk estimates observed for the placebo subgroups when baseline and on-treatment GlycA were examined with the primary endpoint were similar to that for the overall study population; \( P_{\text{linear trend}} \) for baseline and on-treatment GlycA in the fully adjusted model were 0.02 and 0.0005 respectively, the corresponding HRs per SD of GlycA were 1.26 (95% CI, 1.07–1.44; \( P=0.005\)) and 1.35 (95% CI, 1.13–1.62; \( P=0.001\), respectively. Similar magnitudes of effect were observed among the rosvastatin subgroup, although the associations did not reach statistical significance; corresponding \( P_{\text{linear trend}} \) were 0.64 and 0.38 while HRs per SD of GlycA were 1.07 (95% CI, 0.86–1.33; \( P=0.56\)) and 1.06 (95% CI, 0.82–1.37; \( P=0.67\), respectively. There was no statistically significant interaction between either baseline or on-treatment GlycA levels with rosvastatin therapy on the occurrence of the primary events (\( P \) for interaction \( >0.05 \) in all models).

Stronger associations were observed in analysis examining the expanded primary endpoint that included all-cause death...
| Quartile 1 ≤364 μmol/L | Quartile 2 365 to 404 μmol/L | Quartile 3 405 to 449 μmol/L | Quartile 4 >449 μmol/L | L\textsubscript{LINEAR} trend | HR (95% CI, P Values) Per SD | R\textsubscript{INT}* |
|-------------------------|-------------------------------|-------------------------------|-------------------------|-----------------------------|-----------------------------|--------------|
| **Cardiovascular events** |                               |                               |                         |                             |                             |              |
| Overall (No. of events/n:310/12 527)† |                               |                               |                         |                             |                             |              |
| Incidence rate (95% CI) | 0.95 (0.75–1.21)              | 0.98 (0.77–1.25)              | 1.16 (0.93–1.44)        | 1.55 (1.28–1.89)            |                             |              |
| Hazard ratio (95% CI)   | Model 1 1                     | 1.13 (0.80–1.59)              | 1.35 (0.98–1.88)        | 1.85 (1.35–2.54)            | <0.0001                     | 1.27 (1.15–1.40, <0.0001) | <0.0001      |
|                         | Model 2 1                     | 1.05 (0.74–1.49)              | 1.23 (0.88–1.72)        | 1.57 (1.12–2.18)            | 0.004                       | 1.20 (1.08–1.34, 0.0006)  | 0.001        |
|                         | Model 3 1                     | 1.04 (0.73–1.47)              | 1.19 (0.84–1.68)        | 1.45 (1.01–2.10)            | 0.03                        | 1.18 (1.04–1.35, 0.01)   | 0.01         |
| Placebo (No. of events/n:201/6345) |                             |                               |                         |                             |                             |              |
| Incidence rate (95% CI) | 1.18 (0.88–1.59)              | 1.25 (0.92–1.68)              | 1.47 (1.12–1.93)        | 2.02 (1.59–2.57)            |                             |              |
| Hazard ratio (95% CI)   | Model 1 1                     | 1.13 (0.74–1.73)              | 1.40 (0.93–2.11)        | 1.94 (1.30–2.87)            | 0.0004                      | 1.28 (1.13–1.44, <0.0001) | 0.0002       |
|                         | Model 2 1                     | 1.02 (0.66–1.57)              | 1.23 (0.81–1.87)        | 1.55 (1.03–2.34)            | 0.02                        | 1.20 (1.06–1.36, 0.006)  | 0.008        |
|                         | Model 3 1                     | 1.03 (0.67–1.59)              | 1.26 (0.82–1.93)        | 1.63 (1.03–2.57)            | 0.02                        | 1.26 (1.07–1.48, 0.005)  | 0.006        |
| Rosuvastatin (No. of events/n:109/6182) |                             |                               |                         |                             |                             |              |
| Incidence rate (95% CI) | 0.71 (0.48–1.10)              | 0.70 (0.47–1.06)              | 0.83 (0.58–1.21)        | 1.07 (0.77–1.50)            |                             |              |
| Hazard ratio (95% CI)   | Model 1 1                     | 1.14 (0.64–2.02)              | 1.23 (0.71–2.13)        | 1.68 (0.99–2.87)            | 0.048                       | 1.24 (1.04–1.47, 0.02)   | 0.02         |
|                         | Model 2 1                     | 1.13 (0.62–2.03)              | 1.19 (0.67–2.09)        | 1.56 (0.89–2.73)            | 0.11                        | 1.20 (1.002–1.44, 0.047) | 0.06         |
|                         | Model 3 1                     | 1.04 (0.57–1.89)              | 1.04 (0.58–1.86)        | 1.16 (0.62–2.17)            | 0.64                        | 1.07 (0.86–1.33, 0.56)   | 0.56         |
| **Cardiovascular events or all-cause death** |
| Overall (No. of events/n:528/12 527)† |                               |                               |                         |                             |                             |              |
| Incidence rate (95% CI) | 1.48 (1.22–1.79)              | 1.55 (1.28–1.88)              | 1.82 (1.53–2.16)        | 3.09 (2.69–3.54)            |                             |              |
| Hazard ratio (95% CI)   | Model 1 1                     | 1.16 (0.88–1.52)              | 1.37 (1.05–1.78)        | 2.33 (1.83–2.97)            | <0.0001                     | 1.37 (1.28–1.46, <0.0001) | <0.0001      |
|                         | Model 2 1                     | 1.15 (0.87–1.51)              | 1.34 (1.02–1.74)        | 2.08 (1.61–2.67)            | <0.0001                     | 1.30 (1.21–1.39, <0.0001) | <0.0001      |
|                         | Model 3 1                     | 1.12 (0.84–1.47)              | 1.26 (0.96–1.66)        | 1.83 (1.38–2.42)            | <0.0001                     | 1.25 (1.15–1.37, <0.0001) | <0.0001      |
| Placebo (No. of events/n:322/6345) |                             |                               |                         |                             |                             |              |
| Incidence rate (95% CI) | 1.65 (1.28–2.12)              | 1.96 (1.54–2.48)              | 2.08 (1.65–2.61)        | 3.85 (3.24–4.85)            |                             |              |
| Hazard ratio (95% CI)   | Model 1 1                     | 1.28 (0.90–1.81)              | 1.44 (1.02–2.03)        | 2.59 (1.89–3.55)            | <0.0001                     | 1.36 (1.25–1.48, <0.0001) | <0.0001      |
|                         | Model 2 1                     | 1.24 (0.87–1.76)              | 1.37 (0.97–1.95)        | 2.18 (1.57–3.03)            | <0.0001                     | 1.28 (1.17–1.40, <0.0001) | <0.0001      |
|                         | Model 3 1                     | 1.23 (0.86–1.75)              | 1.35 (0.94–1.93)        | 2.10 (1.46–3.02)            | <0.0001                     | 1.28 (1.14–1.44, <0.0001) | <0.0001      |

Continued
in the placebo subgroup; $P_{\text{linear trend}}$ for baseline and on-treatment GlycA in the fully adjusted model were $<0.0001$ and $<0.0001$, respectively, the corresponding HRs per SD of GlycA were 1.28 (95% CI, 1.14–1.44; $P<0.0001$) and 1.41 (95% CI, 1.21–1.64; $P<0.0001$). Likewise, results in the rosuvastatin subgroup trended toward statistical significance; corresponding $P_{\text{linear trend}}$ were 0.054 and 0.21, whereas HRs per SD increase in GlycA were 1.22 (95% CI, 1.06–1.41; $P=0.005$) and 1.30 (95% CI, 1.09–1.56; $P=0.004$), respectively. Similarly, there was no evidence of statistical interaction by rosuvastatin therapy ($P$ for interaction $>0.05$ in all models; Tables 4 and 5).

### Efficacy of Rosuvastatin According to Baseline GlycA

In an analysis that examined participants based on 8 categories that took both treatment assignment and GlycA levels according to quartiles into account, those who were on placebo and had GlycA levels $\leq 364$ μmol/L (first quartile) were considered as the referent. For both placebo and rosuvastatin, there was a suggested trend of increasing risk with increasing baseline GlycA levels; however, at each level, the rosuvastatin group had lower risk (Figure 4A). Consequently, rosuvastatin therapy had similar efficacy regardless of GlycA levels (Figure 4B), with estimates centered around that reported in the original JUPITER trial (HR, 0.56; 95% CI, 0.46–0.69).

### Discussion

In the JUPITER trial, baseline and on-treatment levels of GlycA, a recently characterized biomarker of circulating glycan side chains common to a host of acute-phase glycoproteins, were positively associated with the first occurrence of CVD independent of traditional risk factors and hsCRP. Importantly, there was no evidence of effect heterogeneity by rosuvastatin treatment, and the association did not vary by levels of traditional CVD risk factors.

Among CVD biomarkers assessed at baseline and on-treatment in the current study, GlycA correlated positively with hsCRP ($r=0.5$), similar to the magnitude of correlation of the change in GlycA with change in hsCRP ($r=0.5$), consistent with an inflammatory origin of GlycA. Nonetheless, while rosuvastatin therapy was associated with a meaningful reduction in hsCRP levels compared to placebo, the effect of rosuvastatin on GlycA levels was minimal (median percent decrease of $=2\%$) and may partly relate to the negligibly low contribution of hsCRP to GlycA. Statins may reduce GlycA by inhibiting expression of the acute-phase proteins contributing to GlycA or act more directly by repressing synthesis of the...
Table 5. On-Treatment GlycA in Relation to Incident Events, Overall and by Treatment Group

| Quartile  1 | Quartile 2 | Quartile 3 | Quartile 4 | Hazard ratio (95% CI) Per SD | P_{Linear trend} | HR (95% CI, P Values) Per SD | R^2 sr^* |
|-------------|------------|------------|------------|-----------------------------|-----------------|-----------------------------|---------|
| ≤341 μmol/L | 342 to 379 μmol/L | 380 to 423 μmol/L | >423 μmol/L |                             |                 |                             |         |
| Cardiovascular events | | | | | | | |
| Overall (No. of events/n: 224/10 039)† | | | | | | | |
| Incidence rate (95% CI) | 0.62 (0.45–0.86) | 0.83 (0.62–1.11) | 1.18 (0.93–1.50) | 1.33 (1.06–1.67) | | | |
| Hazard ratio (95% CI) | 1 | 1.46 (0.94–2.25) | 2.10 (1.40–3.16) | 2.47 (1.65–3.70) | <0.0001 | 1.32 (1.18–1.47, <0.0001) | <0.0001 |
| Model 1 | 1 | 1.36 (0.87–2.14) | 2.03 (1.34–3.08) | 2.25 (1.48–3.42) | <0.0001 | 1.27 (1.13–1.42, <0.0001) | <0.0001 |
| Model 2 | 1 | 1.33 (0.85–2.09) | 1.94 (1.26–2.98) | 2.05 (1.28–3.28) | 0.002  | 1.24 (1.07–1.44, 0.004) | 0.006  |
| Placebo (No. of events/n: 143/5113) | | | | | | | |
| Incidence rate (95% CI) | 0.66 (0.41–1.04) | 1.00 (0.70–1.43) | 1.56 (1.17–2.08) | 1.63 (1.24–2.14) | | | |
| Hazard ratio (95% CI) | 1 | 1.73 (0.96–3.12) | 2.73 (1.58–4.72) | 3.10 (1.80–5.35) | <0.0001 | 1.37 (1.20–1.57, <0.0001) | <0.0001 |
| Model 2 | 1 | 1.62 (0.89–2.95) | 2.55 (1.46–4.45) | 2.67 (1.53–4.69) | 0.0003 | 1.27 (1.11–1.45, 0.0005) | 0.001  |
| Model 3 | 1 | 1.65 (0.90–3.02) | 2.65 (1.49–4.70) | 2.88 (1.55–5.35) | 0.0005 | 1.35 (1.13–1.62, 0.001) | 0.002  |
| Rosuvastatin (No. of events/n: 81/4926) | | | | | | | |
| Incidence rate (95% CI) | 0.59 (0.37–0.93) | 0.65 (0.41–1.02) | 0.77 (0.51–1.19) | 0.97 (0.65–1.44) | | | |
| Hazard ratio (95% CI) | 1 | 1.19 (0.62–2.29) | 1.46 (0.78–2.76) | 1.81 (0.97–3.36) | 0.048  | 1.21 (0.996–1.47, 0.054) | 0.069  |
| Model 2 | 1 | 1.09 (0.55–2.17) | 1.46 (0.76–2.80) | 1.72 (0.90–3.30) | 0.07   | 1.18 (0.96–1.44, 0.11) | 0.13   |
| Model 3 | 1 | 1.02 (0.51–2.04) | 1.29 (0.66–2.54) | 1.34 (0.63–2.86) | 0.38   | 1.06 (0.82–1.37, 0.67) | 0.67   |
| Cardiovascular events or all-cause death | | | | | | | |
| Overall (No. of events/n: 315/10 039)† | | | | | | | |
| Incidence rate (95% CI) | 0.84 (0.64–1.12) | 1.04 (0.80–1.34) | 1.59 (1.30–1.95) | 2.10 (1.76–2.52) | | | |
| Hazard ratio (95% CI) | 1 | 1.35 (0.93–1.98) | 2.06 (1.45–2.92) | 2.79 (1.99–3.91) | <0.0001 | 1.47 (1.36–1.60, <0.0001) | <0.0001 |
| Model 2 | 1 | 1.28 (0.87–1.89) | 1.99 (1.40–2.85) | 2.52 (1.77–3.58) | <0.0001 | 1.42 (1.30–1.54, <0.0001) | <0.0001 |
| Model 3 | 1 | 1.20 (0.81–1.78) | 1.76 (1.22–2.55) | 1.99 (1.33–2.94) | 0.0002 | 1.37 (1.22–1.54, <0.0001) | <0.0001 |
| Placebo (No. of events/n: 195/5113) | | | | | | | |
| Incidence rate (95% CI) | 0.91 (0.62–1.35) | 1.17 (0.84–1.64) | 2.03 (1.58–2.61) | 2.47 (1.98–3.09) | | | |
| Hazard ratio (95% CI) | 1 | 1.48 (0.88–2.49) | 2.55 (1.60–4.08) | 3.35 (2.11–5.30) | <0.0001 | 1.49 (1.35–1.65, <0.0001) | <0.0001 |
| Model 2 | 1 | 1.43 (0.84–2.42) | 2.49 (1.55–4.01) | 3.01 (1.88–4.82) | <0.0001 | 1.39 (1.25–1.54, <0.0001) | <0.0001 |
| Model 3 | 1 | 1.38 (0.81–2.35) | 2.34 (1.44–3.82) | 2.67 (1.58–4.52) | <0.0001 | 1.41 (1.21–1.64, <0.0001) | <0.0001 |

Continued
| Quartile | Rosuvastatin (No. of events/n:120/4926) | Incidence rate (95% CI) | Hazard ratio (95% CI, HR (95% CI, P Values) Per SD) |
|----------|----------------------------------|------------------------|---------------------------------------------------|
| ≤341 μmol/L | 0.78 (0.53–1.17) | 1.02 (0.56–1.83) | Model 1 adjusted for age, race, and sex. Model 2 is model 1 plus smoking, blood pressure, body mass index, fasting glucose, LDL-C, high-density lipoprotein cholesterol, triglycerides, and family history of premature coronary disease. Model 3 is model 2 plus high-sensitivity C-reactive protein. SD was 70.28 μmol/L. Incidence rates are reported per 100 person-years. HR indicates hazard ratio; LDL-C, low-density lipoprotein cholesterol. |
| 342 to 379 μmol/L | 0.90 (0.61–1.33) | 1.40 (0.62–2.31) | | |
| 380 to 423 μmol/L | 1.11 (0.78–1.58) | 1.42 (0.77–2.63) | | |
| >423 μmol/L | 1.65 (1.22–2.24) | 1.80 (1.07–3.04) | | |

LRT* comparing model with and without GlycA as a continuous variable. Additionally adjusted for randomized treatment assignment.

Table 5. Continued
**Figure 3.** A, Stratified hazard ratios (95% CIs) per SD of baseline GlycA with the primary endpoint adjusted for age, race, sex, randomization treatment assignment, smoking, blood pressure, body mass index, fasting glucose, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, family history of premature coronary disease, and high-sensitivity C-reactive protein. B, Stratified hazard ratios (95% CIs) per SD of on-treatment GlycA with the primary endpoint adjusted for age, race, sex, randomization treatment assignment, smoking, blood pressure, body mass index, fasting glucose, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, family history of premature coronary disease, and high-sensitivity C-reactive protein. CHD indicates coronary heart disease; HR, hazard ratio.
primary endpoint, they were significant for the expanded primary endpoint that included all-cause death and had a greater number of events. Within this context, the lack of interaction between GlycA levels and rosuvastatin therapy on CVD risk and the stable estimates of rosuvastatin efficacy across varying levels of GlycA suggest that GlycA may mediate residual vascular risk through a pathway that, if targeted, may complement statin therapy for managing the risk of CVD. Given that specific glycan chains unique to a host of acute-phase reactants are captured by GlycA, and presumably the contributing acute-phase proteins bear glycan sequence that do not contribute GlycA, a better understanding of the holo-glycan sequence for each contributing protein may shed light on the potential utility of GlycA for developing diagnostics, and perhaps therapy, that address CVD through inflammation. Research endeavors exploiting glycan biology are already making gains in virology and cancer-preventive research. It is worth noting that heparin, which is broadly indicated in the management of coronary events, is one of the oldest known anticoagulants and was later discovered to be a glycan-based therapeutic.

Merits and limitations of the present analysis warrant mentioning. The present study includes a large number of male and female participants who were recruited internationally and randomly allocated to a potent statin or placebo, had both baseline and on-treatment measurement of GlycA assessed, and were extensively phenotyped for CVD risk factors. Limitations of our study includes lack of generalizability given the entry criteria of JUPITER, which excluded participants with low hsCRP, high LDL-C, high triglycerides, known CVD, or diabetes and a limited follow-up duration. Finally, we expect that missing data on GlycA at 1 year occurred at random in a nondifferential manner that would, if anything, bias our results toward the null.

To conclude, this post-hoc analysis of JUPITER found that baseline and on-treatment GlycA levels were directly associated with the risk of future CVD independent of traditional CVD risk factors, rosuvastatin therapy, and hsCRP. These findings support the contribution of low-grade systemic inflammation to the occurrence of CVD in statin-treated individuals and, importantly, provide a significant biological illustration on the potential role of glycan-based research to address prevailing CVD risk burden.

Sources of Funding

JUPITER was financially supported by AstraZeneca, which collected trial data and monitored sites but had no role in the design or conduct of the present study, including data analysis or interpretation, drafting or editing of this report, or preparation, review, or the decision to submit the manuscript for publication. LipoScience Inc., Raleigh, NC (now LabCorp, Burlington, NC) absorbed the cost of performing the GlycA measurements and performed them in a blinded manner. Research reported in this publication was supported, in part, by the National Heart, Lung, and Blood Institute of the National Institutes of Health under award number R01HL117861 to Dr Mora and a charitable gift from the Molino Family Trust. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Dr Akinkuolie was supported by the National Heart, Lung, and Blood Institute (T32 HL007575).

DOI: 10.1161/JAHA.116.003822
Disclosures
Dr Ridker has received research grant support from AstraZeneca, Novartis, Amgen, and the National Heart, Lung, and Blood Institute and has served as a consultant to Genzyme, Janssen, Aegerion, ISIS, Vascular Biogenics, Boehringer Ingelheim, Pfizer, and Merck. Dr Ridker is listed as a co-inventor on patents held by the Brigham and Women’s Hospital that relate to the use of inflammatory biomarkers in CVD that have been licensed to AstraZeneca and Siemens. Dr Mora has received institutional research support from AstraZeneca, Atherotech Diagnostics, and NHBLI; served as a consultant to Genzyme, Quest Diagnostics, and Cerenis Therapeutics; and received speaker honoraria from AstraZeneca and the National Lipid Association. Drs. Korpela and Perola hold by the Brigham and Women’s Hospital that relate to the use of inflammatory biomarkers in CVD that have been licensed to AstraZeneca and Siemens. Dr Mora has a patent application on the use of NMR-measured GlycA for predicting risk of colorectal cancer. The other authors report no conflicts.

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