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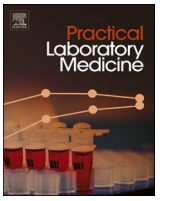

Influence of plasma collection tubes on N-glycome in human blood samples

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ABSTRACT

Background and aims: Quantitative analysis of plasma N-glycome is a promising method for identifying disease biomarkers. This study aimed to investigate the impact of using blood collection tubes with different anticoagulants on plasma N-glycome.
Materials and methods: We used a robust mass spectrometry method to profile plasma N-glycomes in two cohorts of healthy volunteers (cohort 1, n = 16; cohort 2, n = 53). The influence of three commonly used blood collection tubes on fully characterized N-glycomic profiles were explored.
Results: Principal component analysis revealed distinct clustering of blood samples based on the collection tubes. Pairwise comparisons demonstrated significant differences between EDTA and heparin plasma in 55 out of 82 quantified N-glycan traits, and between EDTA and citrate plasma in 62 out of 82 traits. These differences encompassed various N-glycan features, including glycan type, sialylation, galactosylation, fucosylation, and bisection. Trends in N-glycan variations in citrate and heparin plasma were largely consistent compared to EDTA plasma. In correlation analysis (EDTA vs. heparin; EDTA vs. citrate), Pearson's correlation coefficients were consistently higher than 0.7 for the majority of N-glycan traits.
Conclusion: Sample matrix variations impact plasma N-glycome measurements. Caution is crucial when comparing samples from different plasma collection tubes in glycomics projects.

1. Introduction

Glycosylation is one of the most prevalent and pivotal post-translational modifications that can significantly impact on the structural and functional properties of glycoconjugates [1–3]. Given its pervasive occurrence and the capacity to influence nearly every facet of biological processes through variations in glycan structure and abundance, abnormal glycosylation of glycoproteins is

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closely associated with the occurrence, development, and prognosis of various diseases [4–7]. Quantitative analysis of the total blood (serum/plasma) N-glycome is emerging as a potent approach for the discovery of novel disease biomarkers. For instance, the quantitative analysis of plasma N-glycome has been reported to predict cardiovascular risk by reflecting inflammatory pathways [8]. Variations in serum N-glycome were also found to effectively forecast the development of liver cirrhosis and provide ongoing monitoring of fibrotic progression [[9]. Moreover, our recent study reported that plasma N-glycans present itself as a promising prospect for assessing the inflammatory status of patients with chronic thromboembolic pulmonary hypertension [10].

Human blood is the most advantageous biological specimen used by clinicians and researchers due to its accessibility and the wealth of information it represents [11]. About 70% of proteins in the blood are glycoproteins [12]. The repertoire of glycoproteins in the blood primarily encompasses immunoglobulins secreted by plasma cells and glycoproteins synthesized in the liver [13]. Systemic alterations associated with diseases can induce changes in the glycosylation of these glycoproteins, which are detectable in the blood [14]. Such characteristics underscore the suitability of blood as an optimal starting material for N-glycome analysis and the exploration of potential biomarkers.

The attainment of reliable results is paramount to support high-caliber research and informed medical decision-making. Ensuring the attribution of observed alterations to the disease itself, rather than arising from errors within the sampling methodology, is of paramount significance. The pre-analytical phase is the primary contributor to errors within the laboratory testing process and the process of venous blood collection including the correct choice of blood collection tubes represents a critical dimension of the pre-analytical phase, profoundly influencing the overall accuracy of the analysis. There are two types of samples obtained from blood, plasma and serum. Serum is acquired through centrifugation following clotting, enabling the elimination of fibrin clots, blood cells, and associated coagulation factors. In contrast, plasma samples are obtained by introducing anticoagulants (such as EDTA, heparin, and citrate) before centrifugation, facilitating the removal of blood cells. No virtual interferences are anticipated in serum due to postcentrifugal coagulation [15,16]. However, the inclusion of anticoagulants in plasma collection tubes may introduce certain interferents, including enzyme inhibitors and fibrinogen [17].

Various studies have investigated variations in metabolomic and proteomic profiles associated with the utilization of diverse blood collection tubes [18–24]. Conversely, the effects of different blood collection tubes on N-glycomic profiles received relatively little attention. Adamczyk et al. investigated the impact of blood collection conditions, utilizing silicone-coated glass for serum and tubes containing EDTA, Na-heparin, and Li-heparin for plasma samples, on N-glycan analysis [11]. Employing hydrophilic interaction liquid chromatography (HILIC)-high performance liquid chromatography (HPLC) methodology on samples from five healthy volunteers, they identified significant differences in N-glycan peaks between plasma and serum, attributing these distinctions to fibrinogen (a glycoprotein). They also found that specific anticoagulant factors (EDTA, Na-heparin, and Li-heparin) did not influence the N-glycome profiles [11]. Later, another study by Ventham et al. reported that changes in serum sample tubes caused very small intra-individual variation in serum N-glycan profiles in inflammatory bowel disease and healthy controls [25]. The two previous studies, however, used relatively low-sensitive approaches and lost the linkage information of sialylation, which allowed the detection of a limited kind of N-glycans. In addition, the sample size was small for the former (n = 5). The potential influence of blood collection tubes with different anticoagulants on plasma N-glycome has not been fully investigated.

In the present study, we utilized the linkage-specific sialic acids derivatization method in conjunction with a robust high-throughput matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) platform to profile plasma N-glycome, thereby augmenting the effective depth of plasma N-glycome analysis for more expansive coverage of glycan structures. Our primary objective was to characterize, for the first time, the impact of three frequently used plasma collection tubes—EDTA plasma tubes, heparin plasma tubes, and sodium citrate plasma tubes—on fully characterized N-glycomic profiles.

2. Methods

2.1. Plasma samples

In this study, two cohorts (cohort 1, n = 16; cohort 2, n = 53) of apparently healthy volunteers were recruited from the Peking Union Medical College Hospital. For Cohort 1, each donor provided EDTA and heparin plasma utilizing 1.8 mg/mL K_2-EDTA plasma collection tubes (BD 367861, 4 mL) and heparin plasma collection tubes (BD, 367869, 4 mL). In the case of Cohort 2, each donor supplied EDTA and citrate plasma using 1.8 mg/mL K_2-EDTA plasma collection tubes (BD 367861, 4 mL) and 0.109 M 3.2% trisodium citrate plasma collection tubes (BD 363095, 2.7 mL). Plasma samples were processed according to validated standard operating procedures. In short, after the blood draw, samples were inverted six times and centrifuged for 10 min at 2350 g, and plasma was transferred to a clean tube. Samples were drawn, processed, and frozen (at −80 °C) within 3 h and analysis was performed within one month of storage. This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the regional ethics committee of Peking Union Medical College Hospital (No. HS-2402). All donors gave written informed consent.

2.2. Enzymatical release of N-glycans from plasma

N-glycans were released from the human plasma samples with enzymatic treatment as described previously [26,27]. Briefly, 5 μL of plasma sample was firstly denatured by adding 10 μL of 2% SDS and incubation for 15 min at 65 °C. Then, 10 μL of release mixture (1 U PNGase F, 2% NP-40, and 2.5 × PBS) was added to the samples, followed by incubation overnight at 37 °C [26,27].

2.3. N-glycans derivatization and purification

The released N-glycans were subjected to derivatization utilizing the linkage-specific sialic acid stabilization method, facilitating the stabilization and mass-dependent discrimination of sialic acid isomers (specifically, α2,3- and α2,6-linked sialic acids), as described in detail in previous literature [26,27]. In brief, a mixture comprising 1 μL of liberated glycans and 20 μL of derivatization reagent, consisting of 250 mM EDC and 250 mM HOBt in ethanol, underwent an incubation period at 37 °C for 60 min. Subsequently, acetonitrile (ACN) was introduced, and the solution underwent additional incubation at −20 °C. Following these steps, the derivatized N-glycans were subjected to enrichment and purification through HILIC solid-phase extraction (SPE) [28,29].

2.4. MALDI-TOF-MS measurement of N-glycome

MALDI-TOF-MS in reflection positive-ion mode was employed for the profiling of plasma N-glycans [26,27]. Specifically, 1 μL of glycan samples was combined with an equal volume of matrix containing 5 mg/mL super-DHB with 1 mM NaOH in 50% ACN on the AnchorChip target plate (Bruker Daltonics, Bremen, Germany), followed by air-drying the mixture. The N-glycomes were subsequently detected using a rapifleXtreme MALDI-TOF mass spectrometer equipped with a Smartbeam-3D laser, under the control of flexControl 4.0 (Bruker Daltonics). Calibration of the instrument was performed using a peptide calibration standard (Bruker Daltonics), and the mass range was set from *m/z* 1000 to *m/z* 5000. Laser shots were accumulated 5000 times for each spectrum. An automatic acquisition mode and random walk pattern at a laser frequency of 5000 Hz were selected for the acquisition of sample spectra.

2.5. MALDI-TOF-MS data processing and statistical analysis

The comprehensive protocol for processing raw MALDI-TOF-MS data has been previously delineated [26,27]. In short, the initial steps involved baseline subtraction and smoothing of the raw data, followed by conversion into.xy files using flexAnalysis 4.0 (Bruker Daltonics). Subsequent recalibration of the.xy files utilized selected high-intensity glycan signals as calibrants (Supplementary Table S1), a process executed through MassyTools (version 0.1.8.1.2) [30]. Manual assignment and confirmation of mono-isotopic peaks to N-glycan compositions were accomplished using the glyco-Peakfinder tool of GlycoWorkbench (version 1.1.3480) [31] and previously validated glycan compositions [27,28]. A curated N-glycan composition list, encompassing 90 structures, was generated for targeted N-glycan extraction, adhering to criteria such as mono-isotopic peaks with good isotopic patterns, signal-to-noise ratio (S/N) exceeding six, and relative intensity surpassing 0.1%. The peak areas (background-corrected) of putative N-glycans, accompanied by quality parameters, were subsequently extracted into Excel using MassyTools and the N-glycan composition list. Further refinement of the extracted N-glycans ensued, applying criteria of S/N exceeding nine, ppm error less than 20, QC score below 25%, and a minimum presence percentage (greater than 50%) in all spectra of healthy volunteers or quality control plasma samples (replications of a standard plasma sample randomly distributed on the plates). Ultimately, 59 N-glycans out of the initial 90 met the criteria for subsequent statistical analysis (Supplementary Table S2). The sum of the areas of these 59 N-glycans per spectrum was then normalized to one.

As derived N-glycan traits encapsulate glycosylation alterations shared among a set of structurally related N-glycans, thereby facilitating a more nuanced interpretation of the biological implications of glycosylation [13,26,27], we systematically computed 82 derived N-glycan traits (Supplementary Table 3) from the 59 individual N-glycans (directly detected) in RStudio (version 4.0.3). The derivation of the derived traits is guided by specific formulas (Supplementary Table 3) grounded in the common structural elements observed among individual N-glycans. The focal point of each calculation is denoted by the concluding letter, with the preceding letters delineating the group on which the computation is applied. For example, the interpretation of 'A3LF' translates to 'fucosylation (F) within triantennary (A3) N-glycans with α2,3-linked sialic acid (L) ' (Supplementary Table 3).

Principal component analysis (PCA) was employed to acquire a multivariate overview of the N-glycomic data. Paired *t*-test was used for the comparisons of derived N-glycan traits within each cohort (cohort 1, EDTA vs. heparin; cohort 2, EDTA vs. citrate). The reported p-values were adjusted for False Discovery Rate (FDR). P values less than 0.05 indicate statistically significant differences between the groups. In order to describe the data, the pairwise correlations between tubes were also explored. The Pearson's correlation coefficient (r) was calculated for each N-glycan trait to compare EDTA vs. heparin plasma and EDTA vs. citrate plasma. Graphs were generated employing GraphPad Prism 9 and MetaboAnalyst 5.0.

Table 1
The demographic characteristics of the two cohorts comprising healthy volunteers involved in this study.

	Cohort 1 n = 16	Cohort 2 n = 53
Age, mean (SD)	33.1 ± 12.2	38.0 ± 14.9
Male, n (%)	10 (62.5%)	21 (39.6%)
Samples Collected	EDTA and heparin plasma	EDTA and citrate plasma

3. Results

3.1. Demographics of healthy volunteers included in this study

The demographic characteristics of the two cohorts comprising healthy volunteers are detailed in Table 1. In cohort 1, each donor provided both EDTA and heparin plasma samples. The average age of donors was 33.1 ± 12.2 years, with a male proportion of 62.5%. In cohort 2, each donor contributed EDTA and citrate plasma samples. The average age of donors was 38.0 ± 14.9 years, with a male proportion of 39.6%.

3.2. The plasma N-glycome features and data reliability

At the global plasma level, a total of 90 N-glycans were identified across both cohorts, with 59 meeting the established quality criteria for subsequent data analysis. This encompassed diverse N-glycan types, such as high-mannose, hybrid, and complex, as outlined in Supplementary Table S2 which contains a comprehensive list of N-glycans analyzed in this study. The 59 directly detected N-glycans were further grouped into 82 derived N-glycan traits based on shared structural features, including the number of antennas (A), galactosylation (G), bisection (B), fucosylation (F), sialylation (S), and linkage-specific sialylation (E or L), employing specific formulas (refer to the Supplementary Table S3).

The consistent quality and methodological repeatability of the data were evaluated by incorporating standard plasma. The average relative standard deviation (RSD or CV) for all 82 derived N-glycan traits obtained from repeated standard plasma samples was 2.32%.

As derived N-glycan traits effectively encapsulate the collective effects of individual N-glycans sharing similar structural features, thereby aiding the interpretation of biological impacts, and demonstrating heightened repeatability compared to the individual N-glycan traits from which they are derived [27], our subsequent focus in this study centered on the analysis and comparison of derived N-glycan traits.

3.3. Influences of different plasma collection tubes on N-glycome

The median and standard deviation of all N-glycan traits, for each tube type in the two cohorts, are provided in Supplemental Table S4.

Unsupervised PCA showed a clear clustering of blood samples based on the collection tubes (Fig. 1). This suggested significant alterations in the levels of N-glycans, indicative of tube-dependent deregulation. In particular, EDTA and heparin plasma samples present the most pronounced differences (Fig. 1A), whereas EDTA plasma samples and citrate plasma show an intermediate behavior (Fig. 1B).

In detail, pairwise comparisons of samples showed that 55 out of the 82 quantified N-glycan traits displayed statistically significant differences between EDTA and heparin plasma, while 62 out of the 82 showed disparities between EDTA and citrate plasma (Fig. 2, Supplemental Table S4).

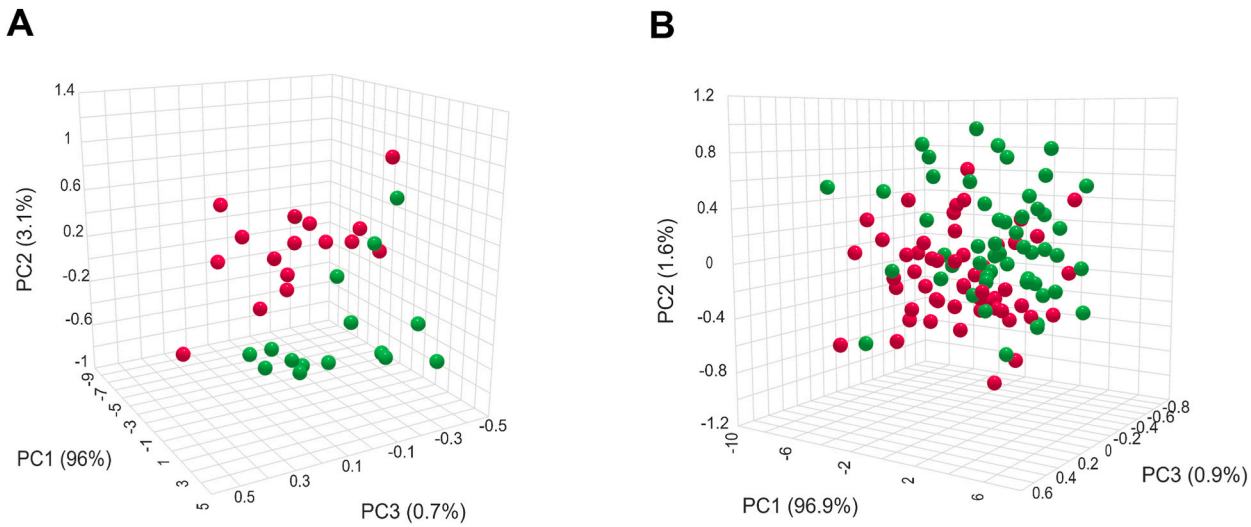

Fig. 1. Principal component Analysis (PCA). PCA was performed on N-glycomic data of (A) EDTA plasma (red) and heparin plasma (green) from participants in cohort 1, and (B) EDTA plasma (red) and citrate plasma (green) from participants in cohort 2. Score plot depicting the first three principal components of PCA derived from N-glycome-related parameters. Each sphere symbolizes an individual sample, with coloring corresponding to the specific blood collection tubes employed. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

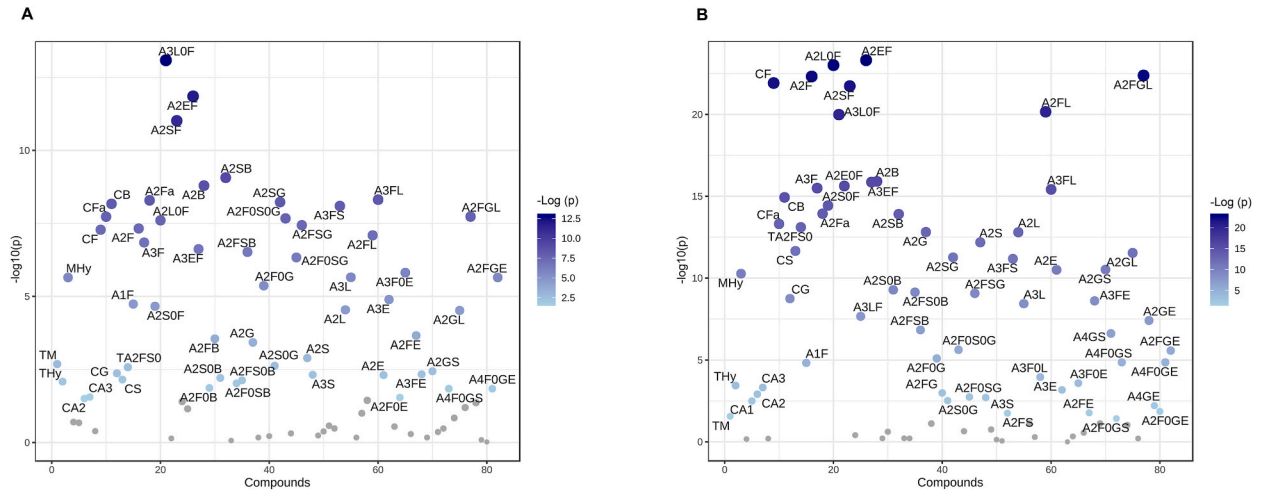

Fig. 2. The N-glycan traits that exhibit statistical significance between (A) EDTA (depicted in red) and heparin (depicted in green) plasma samples, as well as (B) EDTA (depicted in red) and citrate (depicted in green) plasma samples. Blue points represent N-glycan traits that demonstrate statistical differences between the two groups, with deeper shades indicating more pronounced distinctions. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

In comparison to the EDTA plasma, the trends of N-glycans variations in both citrate and heparin plasma were largely consistent. As a general trend, in EDTA plasma as compared with heparin and citrate plasma samples, higher levels of total hybrid type N-glycans (THy), total diantennary N-glycans within complex type N-glycans (CA2), core fucosylation (CF, A1F, A2F, A3F, A2S0F, A2L0F, A3L0F, A2SF, A2EF, and A3EF), total bisecting GlcNAc within complex N-glycans (CB), and total fucosylated, non-sialylated diantennary species (TA2FS0) coupled with reduced levels of total high mannose type N-glycans (TM), the ratio of high-mannose to hybrid N-glycans (MHy), total triantennary N-glycans within complex type N-glycans (CA3), antennary fucosylation (CFa and A2Fa), galactosylation (CG, A2G, A2F0G, A2FG, A2S0G, A2SG, A2F0S0G, and A2FSG), sialylation (CS, A2S, A3S, A3FS, A2GS), and α2,3-linked sialylation (A2L, A3L, A2FL, A3FL, A2GL, and A2FGL) were observed (Figs. 2 and 3, Supplemental Table S4).

The most prominent N-glycan traits exhibiting differences between EDTA plasma and heparin plasma (Top 10) were core and antenna fucosylation within diantennary or triantennary N-glycans (A3L0F, A2EF, A2SF, A2Fa, and A2SF), bisecting GlcNAc within total complex N-glycans and diantennary N-glycans (A2SB, A2B, and CB), sialylation especially α2,3-linked sialylation within fucosylated triantennary N-glycans (A3FL and A3FS), and galactosylation within diantennary N-glycans with sialic acid (A2SG). As for the differences between EDTA and citrate plasma, the most significant N-glycan traits (Top 10) included core fucosylation within total complex N-glycans, diantennary or triantennary N-glycans (A2EF, A2L0F, A2F, CF, A2SF, A3L0F, and A3EF), α2,3-linked sialylation within fucosylated diantennary N-glycans (A2FGL and A2FL), and bisecting GlcNAc within diantennary N-glycans (A2B) (Fig. 3, Supplemental Table S4).

3.4. Pairwise correlation analysis of N-glycan traits between tubes

The comparisons of the EDTA plasma, heparin plasma, and citrate plasma highlight the presence of several statistically significant differences. The Pearson's correlation coefficients were however always higher than 0.7 for most of the N-glycan traits in pairwise correlation analysis (EDTA vs. heparin, EDTA vs. citrate; Fig. 4).

4. Discussion

Standardization plays a pivotal role in the context of any omics technology [32]. Harmonization of procedures to ensure pre-analytical and analytical robustness is necessary in clinical omics. In recent years, numerous reports highlighting N-glycans as potential biomarkers for various diseases have been published [33–39]. The utilization of body fluids in the discovery of N-glycan biomarkers holds significant relevance for identifying circulating disease biomarkers and has garnered extensive support through research employing plasma samples. Considering that most clinical N-glycomics studies include data obtained from different plasma samples with different anticoagulants, including EDTA, heparin, and citrate, the investigation into the potential impact of different plasma collection tubes on N-glycome assumes significant importance for ensuring proper clinical translation. Consequently, this study systematically examined the influence of three commonly employed plasma collection tubes on the N-glycome profile of human blood samples collected from healthy volunteers.

Previously, two studies independently assessed the impact of plasma collection tubes and serum collection tubes on N-glycomic profiling [11,25]. The former study demonstrated anticoagulants of EDTA, Na-heparin, and Li-heparin did not influence the N-glycome profiles [11]. Another study demonstrates minimal inter-individual variation in serum N-glycan profiling using three different serum

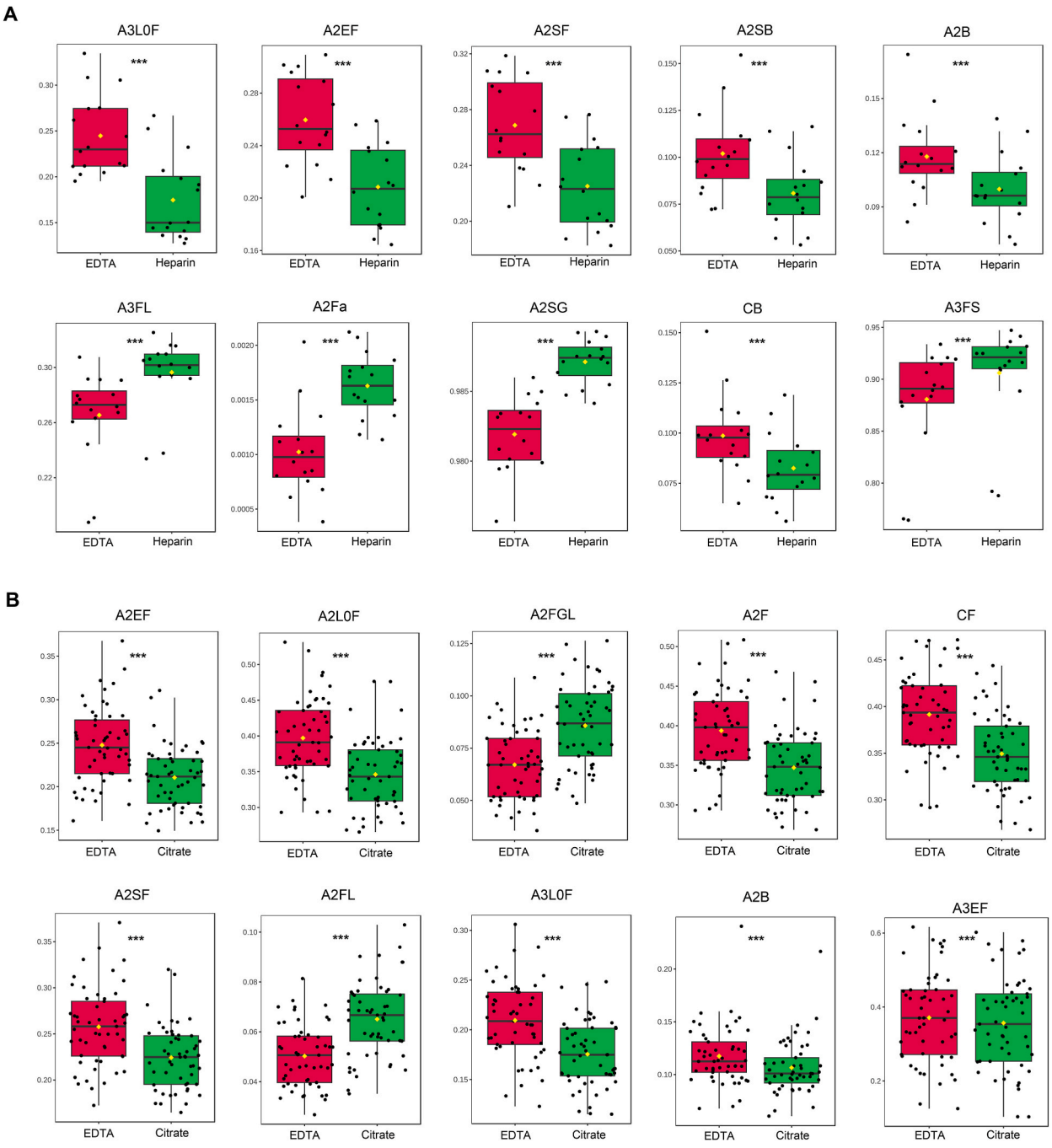

Fig. 3. Boxplots depicting the top 10 most statistically significant N-glycan traits distinguishing (A) EDTA (in red) and heparin (in green) plasma samples, and (B) EDTA (in red) and citrate (in green) plasma samples. The reported p-values were adjusted for False Discovery Rate (FDR): ***$p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

tubes [25]. However, the study assessing plasma collection tubes did not include a comparison with citrate plasma. Extending these previous studies, the present study, for the first time, systematically evaluated the influence of three commonly used plasma collection tubes (EDTA, heparin, and citrate plasma tubes) on the blood N-glycome simultaneously. Additionally, by employing the linkage-specific sialic acids derivatization method in conjunction with a robust high-throughput MALDI-TOF-MS platform for profiling plasma N-glycome, the present study identified multiple N-glycan features that had not been previously identified in earlier investigations. This study demonstrates apparent intra-individual variation in plasm N-glycome profiles following three different methods of plasma tube processing. Compared to EDTA plasma, variations were observed in various N-glycan features in both heparin

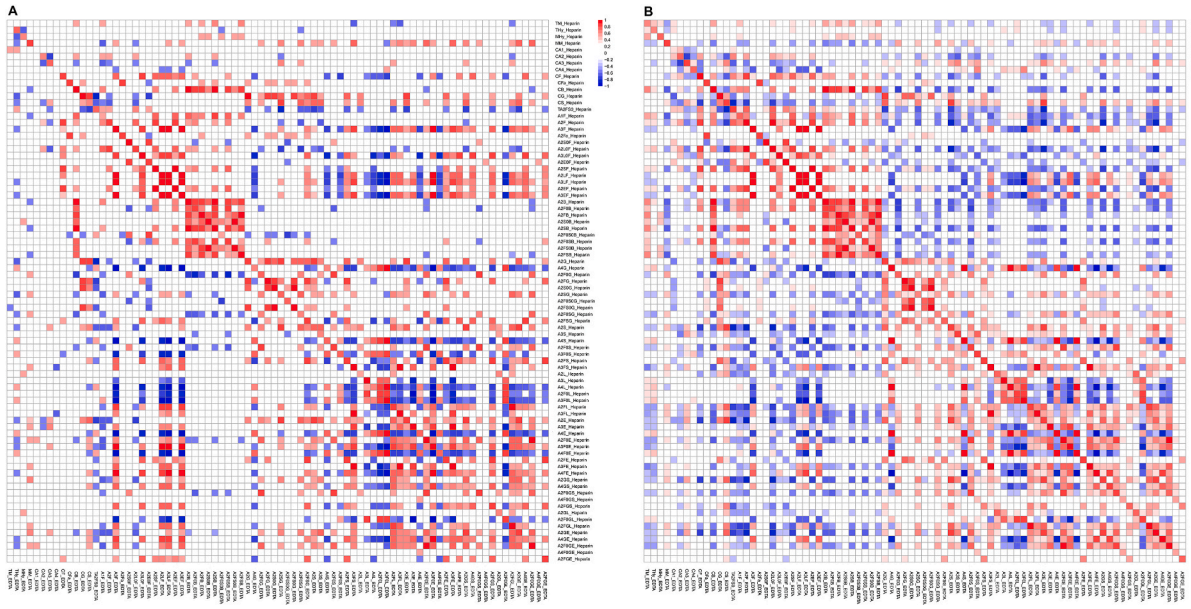

Fig. 4. Heatmap showing correlations of N-glycan traits between (A) EDTA and heparin plasma, and (B) EDTA and citrate plasma.

and citrate plasma. The trends of N-glycan variations in citrate and heparin plasma were predominantly consistent. Generally, our observations indicate that EDTA plasma samples exhibit higher levels of hybrid-type N-glycans, diantennary N-glycans, core fucosylation, and bisection, coupled with reduced levels of high mannose type N-glycans, triantennary N-glycans, antennary fucosylation, galactosylation, and α2,3-linked sialylation. Therefore, our results suggest that background noise, which differs between plasma collection tubes, might affect the profiles of N-glycome.

The correlation analysis conducted among different plasma collection tubes yielded high Pearson's correlation coefficients for the majority of N-glycan traits. These high correlation coefficients suggest that variations in N-glycan levels observed between the collection tubes were primarily attributed to systemic changes caused by the collection matrix across the entire cohort of individuals.

Sodium citrate and K_2-EDTA both target calcium ions to prevent coagulation, as Ca^{2+} serves as a crucial catalyst for numerous procoagulant proteins [40]. Nevertheless, the mechanisms through which these additives interact with calcium differ. Sodium citrate forms a reversible complex with calcium, whereas K_2-EDTA chelates calcium. In contrast, heparin inhibits coagulation by activating the anticoagulant protein antithrombin [41]. The variations in N-glycan characteristics across the three types of plasma samples may be attributed to differences in glycoprotein levels, which arise from the distinct anticoagulant mechanisms associated with their respective collection tubes. For instance, antithrombin is a glycoprotein, and the N-glycans present on it are predominantly of the diantennary complex type, carrying either one (0–30%) or two (70–100%) sialic acids [13]. Differences in the anticoagulant mechanisms can lead to variations in the levels of antithrombin [24]. Moreover, by chelating calcium, EDTA can also induce hypocalcemia, triggering a cellular stress response that leads to elevated levels of other glycoproteins, such as IL-1RA and M-CSF [42]. Consequently, the use of plasma collection tubes with different anticoagulants may result in differences in N-glycomes due to variations in glycoprotein (such as the above mentioned antithrombin, IL-1RA, and M-CSF) levels among different tubes. Moreover, the observed variations in the N-glycome among the three types of plasma tubes may also be attributed to different glycosidase activity. Previous studies have indicated that different anticoagulants can exert distinct effects on enzyme activity [24]. Consequently, the activity of PNGase F in the three types of plasma containing different anticoagulants may vary, potentially leading to discrepancies in the N-glycome profiles. Further investigations are still warranted to explore and elucidate other factors contributing to the variations observed in the N-glycomes across different plasma tubes.

This study has some strengths. Our study represents the first simultaneous assessment of the impacts of three commonly used plasma collection tubes on the plasma N-glycome. Importantly, we employed a robust N-glycomic approach combining the linkage-specific sialic acids derivatization method and a robust high-throughput MALDI-TOF-MS platform for profiling plasma N-glycome. Therefore, we identified a more expansive coverage of N-glycan structures augmenting the effective depth of plasma N-glycome analysis compared to those identified in previous studies, rendering our conclusions more comprehensive and reliable. Several limitations of the present study should be noted. The sample sizes of the cohorts used in the present study are relatively small, studies using larger cohorts are still needed to validate our findings. In addition, due to limitations in sample availability, we collected only two types of plasma for each volunteer (EDTA and heparin, or EDTA and citrate). The comparison between the N-glycome profiles of heparin plasma and citrate plasma was indirect. Further validation of our findings is imperative in more extensive cohorts in the future, where each volunteer contributes samples for all three types of plasma. Despite certain limitations, our study provides valuable insights and serves as a reference for the comparison of N-glycomic data across diverse laboratories and in the context of clinical translational research. Future investigations should delve into the underlying mechanisms elucidating the observed tube-related

impacts on N-glycome composition. Furthermore, the findings of this study should be further validated in larger cohorts in the future and guide the discovery of biomarkers in practical applications.

5. Conclusions

Variations in the sample matrix impact the measurement of plasma N-glycome by MALDI-TOF-MS. The findings from this study underscore the importance of refraining from direct comparisons of samples from different sources, such as various plasma collection tubes, in glycomics projects.

Compliance with ethics guidelines

Zejian Zhang, Xiangyi Cui, Nan Zhou, Lisi Zhu, Yuxiang Zhi, and Shuyang Zhang declare that they have no conflict of interest or financial conflicts to disclose.

Data statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

CRediT authorship contribution statement

Zejian Zhang: Writing – review & editing, Writing – original draft, Validation, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Xiangyi Cui:** Writing – review & editing, Writing – original draft, Visualization, Resources, Formal analysis, Data curation. **Nan Zhou:** Writing – review & editing, Resources, Formal analysis. **Lisi Zhu:** Writing – review & editing, Formal analysis. **Yuxiang Zhi:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Shuyang Zhang:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2024.e00383.

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