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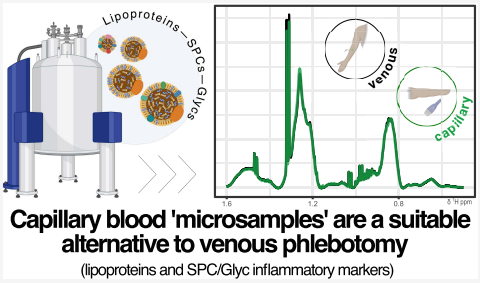
Rapid and Self-Administrable Capillary Blood Microsampling Demonstrates Statistical Equivalence with Standard Venous Collections in NMR-Based Lipoprotein Analysis

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Cite This: *Anal. Chem.* 2024, 96, 4505−4513
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ABSTRACT: We investigated plasma and serum blood derivatives from capillary blood microsamples (500 μL, MiniCollect tubes) and corresponding venous blood (10 mL vacutainers). Samples from 20 healthy participants were analyzed by ^{1}H NMR, and 112 lipoprotein subfraction parameters; 3 supramolecular phospholipid composite (SPC) parameters from SPC_1, SPC_2, and SPC_3 subfractions; 2 *N*-acetyl signals from α-1-acid glycoprotein (Glyc), GlycA, and GlycB; and 3 calculated parameters, SPC (total), SPC_3/SPC_2, and Glyc (total) were assessed. Using linear regression between capillary and venous collection sites, we explained that agreement (Adj. $R^2 \geq 0.8$, $p < 0.001$) was witnessed for 86% of plasma parameters (103/120) and 88% of serum parameters (106/120), indicating that capillary lipoprotein, SPC, and Glyc concentrations follow changes in venous concentrations. These results indicate that capillary blood microsamples are suitable for sampling in remote areas and for high-frequency longitudinal sampling of the majority of lipoproteins, SPCs, and Glycs.



INTRODUCTION

Metabolic phenotyping provides a means to capture an integrated profile of an individual's biological status and reflects the interaction of genes and the environment, especially in response to external stressors.[1,2] Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are the lead platforms to provide high-fidelity dense information about a variety of biomolecules. Robust protocols have been developed, validated, and standardized for various biofluids (e.g., plasma, serum, urine, tissue, etc.). Typically, most clinical studies have relied on conventional venous phlebotomy to acquire samples. However, sampling should be as simple and safe as possible to be clinically relevant. In the case of venous blood collection, a trained phlebotomist is required to perform the tightly regulated procedure to avoid adverse outcomes. To understand the mechanisms of disease or responses to therapeutic interventions, recording the behavior of specific molecules over time contributes valuable information. However, frequent vein puncture must also be avoided, and although a cannula can be used for repeated sampling, it must remain clean and dry, making it ill-suited beyond controlled settings. In contrast, capillary blood microsamples have a collection volumes (typically less than 50 μL, although volumes can extend up to 600 μL)[1] that offer a minimally invasive alternative to traditional intravenous sampling, which supports the evolving nature of healthcare from reactive disease care to care that is predictive, preventive, personalized, and participatory (P4 medicine).[1,3,4] Capillary microsampling is performed by lancing the fingertip for collection of blood drops into a small container or blood collection card, making increased sampling frequencies possible. Many advanced devices are also commercially available with volumes that are suitable for standard metabolic profiling protocols.[1]

^{1}H NMR spectroscopy has long been used for the measurement of variable biochemical signatures of blood plasma and serum to understand health and disease.[5−9] This includes analysis of panels of lipoproteins, supramolecular phospholipid composites (SPCs), and glycoprotein acetyls (α-1-acid glycoproteins) and others, which offer a diagnostic tool that extends beyond the standard clinical assays for low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol. These parameters can be acquired in less than 5 min and require minimal sample preparation.[10] Quantification is achieved by regression of several key regions of a standard NMR spectrum using mathematical models.[11] Using the B.I. LISA analytical model, Lodge et al. previously concluded that 3 mm NMR tubes (that utilize 100 μL of plasma) are well-suited

Received: November 14, 2023
Revised: January 17, 2024
Accepted: January 29, 2024
Published: February 19, 2024



ACS Publications
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https://doi.org/10.1021/acs.analchem.3c05152
Anal. Chem. 2024, 96, 4505−4513

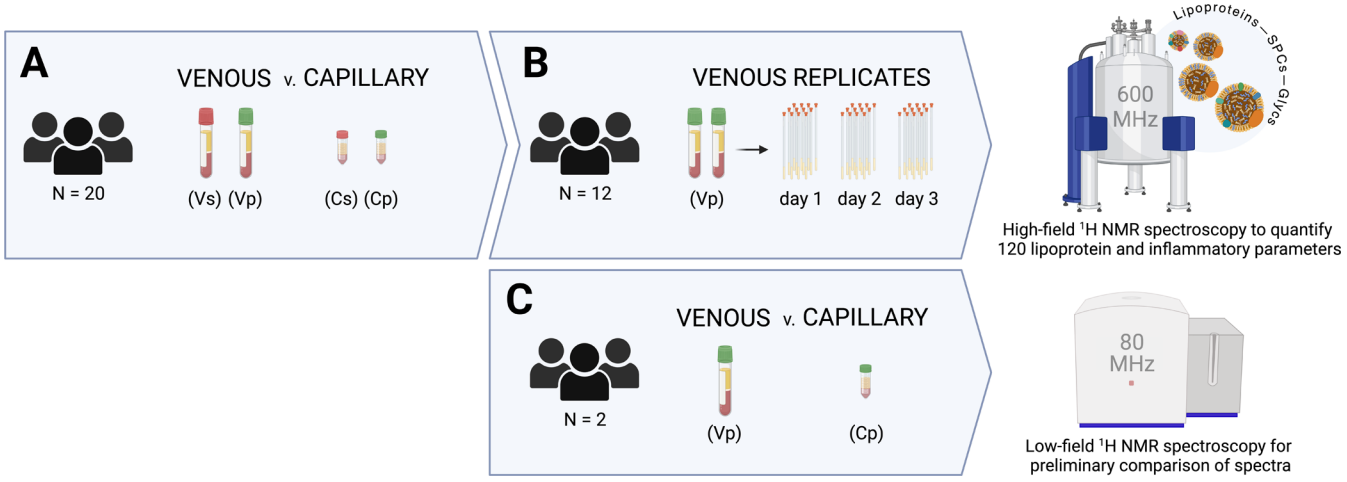


Figure 1. Sample collection workflow for ^{1}H NMR. (A) Evaluating the comparability between venous and capillary blood sampling sites; (B) 3-day assessment of venous technical replicate standard error for 3 mm outer diameter SampleJet NMR tubes; (C) preliminary comparison of low-field spectra: (V) = venous, (C) capillary, (s) = serum, and (p) = plasma. Image created with BioRender.com.

for deriving quantitative measurements, especially when working with samples of limited volume, compared to 5 mm tubes (utilizing 300 μL of plasma).[12] However, the use of 3 mm tubes necessitates extended scan times (15 min per sample per experiment) and spectra with reduced signal-to-noise when compared to 5 mm tubes (4 min). Modified spectroscopic methods have also been proposed for direct measurement of SPCs and Glycs in plasma or serum without the need for complex statistical modeling or peak deconvolution.[7,13−17] Recently, some of those experiments have been successfully translated to benchtop NMR spectrometers.[18]

Lipoprotein assemblies transport water-insoluble lipids such as cholesterol and glycerolipids in blood and are crucial to understanding the etiology and pathophysiology of diseases hallmarked by lipid and lipoprotein metabolism dysregulation, such as cardiometabolic and neurological diseases.[11] Previously, decreased levels of total cholesterol (TC), HDL-C, LDL-C, and increased triglycerides (TGs) have been shown to be positively correlated with cardiovascular disease severity[19] and increased levels of the apolipoprotein B100/apolipoprotein A1 ratio has been associated with atherogenic risk.[20,21] SPC has also been shown to decrease during acute inflammation while GlycA and GlycB demonstrate increases; hence, the SPC/Glyc (total) ratio has been proposed as a marker of inflammation.[22,23] Of the latter two, the SPC signal refers to trimethylammonium residues in supramolecular phospholipid components of lipoproteins, which result in three signal components: SPC_1, SPC_2, and SPC_3, representing the phospholipid content of HDL-4, phospholipids from HDL-1, -2 and -3, and phospholipids from LDL particles, respectively.[24] And Glyc refers to the *N*-acetyl methyl group signals, including GlycA and GlycB, which represent glycosylated amino sugars in side chains of a composite of inter alia five main acute phase glycoproteins: α-1-acid glycoprotein, α-1-antichymotrypsin, α-1-antitrypsin, haptoglobin, and transferrin, all of which are associated with inflammation.[25]

Whole blood, separated into either plasma or serum, is a commonly used blood derivative in metabolic phenotyping research for lipoproteins, SPCs, and Glycs. Previously, *in vitro* proteolysis during the serum clotting process[26,27] and increased osmosis caused by plasma tube anticoagulants[28,29] have been thought to play a role in lipoprotein differences observed between blood collection tubes (i.e., serum vs plasma). Despite only concerted reductions in apolipoprotein B-100,[29] lipoprotein a Lp(a), and antiapolipoproteins (a) and (b)[30] being shown previously, their variation has been thought to be based on small biological differences between plasma and serum blood derivatives. Specifically, plasma is the top protein-rich layer in a suspension derived from the centrifugation of whole blood, below which is a buffy coat of leukocytes and platelets, followed by erythrocytes, whereas serum is the remaining fluid following the removal of the clot from whole blood. Composition-wise, serum excludes the fibrinogens and clotting factors present in plasma, and as such, contains less protein.[28,29] In this context, the recent proton NMR-based lipoprotein studies by Loo et al.[31] demonstrated that the effect of blood collection tubes on lipoprotein profiles was trivial. Thus, highlighting the similar nature of plasma and serum blood derivatives for lipoprotein quantification.

Although the impact of different venous blood collection tubes on the quantification of lipoproteins has been studied,[32] much less is known about the impact of sampling different sites (i.e., fingertip capillary). Capillary sample dilution is hypothesized to occur from the combination of blood serum or plasma with interstitial fluid, likely as a result of sample collection under pressure from the lancing site.[33] For instance, Kupke et al. reported lower levels of lipoproteins[34] and metabolites,[35] respectively, in capillary blood microsamples compared to matched venous samples, whereas Sedgwick et al. previously employed an enzymatic colorimetric assay using EDTA-treated plasma samples and found no notable differences between capillary and venous lipoprotein concentrations.[36] With advancements in high-resolution methodologies capable of lipoprotein subclass quantitation, our study aimed to provide a more accurate and comprehensive understanding of collection site differences.

EXPERIMENTAL DESIGN

Study Participants. Twenty healthy volunteers, 10 men (age: 33 ± 17 (SD) years) and 10 women (age: 40 ± 22 (SD) years), were recruited from Perth, Western Australia, to participate in this study (Figure 1). Participants were included if they were over 18 years old and willing to provide both

https://doi.org/10.1021/acs.analchem.3c05152
Anal. Chem. 2024, 96, 4505−4513

capillary and venous blood samples. Exclusion criteria included conditions where blood collection may be hazardous (e.g., hemophilia). The study was approved by the Human Research Ethics Committee at Murdoch University (Ethics no. 2022/119) and complied with the guidelines from the Declaration of Helsinki. Informed consent forms were obtained from each participant prior to their participation in the study. For the high-field analysis, blood was collected from 20 participants for comparison of capillary and venous collection sites (Figure 1A), and 12/20 participants were on a later date for a venous replicate 3-day assessment (Figure 1B). For the low-field preliminary analysis, two out of 20 participants had a final collection for capillary and venous collection sites (Figure 1C).

Blood Collection Protocol for Human Serum and Plasma. Participants attended the clinic following a 12 h overnight fast for the collection of blood samples. Venous collections included whole blood using a 23-gauge needle into two types of Becton Dickinson (BD) vacutainer tubes taken from the antecubital fossa (1 × 10 mL of serum clot activation tube (CAT) and 1 × 10 mL of lithium heparin (LH) tube). Capillary blood microsampling included 0.5 mL MiniCollect tubes for both CAT and LH that were taken from the index finger (of the same arm venous blood was collected from) using lancets. A hand-warming procedure was used to promote blood circulation and ensure sufficient volume collection for the latter. Here, participants were instructed to immerse their hand in a bucket of warm water for ∼2 min. The hand was removed and dried thoroughly. An alcohol wipe was then used to prepare the index finger for lancing with a single-use AccuChek Safe-T-Pro-Plus lancet set to the greatest depth (2.3 mm). Draws were performed sequentially as follows: venous serum and plasma, followed by capillary serum and plasma. Immediately following collection, all samples were left at room temperature for 1 h (allowing the serum samples to clot). Collection of all samples was completed within <20 min. Samples underwent centrifugation at 4 °C for 10 min at 1300*g*. The resulting plasma/serum layer was aliquoted into cryo-vials and stored at −80 °C until the commencement of sample preparation. Blood samples were processed and frozen within 2 h of collection. A second visit for 12/20 participants collected 2 × 10 mL LH tubes, and a final visit for 2/20 participants collected 1 × 10 mL LH and 1 × 0.5 mL MiniCollect LH tube.

Sample Preparation. Samples were thawed on ice and prepared following an established protocol, as described elsewhere.[13] In brief, samples were centrifuged at 13,000*g* for 10 min at 4 °C to remove any remnant particulate before loading an equal mixture of phosphate buffer (75 mM Na_2HPO_4, 2 mM NaN_3, 4.6 mM sodium trimethylsilyl propionate-[2,2,3,3-$^2H_4$] (TSP) in H_2O/D_2O 4:1, pH 7.4 ± 0.1) into each plasma and serum sample at a 1:1 ratio for a final volume of up to 200 μL. The final volume was transferred to 3 mm NMR SampleJet tubes from the same batch, which were then sealed by adding POM balls to their caps. Samples were manually shaken to remove any bubbles present in the tubes and then stored at 5 °C inside the SampleJet automatic sample changer until measurement (<24 h).

High-Field ^{1}H NMR Spectroscopy Data Acquisition and Data Analysis. NMR analysis was performed using a 600 MHz Bruker Avance III HD spectrometer equipped with a 5 mm BBI probe and fitted with a Bruker SampleJet robotic cooling system set at 5 °C. A full quantitative calibration was completed prior to analysis using a previously described protocol for high-throughput proton NMR spectroscopy on plasma and serum.[13] Measurements for each sample included a standard 1D experiment performed with solvent presaturation (128 scans, 98k data points, spectral width (sw) of 30 ppm), and a J-Edited DIffusional (JEDI) pulsed gradient perfect echo (PGPE) experiment (256 scans, 98k data points, sw 30 ppm).[24] In total, data collection took 31 min per sample, which were processed in automation mode using Bruker Topspin 3.6.3 and ICON NMR to achieve phasing, baseline correction, and calibration to TSP.

Quantification of 112 parameters of the main plasma and serum lipoprotein classes and subclasses using Bruker's IVDr Lipoprotein Subclass Analysis (B.I. LISA, version PL-5009-01/001) method was performed.[11] The platform applied a regression model to ^{1}H NMR spectra with solvent suppression to compute the concentration of triglycerides (-TG), cholesterol (-CH), free cholesterol (-FC), phospholipids (-PL), Apo A1 (-A1), Apo-A2 (-A2), and Apo B100 (-AB) of the main very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL, and HDL classes.[11] The full B.I. LISA parameters and lipoprotein densities have been detailed (Table S1). Four further parameters were measured using Bruker's IVDr PhenoRisk PACS for research use only (RUO) method comprising 3 Glyc parameters and one SPC parameter associated with inflammatory responses.[14,37−39] From the latter, SPC_1, SPC_2, and SPC_3 subfractions and related parameters were measured according to a procedure described elsewhere.[40]

Low-Field ^{1}H NMR Spectroscopy Data Acquisition and Data Analysis. SPC and Glyc comparison was performed manually by acquiring capillary and venous plasma samples in 3 mm 7 inch NMR tubes using an 80 MHz Bruker Fourier 80 (F80) spectrometer. Measurements for each sample included a standard 1D experiment performed with solvent presaturation (96 scans, 23,808k data points, spectral width of 30 ppm) and a JEDI pulsed gradient spin echo (PGSE) experiment (512 scans, 3224k data points, sw 20 ppm).[18] In total, data collection took 45 min per sample and datawere processed using Bruker Topspin 4.3.0 to achieve phasing, baseline correction, and calibration to TSP.

Statistical Analysis. Statistics and data visualizations were performed using R version 3.4.1 and RStudio.[41] For all parameters, the correspondence between capillary and venous plasma samples was determined using simple linear regression. Adjusted R^2 and *p*-values were derived from the linear model to determine the goodness-of-fit between the capillary and venous samples. Adjusted $R^2 \geq 0.8$ was interpreted as strong, values between 0.5 and 0.79 were interpreted as moderate, and values below 0.5 were interpreted as weak. For each parameter, mean value, variance (using the squared difference and adjusting by the degrees of freedom), and standard deviation were computed. Finally, the standard error percentage was calculated by dividing the standard deviation by the mean value and then multiplying by 100. To help with the interpretation of the resulting standard error percentages, those were contrasted with values obtained for technical replicates acquired for 3 consecutive days using venous blood.

RESULTS

To assess if capillary blood microsample collections were amenable for NMR lipoprotein measurement, we compared quantitative concentrations of lipoproteins, SPCs, and Glycs using ^{1}H NMR spectroscopy on two common biofluids derived from whole blood collections (plasma and serum),

https://doi.org/10.1021/acs.analchem.3c05152
Anal. Chem. 2024, 96, 4505−4513

which were collected from capillary and venous collection sites. The demographics for the recruited cohort, including age, sex, weight, height, and BMI, are presented in Table 1. A complete list of the measured lipoproteins, SPCs, and Glycs is reported in Table S1. One sample was removed from the serum data set prior to any statistical analysis due to a failed quality control check, where less than the required 100 μL of plasma was collected. In total, 112 complete lipoproteins and lipoprotein subclasses were quantified as well as a set of inflammatory composite signals for measures of the SPC and Glyc parameters.

Table 1. Participant Demographics

healthy controls	women	men	total
N	10 (50%)	10 (50%)	20 (100%)
age, years [SD]	40 [±22]	33 [±17]	37 [±20]
weight, kg [SD]	61.8 [±9.38]	72.05 [±12.72]	66.93 [±12.08]
height, cm [SD]	160.0 [±8.52]	177.1 [±5.22]	168.6 [±11.15]
BMI [SD]	24.59 [±6.02]	22.95 [±3.76]	23.77 [±4.96]

Evaluating Equivalence and Variation Between Capillary and Venous Collection Sites. The median spectra from the JEDI-PGPE experiments exhibited a high degree of similarity in SPC and Glyc signatures between capillary and venous samples in both plasma and serum (Figure 2) when overlaid. In serum samples, a small difference in the low-frequency portion of SPC was witnessed. This region corresponds to HDL-4, which demonstrated less explained variance between collection sites in the lipoprotein assay, as expected from the 1D spectra and Adj. R^2 (Figure 3—serum). For plasma samples, the difference in SPC intensity between collection sites was smaller, corresponding with a stronger relationship in HDL-4 between capillary and venous collection sites (Figure 3—plasma).

A linear regression model was constructed for each parameter to investigate the relationship between values obtained from different collection sites for both plasma and serum tubes. Additionally, an assessment of analytical variation between capillary and venous collection sites was conducted by calculating the standard error percentage (Figure 3). Explained variance (Adj. $R^2 \geq 0.8$, $p < 0.001$) was witnessed for 86% of plasma analytes (103/120) and 88% of serum analytes (106/120), indicating that the capillary blood analyte concentration follows changes in venous analyte concentration. A further 13% of plasma analytes (15/120) and the remaining 12% of serum analytes (14/120) achieved moderately significant linear relationships ($50\% > R^2 > 79\%$, all $p \leq 0.001$) between collection sites (Figure 3). Notably, the collection site left all main fractions—clinically widely used parameters such as LDL cholesterol (LDCH) and HDL cholesterol (HDCH)—unaffected with goodness-of-fit ranging from Adj. $R^2 = 0.929$ ($p < 0.001$) for plasma HDCH to Adj. $R^2 = 0.969$ ($p < 0.001$) for serum LDCH. The ratio between apolipoprotein B100 and apolipoprotein A1 (ABA1), an important marker for atherogenic risk, also exhibited excellent agreement between the two collection sites in plasma (Adj. $R^2 = 0.968$, $p < 0.001$) and serum (Adj. $R^2 = 0.967$, $p < 0.001$).

Technical Replicates Using Venous Blood in 3 mm ^{1}H NMR SampleJet Tubes. Of the parameters achieving an adjusted $R^2 < 0.8$ between capillary and venous collection sites, smaller errors were witnessed in venous plasma replicate 3 mm measurements of five very high-density parameters of LDL (L6PN, L6CH, L6FC, L6PL, L6AB) and two very high-density parameters of HDL (H3FC, H4FC) over the course of the 3 days of NMR sample preparation (Figure 3—plasma).

Capillary SPC and Glyc Direct Measurement with Benchtop Low-Field 80 MHz NMR. To preliminarily assess the capillary translatability of SPC and Glyc biomarkers of inflammation, we performed the analysis of two sets of capillary and venous plasma samples on benchtop low-field 80 MHz NMR (Figure 4). A small difference between capillary and venous samples for participant one was observed in the 1D experiment. In the JEDI-PGSE experiment, overlap was demonstrated in both participants between their respective capillary and venous plasma samples using the raw traces for SPC and Glyc measurements.

DISCUSSION

We compared 112 quantified lipoproteins and 8 inflammatory SPC and Glyc parameters obtained for plasma and serum samples from venous and capillary blood. Spectra acquired from both capillary and venous sampling sites were shown to be equivalent for all quantitative parameters, specifically, with 86% (103/120) of measurements in plasma and 88% (106/120) in serum, reporting strong significant relationships (adjusted $R^2 \geq 0.8$). Similarly, Sedgwick et al. reported no significant differences between capillary and venous plasma lipid and lipoprotein measurements, albeit delimited to traditional cholesterol, HDL cholesterol, LDL cholesterol, and total triglyceride clinical markers using an enzymatic colorimetric assay.[36] Importantly, markers such as total plasma triglycerides, apolipoprotein A1, and SPCs that have been identified as biomarkers of cardiovascular risk related to both acute and chronic inflammation[40] were found highly correlated between the two sampling sites. These results indicate that self-administrable microcapillary blood collections are a suitable alternative to venous blood collections.

Results from this study revealed that experimental errors observed are similar between capillary and venous plasma collection sites. Eight parameters failed to reach an adjusted R^2 of ≥0.8 and had lower standard error percentages among venous plasma technical replicates than those witnessed between capillary and venous plasma collection sites. Specifically, this concerned two parameters of HDL (H3FC, H4FC) and six parameters of LDL (L2TG, L6PN, L6CH, L6FC, L6PL, L6AB). The former refer to small, high-density parameters within their respective lipoprotein subfractions that are not commonly employed in standard clinical assessments. Recent studies have identified differences in some of these parameters in conditions such as SARS-CoV-2 infection,[22,42] type 2 diabetes mellitus,[43] diet,[44] and the postprandial response.[45] However, only venous collections were utilized, limiting direct comparability to the present findings. These parameters were unable to pass the arbitrary criteria of adjusted $R^2 \geq 0.8$—a threshold we set as a conventional benchmark to ensure a high degree of reliability in the modeling of the explained variance between capillary and venous collection sites. Of these parameters, FC of HDL subfractions 3 and 4 demonstrated lower explained variance (Adj. $R^2 = 0.469$, 0.486, respectively), however, the remaining 6 parameters (all from LDL subfraction 6, plus L2TG) demonstrated moderate explained variance (Adj. R^2 range: 0.658−0.773), suggesting that their measurement in capillary samples is still a biologically valid alternative to traditional venous phlebotomy. It goes beyond the scope of this study to

https://doi.org/10.1021/acs.analchem.3c05152
Anal. Chem. 2024, 96, 4505−4513

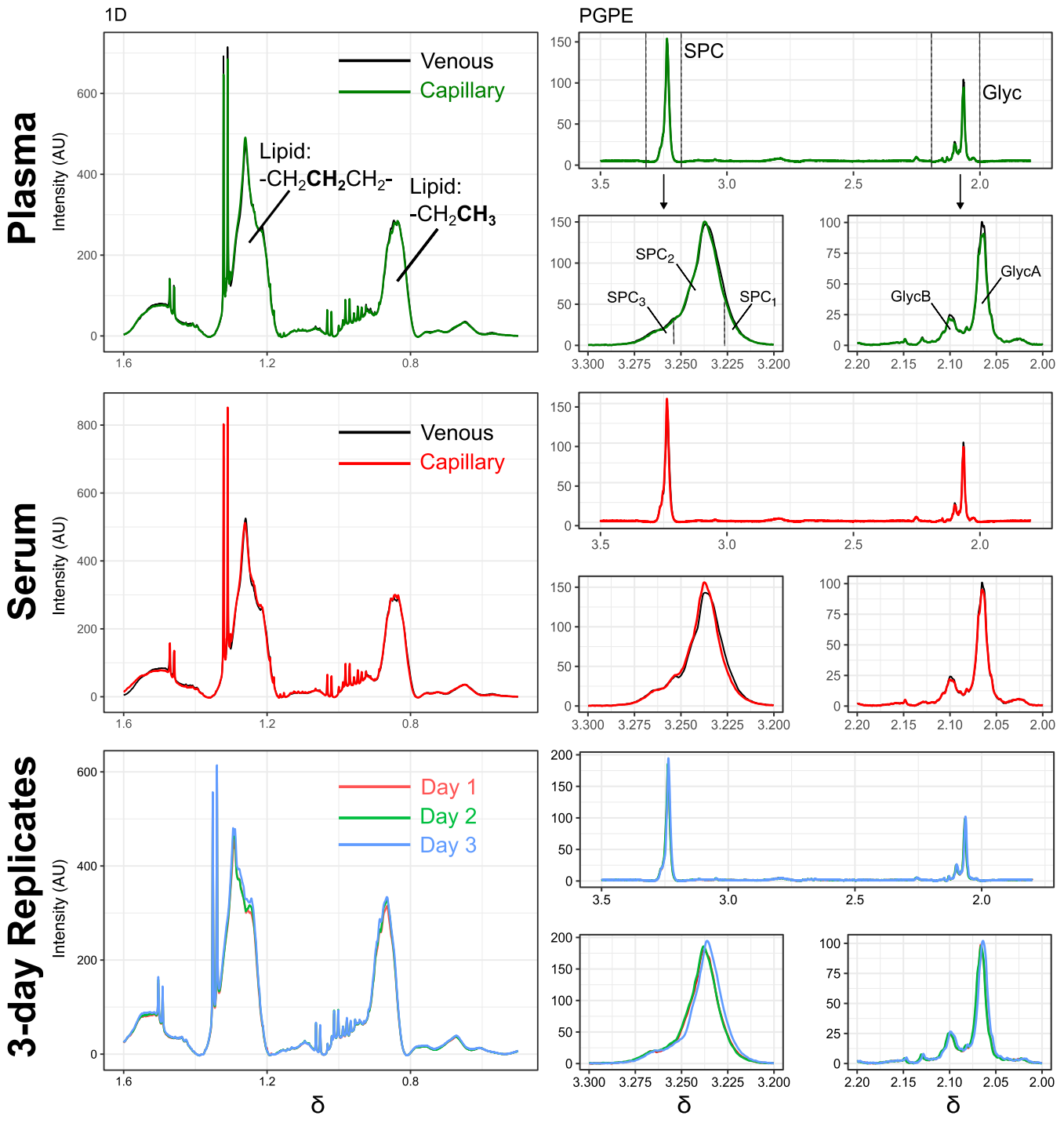


Figure 2. Median proton NMR spectra from all study participants for 1D and JEDI-PGPE experiments. Plasma and serum represent spectra of the capillary and venous samples collected from a clinical visit ($N = 20$). Capillary spectra (green and red traces) are overlaid with venous (black traces) derived in the 1D and PGPE experiments. Expansions of the SPC and Glyc regions from the latter demonstrate an overlap between capillary and venous samples in both plasma and serum. In serum samples, a small difference in the low-frequency portion of SPC_1, corresponding to HDL-4, is witnessed. 3-Day replicates represent spectra of venous plasma collected from two clinical visits ($N = 12$).

determine the exact mechanisms underlying the lower concentration predictability between collection sites, we postulate that these differences are attributed to sample handling and capillary sample dilution,[33] resulting in lower signal-to-noise ratios of parameters derived from smaller, denser subfractions. Considerations to improve sample handling include the use of high-flow lancets that mitigate squeezing of the fingertip, which can cause sample contamination with tissue fluid.

In serum samples, a minor difference was observed between collection sites in the low-frequency portion of SPC (SPC_1), as observed from the respective spectra (Figure 2B). Previously, Masuda et al. identified that SPC_1 is primarily characterized by the presence of HDL-4 and some HDL-3 phospholipid parameters.[40] In the present study, this aligns with the lower

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Anal. Chem. 2024, 96, 4505−4513

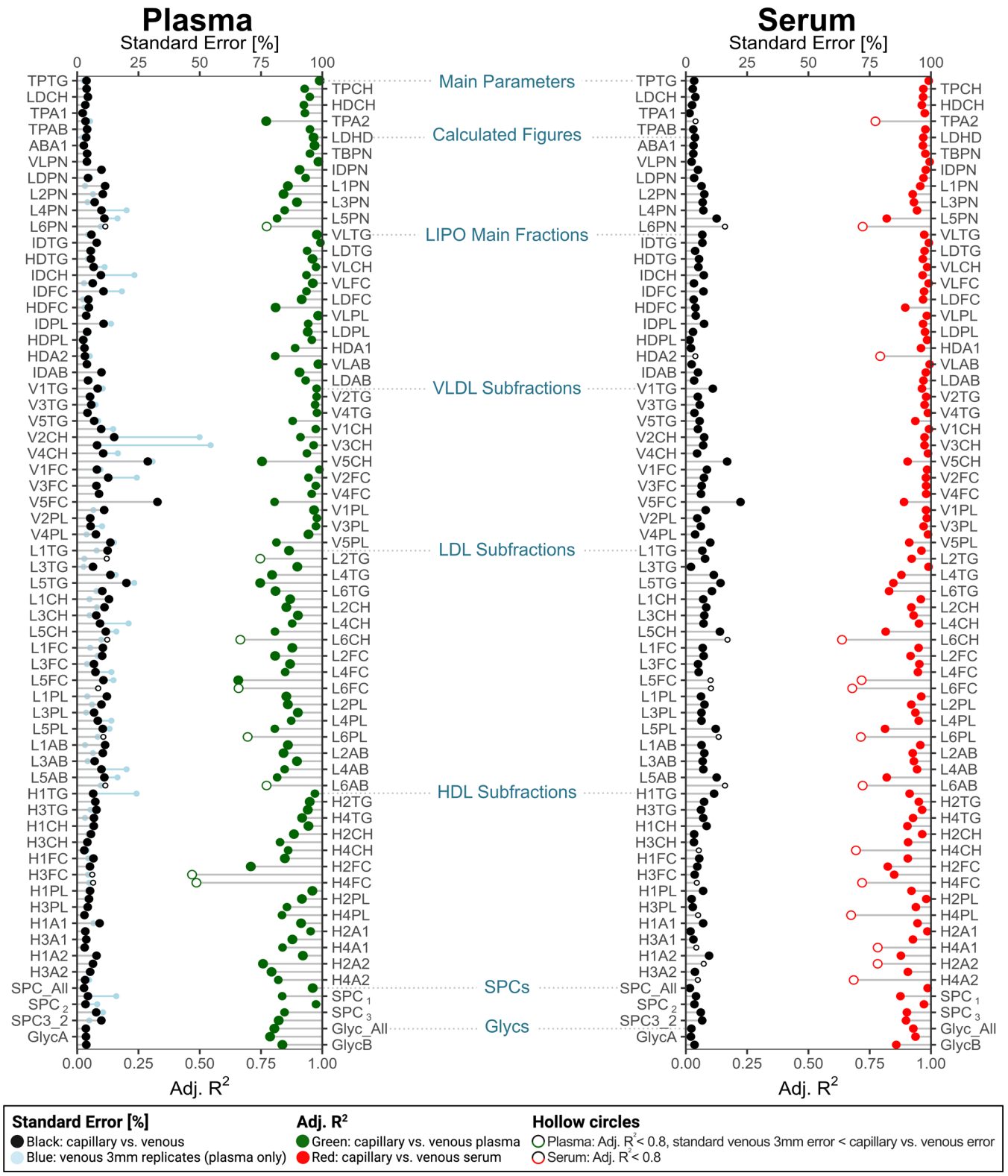


Figure 3. Relationship and variation of plasma and serum analyte concentrations between the different collection sites. Adjusted R^2 for plasma (green) and serum (red) with corresponding standard error percentage (SE%, black) for capillary vs venous lipoproteins (LIPO, VLDL, LDL, HDL), supramolecular phospholipid composites (SPCs), and glycoprotein acetyls (Glycs) parameters. For plasma, collection site SE% is overlaid with SE% of 3-day replicates of venous plasma acquired in 3 mm outer diameter NMR SampleJet tubes (blue). Hollow circles indicate parameters where the standard error percentage is lower for the 3-day 3 mm venous plasma analysis than the error between venous and capillary and Adj. R^2 < 0.80. For serum, only the latter rule applies. A descriptive list of the abbreviated lipoprotein main fractions, subfractions, SPCs, and Glycs (gray labels) are reported in Table S1.

https://doi.org/10.1021/acs.analchem.3c05152
Anal. Chem. 2024, 96, 4505−4513

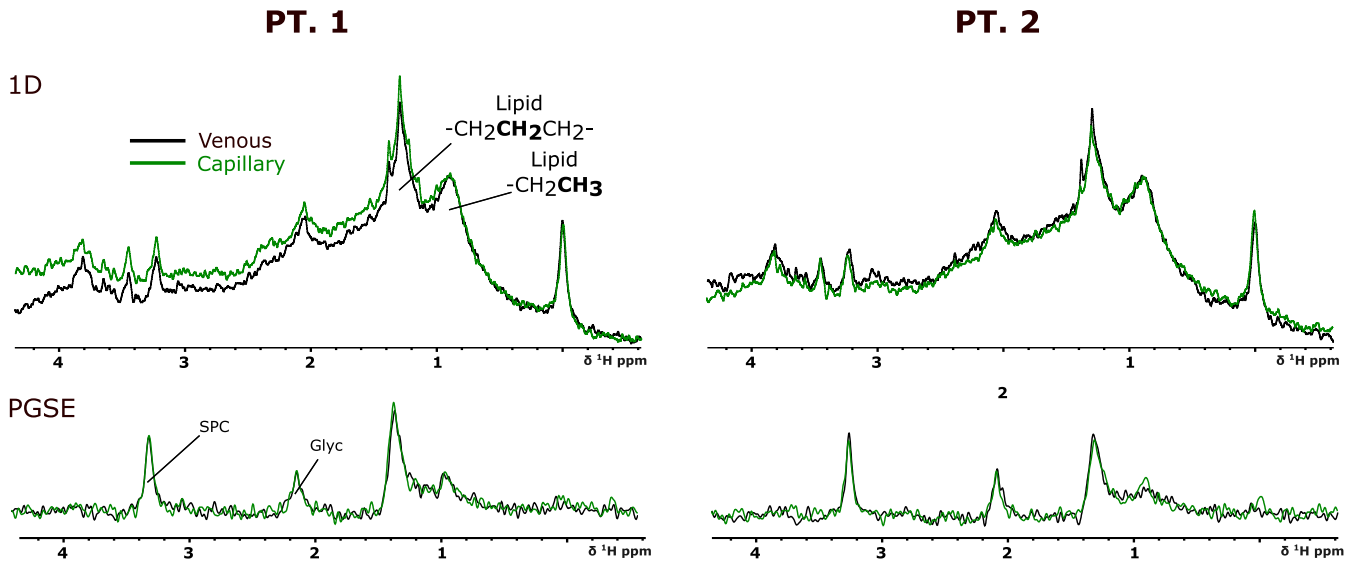


Figure 4. Preliminary direct low-field 80 MHz ^{1}H NMR spectra. 1D and JEDI-PGSE spectra of capillary (green trace) and venous (black trace) plasma samples collected from two participants (PT.1 = left, PT.2 = right). Capillary spectra overlay with venous spectra for SPC and Glyc measurements from the PGSE experiment.

explained variance (i.e., Adj. R^2 < 0.8) between collection sites in the serum lipoprotein assay for HDL-4 subfraction parameters, such as H4PL. Previously, Mallol et al. have reported that the time required for clot formation, which is critical in the collection of serum samples, necessitates attention to lipoprotein binding to the fibrin clot.[46] Importantly, this binding is more variable when samples are maintained at lower temperatures, such as 4 °C, in comparison to clotting occurring after a half-hour incubation at room temperature.[46] Since obtaining serum samples relies on the formation of a clot and capillary samples tend to exhibit increased levels of tissue factor that expedite the clotting process,[47] it is speculated that these distinctions in clotting dynamics may be implicated in the analysis of serum SPC_1 and relevant parameters of the HDL-4 subfraction. The present study did not identify a similar phenomenon for plasma samples collected in lithium heparin (LH) anticoagulant tubes, indicating a stronger relationship in SPC_1-related HDL-4 parameters between those in capillary and venous collections. These data emphasize the appropriate selection of blood tubes, particularly for studies investigating the parameters of SPC, where the selection of plasma over serum may mitigate blood clot-related lipoprotein differences in capillary samples.

The primary goal of the present study was to determine whether the analysis of capillary blood "microsamples" with low-volume 3 mm NMR workflows was representative of traditional venous samples. As a proof-of-concept, direct comparison to clinical measurements or 'ground truth' was beyond the scope of this study. However, establishing a direct relationship with clinical measurements remains an important future direction. All 120 lipoprotein and inflammatory parameters measured in the present study, including concentration means and ranges for each collection site (capillary, venous) and biofluid (plasma, serum), are reported in Table S1.

Preliminary data from this study sheds light on the potential translatability for capillary blood microsample SPC and Glyc biomarker analysis, using a benchtop low-field 80 MHz benchtop NMR. While the JEDI-PGSE experiment demonstrated spectral overlay in both participants, emphasizing the robustness of SPC and Glyc measurements in plasma, variability was identified in the 1D experiment for participant one between the capillary and venous samples (Figure 4). It is noteworthy that small differences in sample collection methods between participants were observed, specifically—sampling under pressure (participant one) vs without (participant two)—with the former potentially influencing tissue fluid contamination during capillary microsampling.[33] Despite this variation, the preliminary data appear to underscore the overall resilience of SPC and Glyc measurements in capillary plasma, suggesting their potential reliability as biomarkers even under different sampling conditions. This preliminary insight into the potential impact of sample collection methods contributes valuable information, laying the foundation for future investigations and emphasizing the need for standardized protocols in capillary microsampling for accurate biomarker assessments.

CONCLUSIONS

This research demonstrates equivalence between venous and capillary sampling for quantifying lipoproteins, supramolecular phospholipid composites (SPCs), and glycoproteins (Glycs), which has not been previously demonstrated for 3 mm NMR-based analytical workflows. This may confer a future diagnostic advantage over traditional venous phlebotomy methods since capillary blood microsampling can be performed longitudinally, at scale, and is suitable for community settings. This supports the extension of lipoprotein measurements, SPCs, and Glycs to remote areas and high-frequency sampling, presenting an opportunity for future translation to field-deployable benchtop instruments. Benchtop, low-field NMR spectrometers represent a cost-effective alternative for measurement of potential biomarkers at point-of-care.[18] Preliminary data from this study indicate that routine measurement of inflammatory SPC and Glyc biomarkers in plasma, using a field-deployable, benchtop 80 MHz NMR approach, could be achieved without the need for trained phlebotomists.

https://doi.org/10.1021/acs.analchem.3c05152
Anal. Chem. 2024, 96, 4505−4513

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.3c05152.

B.I. LISA and PhenoRisk PACS RUO parameters and calculated concentrations (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by a Joint Strategic Research Training Program Scholarship funded by Murdoch University and Bruker Daltonics to higher degree research students for J.L.R. E.H. acknowledges the H2020 EU GEMMA grant for funding. J.L.R. would like to acknowledge N.S.M.

■ ABBREVIATIONS

°C, degrees Celcius; ^{1}H NMR, proton nuclear magnetic resonance spectroscopy; AB, apolipoprotein B100; ABA1, ratio between apolipoprotein B100 and apolipoprotein A1; B.I. LISA, Bruker IVDr lipoprotein subclass analysis; BBI, double resonance broadband probe; BD, Becton Dickinson; CAT, clot activation tube; CH, cholesterol; cm, centimeters; D_2O, deuterium oxide; F80, Fourier 80; FC, free cholesterol; g, g-force; Glyc, glycoprotein; h, hours; H_2O, water; HDL, high-density lipoprotein; IVDr, in vitro diagnostic research; JEDI, J-Edited DIffusional; k, thousand; kg, kilograms; LC, liquid chromatography; LDL, low-density lipoprotein; LH, lithium heparin; Lpa, lipoprotein a; MHz, megahertz; min, minutes; mL, milliliters; mm, millimeters; MS, mass spectrometry; N, number; P4 medicine, predictive, preventive, personalized, and participatory medicine; PGPE, pulsed gradient perfect echo; pH, potential of hydrogen; PL, phospholipids; PN, particle number; ppm, part per million; RUO, for research use only;

https://doi.org/10.1021/acs.analchem.3c05152
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SD, standard deviation; SE%, standard error percentage; SPC, supramolecular phospholipid composite; sw, spectral width; TC, total cholesterol; TG, triglycerides; TSP, sodium trimethylsilyl propionate-[2,2,3,3-$^2H_4$]; VLDL, very-low-density lipoprotein; WHO, World Health Organization; μL, microliters

REFERENCES

(1) Roberts, J. L.; Whiley, L.; Gray, N.; Gay, M.; Lawler, N. G. *Separations* **2022**, *9*, 175.
(2) Holmes, E.; Wilson, I. D.; Nicholson, J. K. *Cell* **2008**, *134*, 714−717.
(3) Flores, M.; Glusman, G.; Brogaard, K.; Price, N. D.; Hood, L. *Pers. Med.* **2013**, *10*, 565−576.
(4) Hood, L. *Rambam Maimonides Med. J.* **2013**, *4*, No. e0012.
(5) Nicholson, J. K.; Foxall, P. J. D.; Spraul, M.; Farrant, R. D.; Lindon, J. C. *Anal. Chem.* **1995**, *67*, 793−811.
(6) Nicholson, J. K.; O'Flynn, M. P.; Sadler, P. J.; Macleod, A. F.; Juul, S. M.; Sönksen, P. H. *Biochem. J.* **1984**, *217*, 365−375.
(7) Bell, J. D.; Sadler, P. J.; Macleod, A. F.; Turner, P. R.; La Ville, A. *FEBS Lett.* **1987**, *219*, 239−243.
(8) Nicholson, J. K.; Buckingham, M. J.; Sadler, P. J. *Biochem. J.* **1983**, *211*, 605−615.
(9) Nicholson, J. K.; Wilson, I. D. *Prog. Nucl. Magn. Reson. Spectrosc.* **1989**, *21*, 449−501.
(10) Lamarche, B.; Tchernof, A.; Moorjani, S.; Cantin, B.; Dagenais, G. R.; Lupien, P. J.; Despre′s, J.-P. *Circulation* **1997**, *95*, 69−75.
(11) Jiménez, B.; Holmes, E.; Heude, C.; Tolson, R. F.; Harvey, N.; Lodge, S. L.; Chetwynd, A. J.; Cannet, C.; Fang, F.; Pearce, J. T. M.; et al. *Anal. Chem.* **2018**, *90*, 11962−11971, DOI: 10.1021/acs.analchem.8b02412.
(12) Lodge, S.; Nitschke, P.; Loo, R. L.; Kimhofer, T.; Bong, S.-H.; Richards, T.; Begum, S.; Spraul, M.; Schaefer, H.; Lindon, J. C.; et al. *J. Proteome Res.* **2021**, *20*, 1415−1423.
(13) Dona, A. C.; Jiménez, B.; Schäfer, H.; Humpfer, E.; Spraul, M.; Lewis, M. R.; Pearce, J. T. M.; Holmes, E.; Lindon, J. C.; Nicholson, J. K. *Anal. Chem.* **2014**, *86*, 9887−9894.
(14) Otvos, J. D.; Shalaurova, I.; Wolak-Dinsmore, J.; Connelly, M. A.; Mackey, R. H.; Stein, J. H.; Tracy, R. P. *Clin. Chem.* **2015**, *61*, 714−723.
(15) Otvos, J. D.; Jeyarajah, E. J.; Bennett, D. W. *Clin. Chem.* **1991**, *37*, 377−386.
(16) Ala-Korpela, M.; Korhonen, A.; Keisala, J.; Hörkkö, S.; Korpi, P.; Ingman, L. P.; Jokisaari, J.; Savolainen, M. J.; Kesäniemi, Y. A. *J. Lipid Res.* **1994**, *35*, 2292−2304.
(17) Bell, J. D.; Brown, J. C. C.; Nicholson, J. K.; Sadler, P. J. *FEBS Lett.* **1987**, *215*, 311−315.
(18) Nitschke, P.; Lodge, S.; Hall, D.; Schaefer, H.; Spraul, M.; Embade, N.; Millet, O.; Holmes, E.; Wist, J.; Nicholson, J. K. *Analyst* **2022**, *147*, 4213−4221.
(19) Lodge, S.; Nitschke, P.; Kimhofer, T.; Coudert, J. D.; Begum, S.; Bong, S.-H.; Richards, T.; Edgar, D.; Raby, E.; Spraul, M.; et al. *J. Proteome Res.* **2021**, *20*, 1382−1396.
(20) Walldius, G.; Jungner, I.; Holme, I.; Aastveit, A. H.; Kolar, W.; Steiner, E. *Lancet* **2001**, *358*, 2026−2033.
(21) Walldius, G. The apoB/apoA-I Ratio is a Strong Predictor of Cardiovascular Risk. In *Lipoproteins*; Sasa, F.; Gerhard, K., Eds.; IntechOpen, 2012, Chapter 5.
(22) Rössler, T.; Berezhnoy, G.; Singh, Y.; Cannet, C.; Reinsperger, T.; Schäfer, H.; Spraul, M.; Kneilling, M.; Merle, U.; Trautwein, C. *Metabolites* **2022**, *12*, No. 1277, DOI: 10.3390/metabo12121277.
(23) Kazenwadel, J.; Berezhnoy, G.; Cannet, C.; Schäfer, H.; Geisler, T.; Rohlfing, A.-K.; Gawaz, M.; Merle, U.; Trautwein, C. *Commun. Med.* **2023**, *3*, 145.
(24) Masuda, R.; Lodge, S.; Whiley, L.; Gray, N.; Lawler, N.; Nitschke, P.; Bong, S.-H.; Kimhofer, T.; Loo, R. L.; Boughton, B.; Zeng, A. X.; Hall, D.; Schaefer, H.; Spraul, M.; Dwivedi, G.; Yeap, B. B.; Diercks, T.; Bernardo-Seisdedos, G.; Mato, J. M.; Lindon, J. C.; Holmes, E.; Millet, O.; Wist, J.; Nicholson, J. K. *Anal. Chem.* **2022**, *94*, 4426−4436.
(25) Mallagaray, A.; Rudolph, L.; Lindloge, M.; Molbitz, J.; Thomsen, H.; Schmelter, F.; Alhabash, M. W.; Abdullah, M. R.; Saraei, R.; Ehlers, M.; Graf, T.; Sina, C.; Petersmann, A.; Nauck, M.; Gunther, U. L. *Angew. Chem.* **2023**, *62*, No. e202306154.
(26) Anderson, N. L.; Anderson, N. G. *Mol. Cell. Proteomics* **2002**, *1*, 845−867.
(27) Yu, Z.; Kastenmüller, G.; He, Y.; Belcredi, P.; Möller, G.; Prehn, C.; Mendes, J.; Wahl, S.; Roemisch-Margl, W.; Ceglarek, U.; et al. *PLoS One* **2011**, *6*, No. e21230.
(28) Leeman, M.; Choi, J.; Hansson, S.; Storm, M. U.; Nilsson, L. *Anal. Bioanal. Chem.* **2018**, *410*, 4867−4873.
(29) Collins, L. A.; Olivier, M. *Proteome Sci.* **2010**, *8*, 42.
(30) Lippi, G.; Giampaolo, L.; Guidi, G. *Eur. J. Clin. Chem. Clin. Biochem.* **1996**, *34*, 251−255.
(31) Loo, R. L.; Lodge, S.; Kimhofer, T.; Bong, S.-H.; Begum, S.; Whiley, L.; Gray, N.; Lindon, J. C.; Nitschke, P.; Lawler, N. G.; et al. *J. Proteome Res.* **2020**, *19*, 4428−4441.
(32) Vignoli, A.; Tenori, L.; Morsiani, C.; Turano, P.; Capri, M.; Luchinat, C. *J. Proteome Res.* **2022**, *21*, 1061−1072.
(33) *Capillary Sampling − Guidelines on Drawing Blood: Best Practices in Phlebotomy* WHO: 2010.
(34) Kupke, I. R.; Zeugner, S.; Gottschalk, A.; Kather, B. *Clin. Chim. Acta* **1979**, *97*, 279−283.
(35) Kupke, I. R.; Kather, B.; Zeugner, S. *Clin. Chim. Acta* **1981**, *112*, 177−185.
(36) Sedgwick, M. J.; Morris, J. G.; Nevill, M. E.; Barrett, L. A. *Atherosclerosis* **2011**, *218*, No. e10.
(37) Lodge, S.; Nitschke, P.; Kimhofer, T.; Wist, J.; Bong, S.-H.; Loo, R. L.; Masuda, R.; Begum, S.; Richards, T.; Lindon, J. C.; et al. *Anal. Chem.* **2021**, *93*, 3976−3986.
(38) Kimhofer, T.; Lodge, S.; Whiley, L.; Gray, N.; Loo, R. L.; Lawler, N. G.; Nitschke, P.; Bong, S.-H.; Morrison, D. L.; Begum, S.; et al. *J. Proteome Res.* **2020**, *19*, 4442−4454.
(39) Cutler, D. M. *JAMA Health Forum.* **2022**, *3*, e221809.
(40) Masuda, R.; Lodge, S.; Whiley, L.; Gray, N.; Lawler, N.; Nitschke, P.; Bong, S.-H.; Kimhofer, T.; Loo, R. L.; Boughton, B.; et al. *Anal. Chem.* **2022**, *94*, 4426−4436.
(41) *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2022 https://www.R-project.org/.
(42) Lodge, S.; Lawler, N. G.; Gray, N.; Masuda, R.; Nitschke, P.; Whiley, L.; Bong, S. H.; Yeap, B. B.; Dwivedi, G.; Spraul, M.; et al. *Int. J. Mol. Sci.* **2023**, *24*, No. 1614, DOI: 10.3390/ijms241411614.
(43) Yuan, L.; Li-Gao, R.; Li-Gao, R.; Verhoeven, A.; van Eyk, H. J.; Bizino, M. B.; Rensen, P. C. N.; Giera, M.; Jazet, I. M.; Lamb, H. J.; Rabelink, T. J. *Diabetes Obes. Metab.* **2023**, *25*, 2374−2387.
(44) Okami, Y.; Chan, Q.; Miura, K.; Kadota, A.; Elliott, P.; Masaki, K.; Okayama, A.; Okuda, N.; Yoshita, K.; Miyagawa, N.; et al. *J. Atheroscler. Thromb.* **2023**, *30*, 884−906.
(45) Stănciulescu, L.-A.; Scafa, A.; Duduianu, C.; Stan, R.; Nicolescu, A.; Deleanu, C.; Dorobantu, M. *Diagnostics* **2022**, *12*, No. 1675, DOI: 10.3390/diagnostics12071675.
(46) Mallol, R.; Rodriguez, M. A.; Brezmes, J.; Masana, L.; Correig, X. *Prog. Nucl. Magn. Reson. Spectrosc.* **2013**, *70*, 1−24.
(47) Fliervoet, L. A. L.; Groenestege, T.; Huisman, W. M. *Clin. Biochem.* **2022**, *104*, 30−35.

https://doi.org/10.1021/acs.analchem.3c05152
Anal. Chem. 2024, 96, 4505−4513