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A Novel Molecular Link Between IgG N-Glycosylation and Specialized Pro-Resolving Mediators

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Abstract

Immunoglobulin G (IgG) glycosylation is an important modulator of immune function, while specialized pro-resolving lipid mediators (SPMs) promote inflammation resolution; however, their molecular relationship remains unclear. We hypothesized that IgG glycosylation is molecularly linked to SPM pathways, revealing a previously unrecognized connection between adaptive immune glycosylation and inflammation resolution.

We integrated data on 24 circulating IgG N-glycans and 14 SPMs. Pairwise association analysis identified 11 significant glycan–SPM associations (FDR < 0.05), predominantly involving eicosapentaenoic acid (EPA) and its lipid mediator derivative 5-hydroxyeicosapentaenoic acid (5-HEPE). AI-driven network integration prioritized 5-HEPE and a complex, disialylated IgG glycan as central nodes linking the two molecular layers. Both 5-HEPE and the glycan showed evidence of polygenic regulation. Because IgG glycans are synthesized through enzymatic pathways in B cells, we performed *in vitro* experiments exposing human B cells to EPA or a control. EPA exposure resulted in conversion of EPA to 5-HEPE and related SPMs and increased the abundance of complex IgG glycans.

Together, these findings identify a previously unrecognized molecular link between EPA-related pro-resolving lipid mediator pathways and IgG glycosylation and provide experimental evidence that EPA exposure can modulate immune glycan remodeling in human B cells.

Introduction

Immunoglobulin G (IgG) N-glycosylation and specialized pro-resolving lipid mediators (SPMs) are important regulators of immune function and inflammation, yet potential crosstalk remains largely unexplored. IgG N-glycans modulate antibody effector functions through variations in core-

fucosylation, bisection, galactosylation, and sialylation, thereby influencing pro- and anti-inflammatory activity¹. In parallel, SPMs are derived primarily from omega-3 and omega-6 fatty acids, which actively promote the resolution phase of inflammation by limiting excessive immune activation and facilitating tissue homeostasis.

IgG glycosylation is a key determinant of antibody function and has been implicated in aging, chronic inflammation, autoimmune diseases, infections, and cardiometabolic disorders². Likewise, SPMs have emerged as critical regulators of inflammatory resolution and have been increasingly studied in chronic conditions such as cardiovascular disease, diabetes, and autoimmunity^{3,4}. Although glycans and SPMs have each been independently associated with immune-mediated diseases and inflammatory processes, their potential molecular interrelationship has not been examined. We hypothesized that IgG N-glycosylation is molecularly linked to SPM pathways, representing previously unrecognized crosstalk between adaptive immune glycosylation and inflammation resolution. Integrating these molecular layers may provide new insights into mechanisms linking lipid-mediated resolution pathways with glycan-driven immune function.

Here, we present an integrative analysis of IgG N-glycans and SPMs measured in human plasma samples from JUPITER, progressing from pairwise association analyses to AI-driven causal network integration to characterize molecular relationships and generate mechanistic hypotheses. We further integrated genetic data to infer directional dependencies and strengthen causal network inference. This approach identified key molecules within the glycome–lipid mediator axis.

To obtain experimental evidence supporting selected findings from the plasma analysis, we performed a proof-of-concept experiment in human B cells, exposing cells to EPA and assessing changes in SPM production and IgG glycan complexity. This work provides a systematic characterization of relationships between IgG glycans and SPMs and experimental evidence that EPA exposure can modulate IgG glycosylation, providing a foundation for further investigation of lipid mediator–glycan crosstalk.

Results

The glycan cohort included 456 participants with glycan measurements from JUPITER human plasma samples, of whom 368 had available SPM measurements and were included in the integrated glycan–SPM analyses. Participant characteristics are summarized in Table 1. The distribution and missingness of the molecular measurements before imputation are provided in Supplementary Table S1.

Table 1. Participant characteristics

Characteristic	Glycan cohort (n=456)	SPM subset (n=368)
Age, years, mean $\pm$ SD	70 $\pm$ 8	70 $\pm$ 8
Female, n (%)	132 (29%)	103 (28%)
White, n (%)	376 (82%)	309 (84%)
BMI, kg/m ² , mean $\pm$ SD	28 $\pm$ 6	28 $\pm$ 6
LDL-C, mg/dL, mean $\pm$ SD	103 $\pm$ 19	104 $\pm$ 19
HDL-C, mg/dL, mean $\pm$ SD	51 $\pm$ 15	51 $\pm$ 15

Characteristic	Glycan cohort (n=456)	SPM subset (n=368)
CRP, mg/L (median, IQR)	4 (2–8)	4 (2–7)
Triglycerides, mg/dL, mean $\pm$ SD	135 $\pm$ 74	132 $\pm$ 72

Values are presented as mean $\pm$ SD for continuous variables or n (%) for categorical variables, except CRP (high-sensitivity C-reactive protein), which is presented as median (IQR). The SPM subset comprises 368 participants from the 456 participants in the glycan cohort.

The study design, analytical approaches, and sample sizes are reviewed in Figure 1.

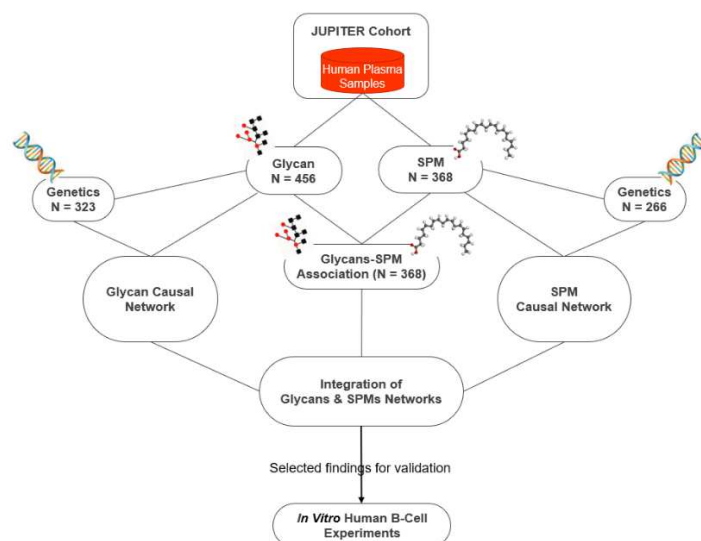


Figure 1. Study workflow and sample sizes. This study comprises two parts: first, the analysis of human plasma samples, followed by *in vitro* human B-cell experiments guided by the plasma findings. In the first part, specialized pro-resolving mediators (SPMs) and immunoglobulin G (IgG) N-glycans are measured in human plasma, and network analysis is used to characterize the connectivity among these molecules. Findings from the first part are then evaluated in *in vitro* human B-cell experiments, which constitute the second part of the study.

Using the 368 samples with both glycan and SPM measurements, we performed pairwise association analysis between 24 glycans and 14 SPMs using linear regression models adjusted for age, sex, race, batch effect, and cardiovascular disease status. The analysis revealed 11 significant associations between several IgG glycan peaks (GPs) and SPMs (FDR < 0.05) (Table 2).

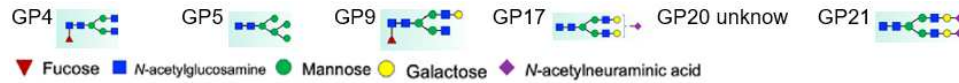
Table 1. The significant associations (FDR < 0.05) between IgG glycan peaks (GPs) and specialized pro-resolving mediators (SPMs) measured in human plasma using pairwise associations.

	Effect size	SE	p-value	FDR
GP21, 5-HEPE	0.34	0.049	3.6 e-11	1.2 e-8
GP21, EPA	0.33	0.052	1.3 e-9	2.1 e-7
GP20, EPA	0.28	0.051	9.2 e-8	1.0 e-5
GP20, 5-HEPE	0.23	0.050	3.9 e-6	3.2 e-4

GP21, 18-HEPE	0.21	0.050	3.1 e-5	2.1 e-3
GP17, 5-HEPE	0.21	0.054	7.2 e-5	4.0 e-3
GP9, EPA	0.20	0.053	2.1 e-4	1.0 e-2
GP17, EPA	0.20	0.056	2.7 e-4	1.1 e-2
GP5, EPA	0.19	0.054	7.8 e-4	1.8 e-2
GP21, DHA	0.25	0.078	1.3 e-3	4.4 e-2
GP4, 5-HEPE	-0.16	0.051	1.6 e-3	4.9 e-2

GP: glycan peak; HEPE: hydroxyeicosapentaenoic acid; EPA: eicosapentaenoic acid; FDR: false discovery rate; SPM: specialized pro-resolving mediator; SE: standard error
 Traits: GP4 & GP9 (asialylated), GP5 (oligomannose), GP17 (monosialylated), GP20 (sialylated or unknown), GP21 (disialylated)

Glycan structure:



The associations are ordered first by GPs and then by FDR

Among these associations, only GP4 and 5-HEPE were inversely associated, and the rest were positively associated.

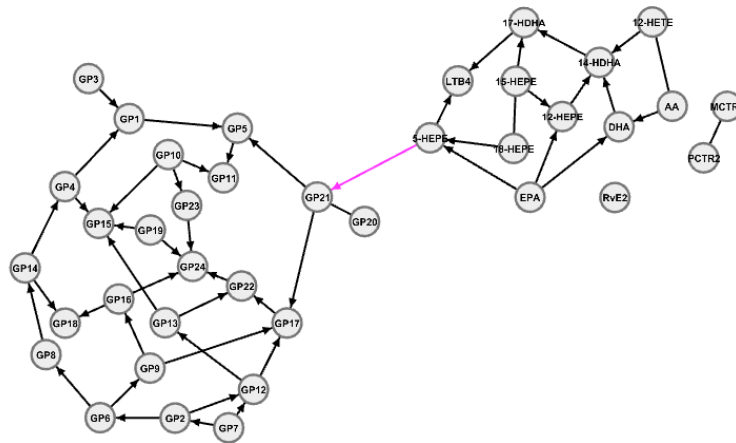
To evaluate whether the observed glycan–SPM associations in human plasma were explained by systemic inflammation, we repeated the pairwise association analyses after adjusting for high-sensitivity C-reactive protein (CRP). The direction and magnitude of the associations were broadly consistent with the primary analysis, with 10 of the 11 associations reported in Table 1 remaining significant after CRP adjustment (FDR <0.05 and all at FDR < 0.1) (Supplementary Table S2). These findings indicate that the observed glycan–SPM associations were largely robust to adjustment for CRP.

We then identified the SPM connectivity network (sample size $n = 368$ participants) and glycan connectivity network (sample size $n = 456$ participants) at level $\alpha = 1 \times 10^{-3}$ (Methods). These networks revealed the connectivity among molecules within each omics layer. The edges in the networks represent relationships between the two omics layers that cannot be fully explained by any subset of the other molecules⁵. We also integrated the glycan network with the SPM network at level $\alpha = 1 \times 10^{-2}$, which revealed the connectivity between the two pathways, which was through molecules 5-HEPE (an EPA-derived SPM) and GP21 (a disialylated IgG glycan) (**Figure 2**).

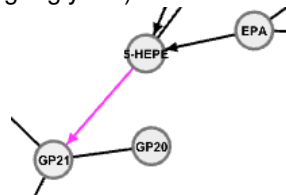
To illustrate how to interpret results, we zoomed in on **Figure 2a** around the two linking molecules (**Figure 2b**). From the 11 pairwise associations presented in Table 2, EPA was associated with both GP21 and GP20. However, in the joint analysis of all measured molecules, these associations were no longer retained as direct edges in the inferred network. Specifically, the EPA–GP21 association was explained by 5-HEPE, and the EPA–GP20 association was explained by GP21 and 5-HEPE. In contrast, 5-HEPE and GP21 remained directly connected, indicating a conditional relationship that could not be explained by other measured variables in this analysis.

We note that these findings reflect conditional dependencies within the set of observed molecules and do not exclude the possibility of residual confounding or latent factors arising from unmeasured variables or other omics layers.

As expected, we observed that the connectivity within each omics layer is stronger than between the two omics layers (**Figure 2c**).

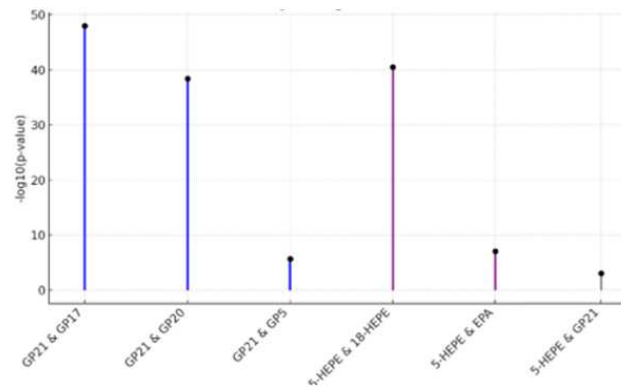


- a. The two linking molecules between the glycan and SPM are 5-HEPE (an EPA-derived lipid mediator) and GP21 (a disialylated IgG glycan).



- b. 5-HEPE and GP21 remained directly connected, whereas the pairwise associations of EPA with GP21 and GP20 were explained by subsets of 5-HEPE and GP21.

Connectivity strength around bridging molecules linking IgG-glycan and SPM omics



- c. The strength of connectivity around the two linking molecules 5-HEPE and GP21.

Figure 2. Underlying connectivity among glycans and SPMs measured in human plasma and between the two networks. **a.** The connectivity within each omics was identified at level $\alpha = 1e-3$. Then, the networks were integrated at level $\alpha = 1e-2$. The molecules 5-HEPE and GP21 were identified as linking nodes between the two molecular networks. **b.** Zoom in on the network. 5-HEPE and GP21 remained directly connected, indicating a conditional relationship that could not be explained by other measured variables in this analysis. In contrast, several pairwise associations disappeared, such as the pairwise associations of EPA with GP21 and GP20, indicating that the associations were explained by subsets of 5-HEPE and GP21. Therefore, EPA was not directly connected to GP21 or GP20. **c.** The connectivity strength of linking molecules 5-HEPE and GP21 with directly connected molecules was measured by $-\log_{10}(p\text{-value})$. The strengths within each network, such as GP21 with GP17, were much larger than those between the networks, 5-HEPE and GP21. Abbreviations: GP: glycan peak, SPM: specialized pro-resolving mediator, EPA: eicosapentaenoic acid

Using genetic variation as a directional anchor, we identified directions in each network (Methods). We observed that both linking molecules, 5-HEPE and the GP21 glycan, were under polygenic control. To identify the direction of influence between 5-HEPE and GP21, we evaluated residual independence under competing regression directions. Regressing GP21 on 5-HEPE yielded residuals that were independent of 5-HEPE (p-value = 1.0; failing to reject the null hypothesis of independence), supporting the 5-HEPE → GP21 direction. In contrast, the reverse orientation (GP21 → 5-HEPE) yielded weaker evidence for residual independence (p-value = 0.3). Although these tests do not establish causality on their own, the stronger evidence for residual independence in the 5-HEPE → GP21 direction is more consistent with 5-HEPE influencing GP21 than with the reverse direction.

Because shared upstream factors may influence both omics layers in cross-sectional data, we additionally evaluated potential sources of shared variation, including BMI and CRP, using age- and sex-adjusted partial Spearman correlation analyses (Table S2X). Some molecules showed associations with BMI and/or CRP. To further assess the robustness of the molecular networks to these potential upstream influences, we adjusted each molecular measure for BMI and CRP, in addition to the covariates included in the primary analysis, and repeated the causal network integration analysis. Adjustment for BMI and CRP did not alter network connectivity.

Extracting information from Causal Networks

We first review key causal network criteria⁶. Broadcaster nodes (shown in orange) are nodes with predominant outgoing influence (Out-degree > In-degree), including absolute broadcasters (In-degree = 0). Receiver nodes (shown in purple) are nodes with predominant incoming influence (In-degree > Out-degree), including absolute receivers (Out-degree = 0). The other nodes remain uncolored to preserve visual clarity.

The IgG glycan network. We observed that IgG glycans belonging to the trait core fucosylation (GP24, GP18, GP15, and GP11) functioned as receivers (**Figure 3a**). Receivers are not strongly connected to the network, regardless of their total number of connections. For example, GP15 and GP24 have the highest connectivity in the network (degree = 4); however, because their connections are dominated by incoming edges, their overall connection strength was among the lowest (**Figure 3b**).

This is opposite for broadcasters, including GP21, GP10, GP19, and GP7 (related to core fucosylation, disialylation, and monogalactosylation), which exert outgoing influence on downstream molecules and generally exhibit strong connection strength (Figure 3b, orange needles). This trend is consistent in the SPM network (Figure 4b) and aligns with previous observations in metabolomics networks⁷. In addition, IgG glycan pairs showing the strongest connectivity (Figure 3b; e.g., GP2–GP7, GP19–GP24, GP7–GP12, and GP17–GP21) occupy sequential positions in the biosynthetic cascade of glycosylation.

The IgG glycans GP20 (with an unknown chemical structure) and GP21 (a disialylated glycan) show strong statistical evidence of dependence across all tested conditioning sets of IgG glycans. The highest p-value from the conditional independence test between GP20 and GP21 remained

very low (p -value = $2e-30$), whereas the corresponding p -values for GP20 with other IgG glycans were substantially weaker ($p > 0.05$). Accordingly, GP20 was connected only to GP21 in the network. These findings may provide clues about the composition of GP20.

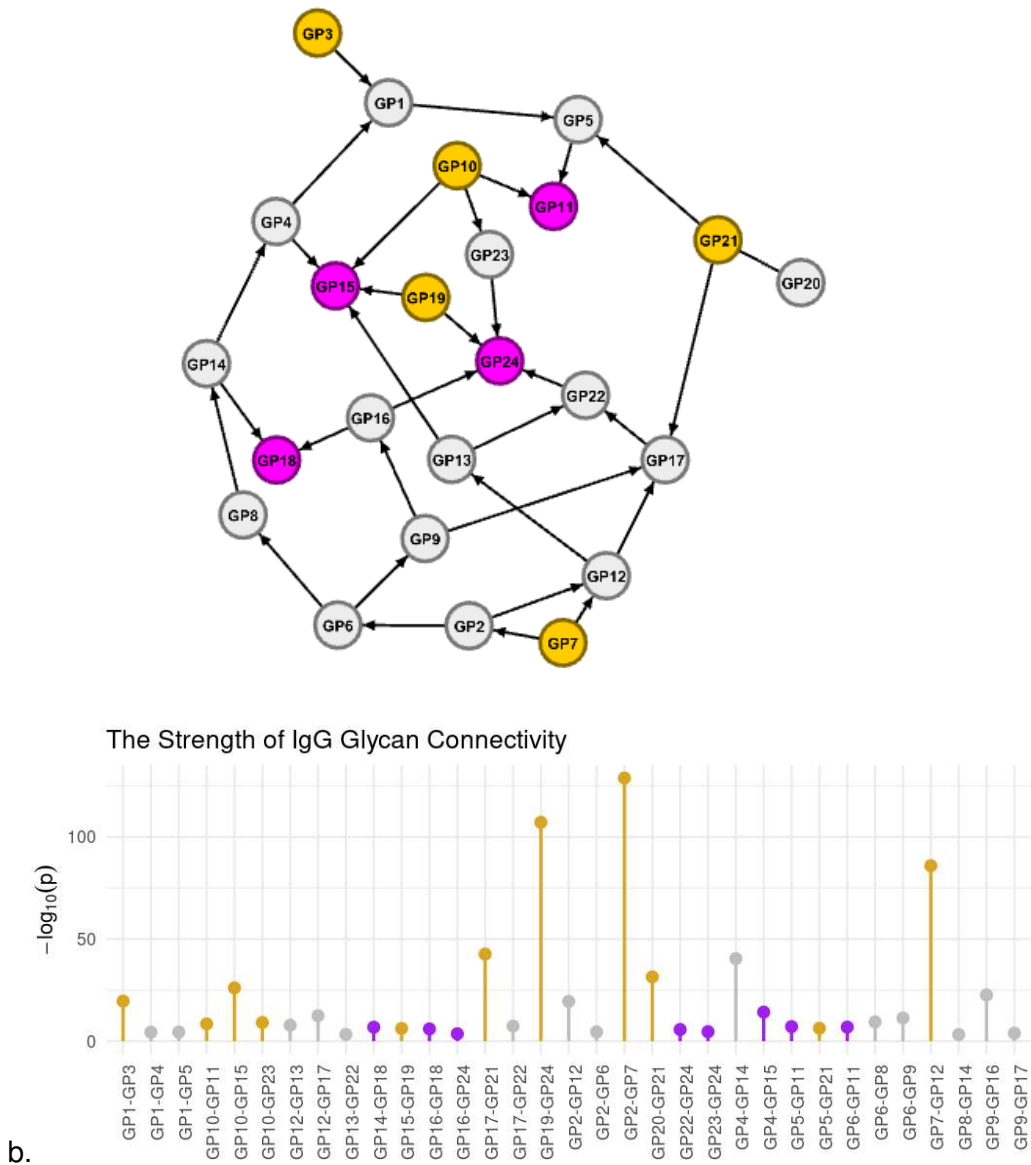


Figure 3. The IgG N-glycan Causal Network. **a.** The network connectivity of IgG glycans at level $\alpha = 1e-3$. The directions are identified by genetic factors (not depicted in the network). The nodes in orange are broadcasters, and the nodes in purple are receivers. **b.** The strength of the connections in the IgG glycan network. The highest strengths belong to broadcasters (yellow needles), while receivers have the lowest strength. The strongest connectivity (GP2–GP7, GP19–GP24, GP7–GP12, and GP17–GP21) occurs in sequential steps of the glycosylation biosynthetic pathway.

The SPM network. In the SPM network, leukotriene B-4 (LTB₄) had the properties of an absolute receiver (out-degree = 0), and EPA had the properties of an absolute broadcaster (in-degree = 0) (**Figure 4a**). LTB₄ was connected to the network with the lowest strength (**Figure 4b**), and the highest strength of connectivity belonged to the broadcaster 15-HEPE.

Two SPMs, maresin conjugates in tissue regeneration 2 (MCTR2) and protectin conjugates in tissue regeneration 2 (PCTR2), were directly connected but independent of the SPM network at level $1e-3$. Resolvin E2 (RvE2) was also independent of the network and other SPMs.

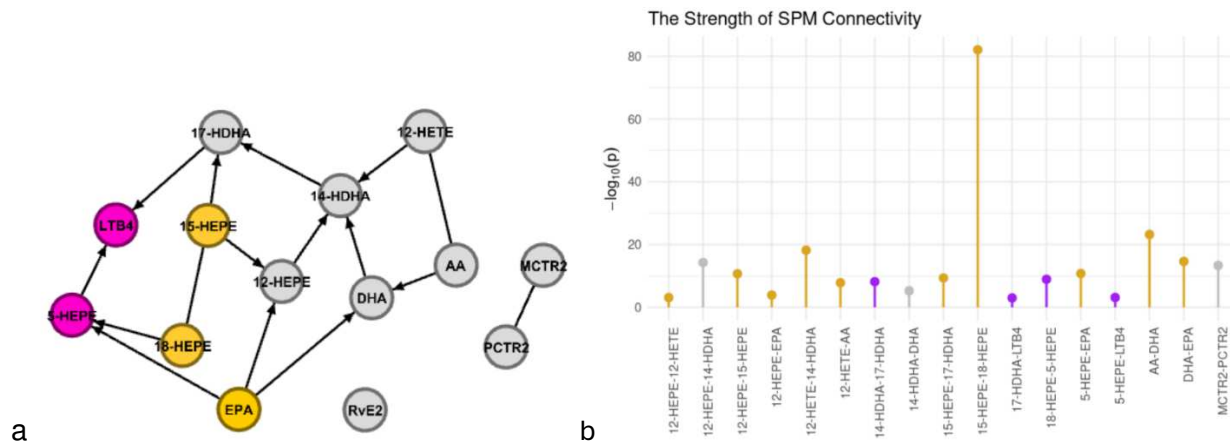


Figure 4. The SPM Causal Network. a. Network connectivity of SPM lipids at $\alpha = 1 \times 10^{-3}$. When the threshold is relaxed to $\alpha = 0.01$, RvE2 becomes connected to 5-HEPE; however, MCTR2 and PCTR2 remain unconnected to the other SPMs. Directions were identified using genetic factors (not depicted in the network) based on principles of Mendelian randomization. Selected broadcaster nodes are shown in orange and selected receiver nodes in purple. b. The strength of connections in the SPM network. 15-HEPE, a broadcaster, had the highest connection strength, whereas LTB₄, an absolute receiver, had the lowest.

Experimental study: Incubation of human-derived B cells *in vitro* with EPA versus cells alone. Since IgG glycans are synthesized via enzymatic pathways in B cells, we conducted an exploratory study to assess and validate potential links between EPA-derived mediators and glycosylation; human B cells (ARH-77 cell line) were incubated with EPA in the presence or absence of zymosan as an agonist and taken immediately for LC-MS-MS analyses and not stored before LC-MS-MS. Following EPA treatment, we observed that B cells converted EPA and increased the amounts of 5-HEPE, 18-HEPE, 17-HDHA, and 14-HDHA. In parallel incubations without cells, EPA did not undergo oxidation, and neither RvE2, 5-HEPE, nor 18-HEPE were produced without B cells in these incubations.

Fig 5.

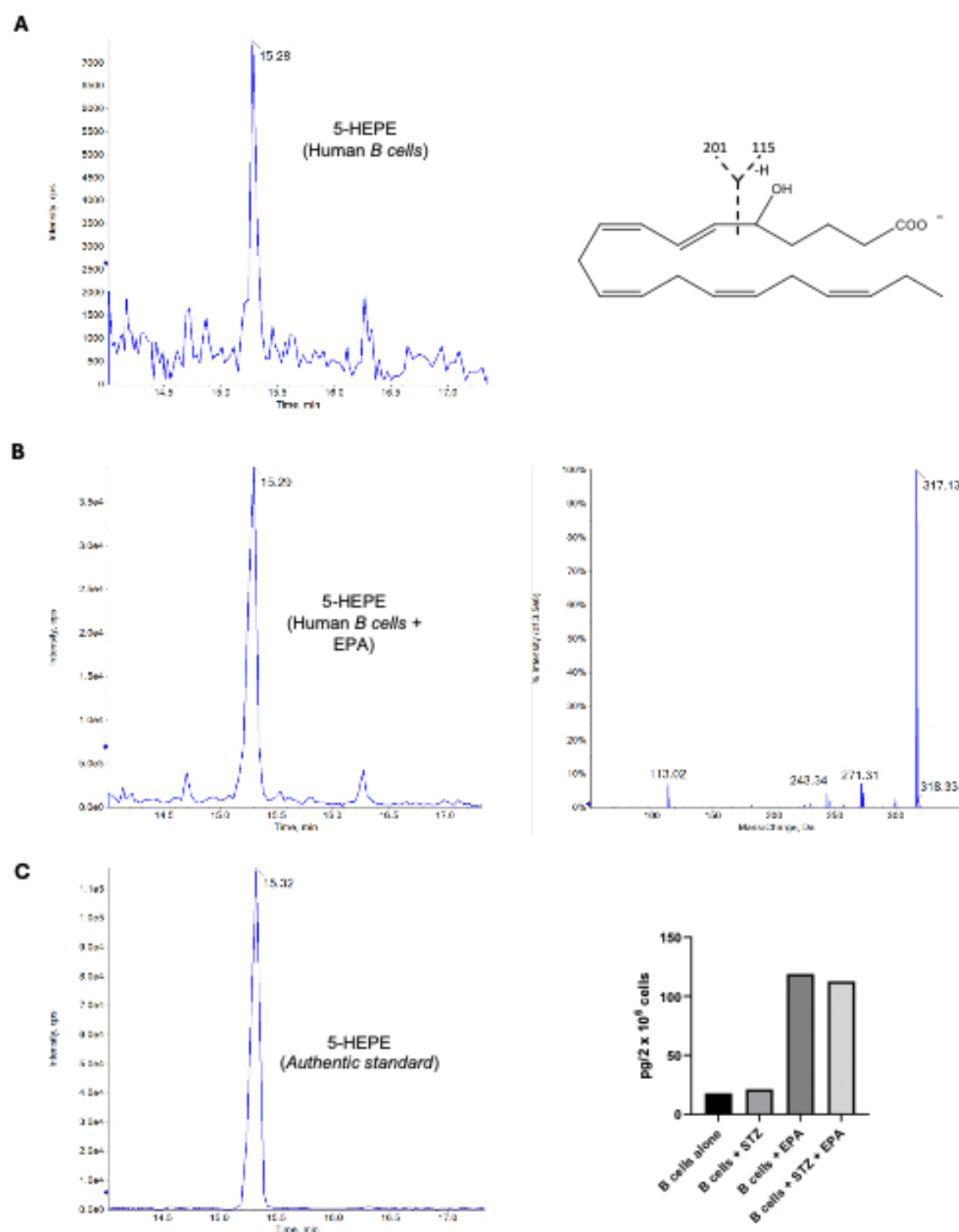


Figure 5. Identification of 5-HEPE in B-cell incubations: Targeted liquid chromatography tandem mass-spectrometry (LC-MS/MS). Human B cells (2×10^6) were incubated with 100 ng EPA in PBS+/-, pH 7.45, for 30 min at 37°C, then stimulated with serum-treated zymosan (STZ) for an additional 30 min at 37°C. Incubations were stopped with 2 volumes of ice-cold methanol containing deuterium-labeled internal standards and immediately (not stored) subjected to solid-phase extraction. Lipid mediator identification

and quantitation were carried out using liquid chromatography and tandem mass spectrometry (LC-MS/MS) on SCIEX Triple Quad 7500 (see Methods). (A) Multiple reaction monitoring (MRM) chromatogram showing the retention time of 5-HEPE ($T_R = 15.28$ min) identified in B cells alone (S/N=18.0). (B) Left, MRM chromatogram of 5-HEPE ($T_R = 15.29$ min) identified in B cells incubated with EPA (S/N=165.4). Right, corresponding MS/MS spectrum of 5-HEPE showing fragment ions including m/z 317 = M-H, m/z 299 = M-H-H₂O, and m/z 273 = M-H-CO₂. (C) Left, MRM chromatogram of 5-HEPE ($T_R = 15.32$ min) authentic standard (S/N=516.6). Right, Quantitation values of 5-HEPE.

In an exploratory additional experiment, IgG N-glycans were subsequently measured in the EPA-treated and control samples, with all 24 glycan peaks quantified. In EPA-treated cells, less complex glycans (agalactosylated) decreased by 8.84%, whereas more complex glycans, digalactosylated and sialylated, increased modestly by 4.97% and 6.15%, respectively. The observed shift toward greater glycan complexity was consistent with the network analysis.

Methods

Data Availability.

The study population was derived from JUPITER⁸ (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin; NCT00239681), an international, randomized, placebo-controlled trial of rosuvastatin (20mg/day) in the primary prevention of cardiovascular disease conducted among apparently healthy men and women⁸. Here, the focus was on a subset of participants with baseline measurements of the omics biomarkers.

Samples were collected and processed according to the established JUPITER protocol and stored at -170°C until analysis.

Requests to access the dataset from qualified researchers trained in human subject confidentiality protocols should be sent to the Steering Committees of the parent trial. Supporting LC-MS/MS raw data files will be available in the BioStudies database (<https://www.ebi.ac.uk/biostudies/>) upon publication.⁹

Omics Profiling. IgG N-glycans were measured at Genos, Inc. (Zagreb, Croatia. IgG was isolated from individual human plasma samples using CIM r-Protein G LLD 0.2 mL Monolithic 96-well plate (2 μm channels; catalog no. 120.1012-2, Sartorius BIA Separations)¹⁰, while N-glycans were released by peptide N-glycosidase F (PNGase F, catalog no. V4831, Promega) and labeled with a fluorescent dye, 2-aminobenzamide (catalog no. A89804, Sigma-Aldrich). For details see¹¹. Prepared samples were sent to the processing laboratory, where they were stored at -20°C until ultra-high-performance liquid chromatography analysis was performed on a Waters ACQUITY ultra-high-performance liquid chromatography H-Class instrument. All chromatograms were separated in the same manner into 24 IgG glycan peaks (IgG GPs), and the amount of glycans in each peak was expressed as the percentage of the total integrated area¹².

SPM levels were identified in human plasma samples using non-targeted liquid chromatography-mass spectrometry (LC-MS); their identification was confirmed using internal standards at Sapient Bio (San Diego, CA)¹³. The samples were spiked, SPM standardized, and then purified. The SPM peaks were manually identified using exact mass-to-charge ratios (m/z), retention times, and peak shapes, complemented by MS/MS fragmentation patterns to confirm their identity. This approach ensured accurate and reliable identification of SPMs in human plasma.

Genotyping was performed using the Illumina Omni 1M Quad platform (Illumina, San Diego, CA). After standard quality control procedures, 99.71% of loci yielded high-quality genotype calls,

details described elsewhere¹⁴. All samples had successful genotyping for > 98.5% of the final single-nucleotide polymorphisms (SNPs). A total of 1,006,348 SNPs passed QC filters, of which 814,418 had a minor allele frequency >1%. These genetic variants were used for imputation via the Michigan Imputation Server¹⁵ using the 1000 Genomes Project pilot reference panel¹⁶, with Hardy–Weinberg equilibrium filtering set at $p > 10^{-6}$.

Analytical Approach and Experimental Laboratory Studies

Linear Regression. To integrate SPMs and IgG glycans, we focused on 368 individuals with measurements for 24 IgG glycans and 14 SPMs. Of the 14 SPMs included in the analysis, all had <10% missing values (Table S1) and were imputed using the mean of the observed values. An additional 13 SPMs with >50% missing values were excluded from the analysis.

We first performed linear regression analyses, fitting each IgG glycan to each SPM molecule. Data preprocessing for IgG glycans included total area normalization, winsorization, and standardization (mean = 0 and standard deviation = 1). The winsorization function calculated the 5th and 95th percentiles and replaced values outside this range with the corresponding thresholds. This method limits the impact of outliers while preserving the overall distribution of the data.

For SPMs, we applied winsorization and standardization and adjusted them for batch effects (see Supplementary for batch correction). Then, we modeled IgG glycans on SPMs. All models were adjusted for age, sex, race, IgG glycan batch effect, and whether participants subsequently experienced a cardiovascular disease event. We selected the significant associations using the Benjamini-Hochberg method, FDR < 0.05. The results of this analysis are provided in Table 2. As a sensitivity analysis, we additionally adjusted for CRP to assess the robustness of the observed associations to systemic inflammation, with results provided in Supplementary Table S2.

AI-Driven Causal Network Integration. We first learn the relationships within each omics layer and subsequently integrate the networks across omics layers to identify cross-omics connectivity. This approach extends our previous work on identifying causal networks¹⁷. In cross-sectional studies, each omics layer may already reflect existing variation in the other omics layers. We therefore assume that the network structure within each omics layer can be estimated independently before identifying cross-omics connectivity:

$$str_{omics\ i} \perp str_{omics\ j}, \text{ for } \forall i \neq j, \quad (1)$$

where the structure (str) of omics i is independent of the structure of omics j , for all i not equal to j .

We first normalized and then adjusted the molecules at each omics for batch effects, sex, age, race/ethnicity, cardiovascular disease events, and statin use. We identified a network structure of 24 adjusted IgG glycans on 456 samples, and a network structure of 14 adjusted SPMs on 368 samples. Both network structures were identified using the PC algorithm, a probabilistic graphical-model approach for identifying conditional dependencies among variables¹⁸. We identified networks at level $\alpha = 1e - 3$ which controls the sparsity of the inferred network. We examined sparsity parameters ranging from $\alpha = 1e - 1$ to $1e - 7$ using the SPOT (Sparse Optimal Topology) method¹⁹, which determines the optimal data-driven sparsity level by identifying the inflection point at which network topology changes most substantially. This analysis identified $\alpha = 1e - 3$ as the optimal sparsity parameter. Therefore, the network structures are identified at this level. See Supplement for the visualization of the networks at three different levels and optimization of the network sparsity parameter.

Finally, we extended the PC algorithm under the assumption reviewed in formula (1) and integrated the glycan network with the SPM network to uncover the connectivity between the two omics layers, IgG glycans and SPMs, at $\alpha = 0.01$. As a sensitivity analysis, we also evaluated the integrated network at $\alpha = 0.05$. The cross-layer edge between 5-HEPE and GP21 was retained at both $\alpha = 0.01$ and $\alpha = 0.05$, indicating that the inferred connection was robust across these thresholds.

For visualization of the relative connectivity strength around the linking molecules, we quantified connectivity strength as $-\log_{10}(p)$, where p is the p -value from the corresponding conditional-independence tests used for network construction. Higher values indicate stronger evidence against conditional independence. The weaker conditional associations were observed between omics layers compared with within-layer associations.

To evaluate potential shared upstream influences on the two omics layers, we first assessed associations of each molecular measure with BMI and CRP using age- and sex-adjusted partial Spearman correlation analyses (Supplementary Table S3). We then performed a sensitivity analysis in which each molecular measure was adjusted for BMI and CRP, in addition to the covariates included in the primary analysis. The BMI- and CRP-adjusted molecular measures were subsequently used to repeat the causal network construction and integration analyses using the same analytical procedures and parameters as in the primary analysis.

Direction Identification.

Using previously identified associated genetic variants within each omics layer, we generated genetic factors by extracting shared genetic variation across variants. We used genetic factors and the G-DAG algorithm to identify the causal networks¹⁷. Each genetic factor captures variation across multiple individual genetic variants and may show a stronger association with molecules in the study, helping to avoid excluding molecules from causal network identification due to the lack of strongly associated genetic variants for each molecule. The G-DAG algorithm and its extensions have been previously applied to identify causal relationships and novel biological findings that were subsequently supported by clinical or experimental validation, e.g.,^{20,21}.

Previous work has shown that PCA-derived genetic factors can improve the stability of genetic-instrument-based analyses compared with using individual SNPs, particularly when multiple correlated variants are included^{17,22}. Genetic factors also reduce the need to rely on a small number of individual variants and thereby allow more of the available genetic variation to be incorporated. Furthermore, using genetic factors provides more robust directionality compared to seemingly arbitrary choices in the variable selection step¹⁷. If the direction between two molecules cannot be identified, the G-DAG algorithm leaves the link undirected.

To generate genetic factors, mathematical approaches for dimension reduction can be used, such as the application of principal component analysis¹⁷ or multiple correspondence analysis²³; in the present study, we used principal component analysis.

For generating genetic factors, we took the steps below for each omics separately (for details see Supplementary, Section 3):

1. Genetic variants associated with each omics layer (IgG glycans and SPMs) were extracted from the GWAS Catalog (<https://www.ebi.ac.uk/gwas/>). These variants were then identified in the imputed genotype data from the JUPITER cohort. In total, we retrieved 455 genetic variants for IgG glycans and 750 for SPMs.

2. Linear regression of each molecule was performed on each genetic variant, adjusting for the first five principal components, sex, age, and race. Variants with p -value < 0.05 were retained, yielding 81 unique variants associated with SPMs and 116 with IgG glycans.
3. Genetic factors were derived by applying principal component analysis to the selected variants (step 2) within each omics layer to capture underlying genetic variation. These PCA-derived genetic factors were subsequently used as candidate genetic anchors in G-DAG; factors that were not associated with any molecules were not retained for directional inference.
4. Importantly, not all PCA-derived genetic factors contribute to the final G-DAG network. Genetic factors that are not associated with any of the molecules are not retained for directional inference. In addition, genetic factors that are conditionally dependent on two molecules are excluded from directional inference by the G-DAG algorithm because of potential pleiotropy.
5. To evaluate the robustness of our network directional conclusions, sensitivity analyses using more stringent SNP-selection thresholds ($\alpha = 0.01$ and 0.001) were performed and revealed high directional stability, with zero edge reversals and retention of 88.4% and 81.4% of base directed edges, respectively (Supplementary Section 3.3).
6. Evaluated network stability using the Variable Reduction Test²³: In a directed network, a node with only incoming edges (i.e., a “receiver”) does not influence other molecules. Therefore, removing such a node and re-estimating the network on the reduced set should preserve the directionality among the remaining nodes. Consistency of edge directions before and after removal was used as an indicator of network stability (see Supplementary, Section 3.4 Assessing the stability of networks).

To assess the directional orientation of the established edge between 5-HEPE and GP21, we applied residual independence testing within an additive noise model framework. Under this framework, the null hypothesis (H_0) states that the regression residuals are statistically independent of the predictor variable (X). For the hypothesized orientation 5-HEPE $\rightarrow$ GP21, the residuals from regressing GP21 on 5-HEPE were independent of 5-HEPE ($p = 1.0$; failing to reject H_0), providing strong support for this directional model. Conversely, for the reverse orientation GP21 $\rightarrow$ 5-HEPE, the residuals showed weaker evidence of independence ($p = 0.3$), providing less support for the reverse direction.

Experimental Laboratory Study

Lipid mediator metabololipidomics. B cells from a human B-lymphoblast cell line (ATCC Cat. No. ARH-77) were cultured in RPMI media with 10% FBS and 1% penicillin-streptomycin, then collected and washed with calcium-free, magnesium-free phosphate-buffered saline (PBS^{-/-}, pH 7.45). The B cells (2×10^6 cells) were then incubated with 100 ng of eicosapentaenoic acid (EPA) (Cayman Chemical, Ann Arbor, MI) for 30 min at 37 °C in phosphate-buffered saline with calcium and magnesium (PBS^{+/+}, pH 7.45), then stimulated with serum-treated zymosan (100 μ g) for an additional 30 min at 37 °C in PBS^{+/+}. Incubations were stopped using two volumes of ice-cold and LC-MS-grade methanol (Thermo Fisher Scientific, Waltham, MA) containing 500 pg of deuterated internal standards d8-5S-HETE, d8-12S-HETE, d8-15S-HETE, d8-17S-HDHA, d4-LTB₄, d4-PGD2, d4-PGE2, d4-PGF2 α , d5-LXA4, d5-17R-RvD1, d5-RvD2, d5-RvD3, d5-MaR1, d5-MaR2, d5-PD1, d8-AA, d5-EPA, and d5-DHA (Cayman Chemical, Ann Arbor, MI). Samples were placed at -20 °C for at least 30 min to allow protein precipitation, not stored, and taken immediately for solid-phase extraction to avoid potential auto-oxidation, as described earlier²⁴.

Next, the extracted fractions were immediately subject to liquid chromatography-tandem mass spectrometry (LC–MS/MS) data acquisition in negative ionization mode. These experiments were conducted on a Triple Quadrupole 7500 (Sciex, Framingham, MA) equipped with an ExionLC HPLC system (Sciex, Framingham, MA) and a Kinetex® 2.6 µm Polar C18 100 Å, 100 × 3.0 mm column (Phenomenex, Torrance, CA) maintained at 50 °C. Details on the mobile phase composition and gradient used in the Serhan laboratory lab for these B- cell incubations, as well as gas and source parameters, are described in²⁴.

Identification of lipid mediators was performed by matching chromatographic retention times and tandem MS/MS spectra prominent ions to those of authentic reference standards, in accordance with our published criteria²⁴. Of note, the mass accuracy on the Triple Quad 7500 instrument is ± 0.1 atomic mass units (a.m.u.). The additional digits presented in MS/MS spectra are due to default manufacturer settings. These data were acquired and analyzed using SCIEX OS-Q 3.4.0.19154 and are presented as screen captures. For details, see Supplement, section 4.1.

IgG glycosylation in ARH77 cells. Following EPA treatment, IgG was isolated from cell supernatants using protein G agarose beads as described previously. Purified IgG was denatured, and N-glycans were enzymatically released using PNGase F. Released glycans were fluorescently labeled with procainamide, purified by HILIC-SPE, and separated by HILIC-UPLC with fluorescence detection. Glycan peaks (GP1–GP24) were quantified as relative abundances (% total area). For details, see Supplement, section 4.2.

Discussion

In this study, we identified a novel molecular link between EPA-related pro-resolving lipid mediators and IgG glycosylation. We leveraged both classical statistical rigor and the Causal Network Integration framework based on genetic data. We uncovered polygenic molecules and structure within the glycome–lipidome axis and provided mechanistic insights into pathways linking SPMs and IgG glycans. We identified a novel link between EPA-related pro-resolving lipid mediators and IgG glycosylation. To our knowledge, this is the first integrative evidence linking IgG N-glycosylation with EPA-related SPMs, two key pathways of immune function and inflammation resolution.

By combining multi-omics (glycomics, lipidomics, and genetic analyses) with the AI-driven causal networks, we extracted information about these molecules and their underlying connectivity. We identified a coordinated network centered on EPA-derived SPMs and sialylated IgG glycans. Our exploratory *in vitro* human B-cell model provided experimental evidence that B cells convert EPA, resulting in increased levels of 5-HEPE and related SPMs.

In the network of SPMs measured on plasma samples, EPA was identified as a broadcaster, a molecule that influences other molecules and is not influenced by them. Then, in B-cells in an experimental study, we observed the conversion of EPA to 5-HEPE. In the network integration of SPMs and glycans measured in plasma samples, EPA → 5-HEPE. Both 5-HEPE and EPA are known to regulate metabolic and inflammatory pathways, suggesting a potential link to immune function²⁵; however, their relationships with IgG glycosylation have not been previously reported. These findings suggest that pro-resolving lipid pathways may interact with antibody glycosylation processes that favor anti-inflammatory or pro-resolution immune responses, revealing a previously unexplored molecular axis connecting lipid-mediated resolution with glycan-driven modulation of IgG function. Specifically, we found that the two molecules 5-HEPE (an EPA-derived

lipid mediator) and GP21 (a disialylated IgG glycan) were key linking molecules between the two networks, and both 5-HEPE and the GP21 glycan were under polygenic control, which may imply that their levels in the human body are influenced by genetic variation. Recent studies have also shown that IgG glycans are regulated by a complex network of genes, as demonstrated by several large genetic studies^{26,27}.

Interestingly, both 5-HEPE and complex galactosylated and sialylated IgG glycans are generally considered protective in the context of inflammation². We found that higher levels of SPMs may be associated with more complex, galactosylated, and sialylated IgG glycans. These glycan patterns are more abundant in young and healthy people^{1,2}. In addition, the current study found evidence that lipid mediators may influence more complex IgG glycans, i.e., those glycans with galactose and sialic acid structures. The loss of these glycans has been associated with various cardiometabolic and autoimmune conditions, diseases in which SPMs, such as 5-HEPE, have also been shown to play a protective role²⁸. In this study, we found that IgG glycans containing galactose and sialic acid were positively associated with multiple SPMs. Conversely, GP4, an agalactosylated, core-fucosylated glycan elevated in both cardiometabolic and autoimmune diseases¹², exhibited a negative association with 5-HEPE in this study and was the only glycan showing a significant negative association with SPMs.

In the network of plasma SPMs, RvE2 was directly connected with 5-HEPE at a significance level of 0.01 (Supplementary Section 2). Although RvE1 was not included in our analysis, a population study of 978 healthy 27-year-olds reported an inverse association between plasma RvE1 and adiposity, including BMI and abdominal subcutaneous fat volume²⁹. Although the two SPMs, PCTR2 and MCTR2, were strongly connected, neither was connected to the other SPMs in the analysis, even at $\alpha = 0.05$. PCTR2 and MCTR2 arise from distinct biosynthetic pathways and therefore may represent SPMs with biosynthetic origins distinct from the EPA- and AA-derived mediators examined in this study. These two SPMs did not show any connectivity with glycans.

The strong connectivity of IgG glycans corresponding to successive steps in glycan biosynthesis suggests that network structure can capture underlying biology within the omics layer. For example, GP2 can be converted to GP7 in a single step by galactosyltransferase activity. Thus, Causal Network Integration can generate testable hypotheses within and across omics layers, including relationships not apparent from pairwise associations, as demonstrated in our previous study that identified and experimentally validated a correction to a mischaracterized IgG biosynthetic pathway³⁰. GP24 was identified as a receiver, a molecule influenced by several glycans. This finding is consistent with its highly processed structure, FA2BG2S2, which incorporates bisecting GlcNAc, galactosylation, and terminal sialylation¹⁰.

5-HEPE is an EPA-derived 5-lipoxygenase metabolite. In our network topology, 5-HEPE connects EPA and GP21, suggesting a prospective EPA $\rightarrow$ 5-HEPE $\rightarrow$ GP21 directional relationship. This interpretation is biochemically plausible given the established metabolic relationship between EPA and 5-HEPE; however, the network topology represents a statistical hypothesis rather than a validated mechanistic pathway. Although the inferred direction is more consistent with 5-HEPE influencing GP21 than the reverse, establishing the direction and mechanism of this relationship will require targeted functional experiments.

All biospecimens were collected at trial baseline prior to randomization and treatment initiation; thus, participants were statin-naïve at sampling. However, because the JUPITER cohort selected for individuals with elevated CRP (≥ 2 mg/L) and normal LDL-C (< 130 mg/dL), this clinical trial

context reflects a specific sub-population. Second, our cohort comprised older adults (mean age ~70 years) with a higher proportion of male (75.4%) and White (87.4%) participants. Because age, biological sex, and ancestry strongly influence systemic metabolic profiles and IgG N-glycosylation, our findings warrant future validation in younger, more racially diverse, and unselected community-based cohorts. Finally, in vitro observations using transformed cell lines (such as ARH-77) represent exploratory model systems and require further functional validation in primary human plasma cells.

Together, these findings support a previously unrecognized molecular connection between EPA-related pro-resolving lipid mediator pathways and IgG N-glycosylation. The observed relationships and exploratory B-cell findings provide a basis for future studies to determine whether lipid mediator metabolism directly influences IgG glycan remodeling and to define the functional consequences of this potential axis in inflammation and immune regulation.

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