Comparison of metabolomic profiles in plasma versus dried blood spot cards

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Introduction

Plasma is an extremely well-characterized matrix using Metabolon's global untargeted Precision Metabolomics™ platform. The gold standard storage/shipping conditions for plasma (*i.e.* dry ice or ultracold freezers) are unfortunately not always achievable in real world situations where blood analysis is desirable (*i.e.*, field studies or at-home collection) or when repeated measurements make plasma collection unfeasible (*i.e.*, pharmacokinetic studies) (1-3). Given the popularity and large body of metabolomics research performed on plasma, it is of interest to understand how dried spot cards compare as an alternative sample type. *It is important to note that plasma and DBS cards are inherently two different matrices*, as DBS cards contain whole blood, with roughly equal parts plasma and cell material. This serves to dilute the signal from plasma specific biochemicals, but also introduces cell-specific metabolites that are present in low levels (or not at all) in plasma. They are also dried blood on a paper matrix rather than liquid, which can cause oxidation and hydrolysis of susceptible biochemicals, but also serves to preserve the stability of biochemicals at room temperature. The inclusion of a paper matrix in the sample also presents a potentially confounding factor in the extraction process. Lastly, the inherent differences between collection methods (capillary puncture versus venipuncture) results in differences in the amount of sample that is available for extraction. To better understand how DBS cards compare to plasma samples, we have directly tested how metabolite profiles from DBS cards and liquid EDTA-plasma samples compared to each other using Metabolon's Precision Metabolomics™ platform. Here, we present our findings on the numbers and types of biochemicals that are detected in both matrices, as well as on how known signatures of various physiological states are represented in DBS cards.

Study Design

EDTA-anticoagulated whole blood was collected in-house (under approved IRB protocol) via venipuncture from 49 self-reported healthy volunteers with an even distribution of fasted/fed status and gender. An aliquot of whole blood was taken for preparation of multiple 50 µL dried blood spots per individual, while the remainder was used to generate plasma that was frozen and stored at -80°C. The DBS samples were dried ~4 hours at room temperature (RT) and stored in gas-impermeable bags with desiccant at -80°C. *It is noted that we have found minimal differences between DBS cards prepared via pipetting venous whole blood compared to those prepared via finger-stick blood drop collection.* Both dried blood spots and plasma samples were processed and extracted for metabolomic analysis using our standard protocol for each matrix (4). Correlations, Z-scores, and fed versus fasted metabolomic signatures were used to compare and assess agreement between DBS and plasma analytical measurements.

Results

As a first direct comparison, we tabulated the numbers of named biochemicals that were detected in at least 70% of the 49 donors in each set of samples (DBS and plasma). **Table 1** shows the

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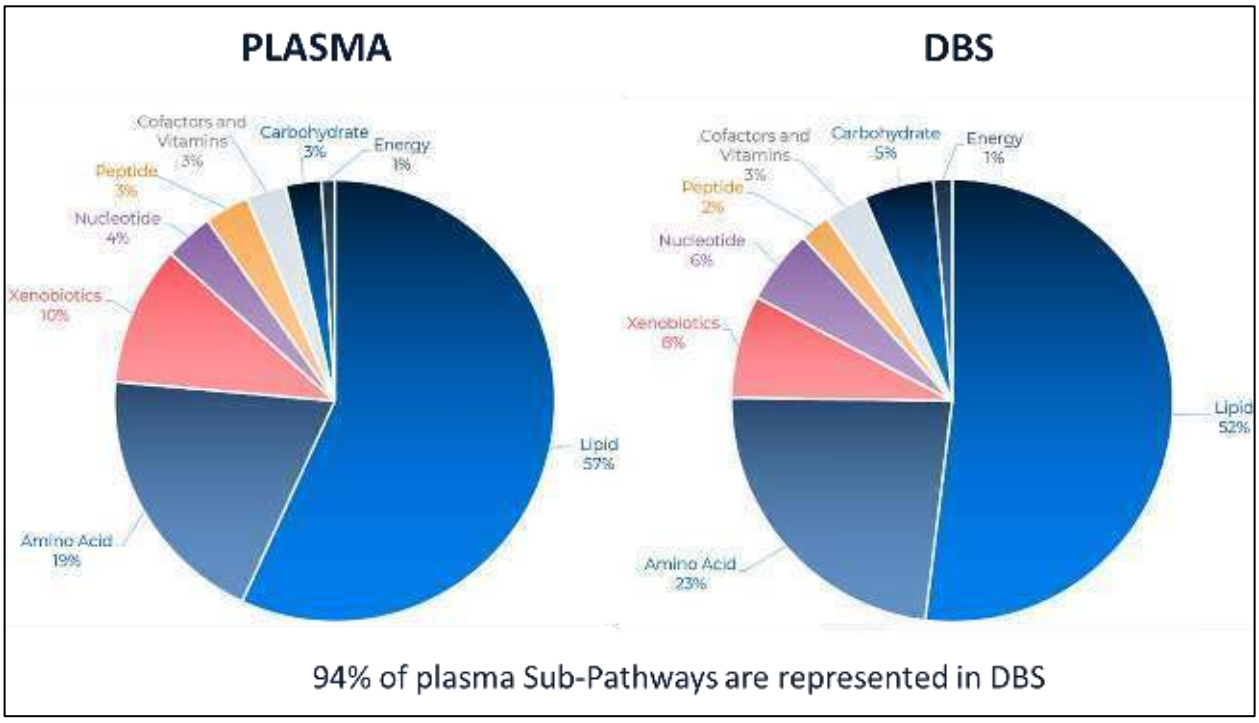

Figure 1 - Metabolomic profiles of plasma and DBS broken down by Super pathway, for biochemicals detected in at least 70% of samples from each matrix in a cohort of 49 donors.

number of metabolites in each super pathway, demonstrating that even with ~4-fold less volume extracted, >550 named metabolites were detected using DBS. Due to the sampling and extraction process, we estimate that ~22 µL of blood is extracted for DBS, compared to 100 µL for plasma extraction. **Figure 1** illustrates the similarity in Super Pathway composition between plasma and DBS samples. It is noted that this conservative detection threshold (70%) was applied to compare *routinely* detected biochemicals. However, the relative pathway compositions of Super Pathways remains the same regardless of the detection criteria used. Looking deeper, the data shows that *94% of the 103 sub pathways represented in plasma were also represented in DBS* in some fashion: by the same biochemicals, unique ones (*i.e.*, surrogate biochemicals), or a combination of these. That is, even though some biochemicals differ between plasma and DBS cards compared, these differences are dispersed across all pathways so that the large majority of sub-pathways are still represented by at least one biochemical in DBS cards.

Super Pathway	Plasma (100 µl)	DBS (2 x 6 mm spots)
Amino Acid	179	136
Peptide	30	13
Carbohydrate	24	30
Energy	9	8
Lipid	523	304
Nucleotide	33	32
Cofactors and Vitamins	27	18
Xenobiotics	94	44
Total	**919**	**585**

Table 1 – Breakdown by super pathway of the biochemicals detected in at least 70% of samples in each matrix.

Next, we correlated the counts as a percent of the total for each matrix to itself, as well as between plasma and DBS cards. **Figure 2** shows that >87% of biochemicals correlated fairly well (r>0.6) when each matrix was compared with itself (91%

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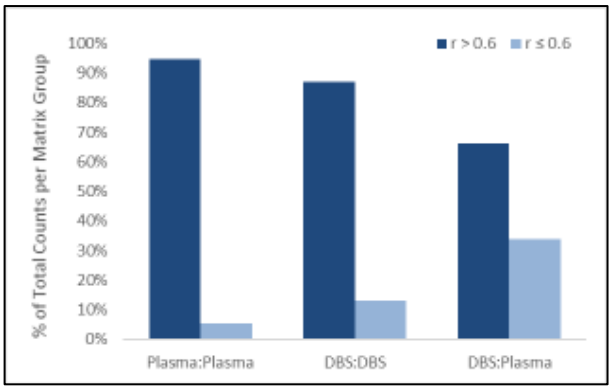

Figure 2 - Correlation comparison between matrices. Counts of each biochemical as a percentage of the total counts were compared in each matrix and between matrices, with the indicated percentage of biochemicals having correlation values (r) less than (light blue) or greater than (dark blue) 0.6.

plasma:plasma; 87% DBS:DBS). When plasma and DBS were compared, the median correlation coefficient of >500 biochemicals was 0.75, with approximately 65% having r values greater than 0.6. Of these, 20% showed moderate correlation (r between 0.6 and 0.8) and 45% showed strong correlation (r>0.8). Xenobiotics and Cofactors/Vitamins correlated the best, while the Energy and Peptide pathways had the lowest correlations, with 60–70% of each containing biochemicals with correlation values greater than 0.6. The latter is neither surprising (biochemicals in these pathways, for example TCA cycle metabolites, are enriched in red blood cells) nor significantly impactful on the overall correlation since these are among the smallest pathways, with only 4–5 biochemicals that were poorly correlated.

Lastly, it is noted that the precision of internal standards, retention standards, and endogenous biochemicals detected in each quality control matrix were comparable in the two samples types, with median relative standard deviations (RSDs) ranging from 4–10% for DBS cards and 4–7% for plasma samples, thus indicating no inherent issues with DBS cards as a sample matrix.

Detection of Fed vs Fasted signatures in plasma vs DBS cards

Using the same DBS and plasma sets described, an ANCOVA (analysis of covariance) was used to compare the metabolic signatures of fed versus fasted individuals in DBS to those in plasma, adjusted for gender and age. Some metabolic signatures were only evident in one matrix, a result that is not surprising *given they are inherently different matrices*, but overall, there was good agreement. Approximately half of the top 50 signatures in plasma were also significant in DBS ($p \leq 0.07$). Conversely, of the 50 strongest metabolic signatures in DBS ($p<0.02$; $q<15\%$) that were also detected in plasma, 41 were significant ($p<0.05$) and another 4 were trending towards significance ($0.05<p<0.10$). All 45 biochemicals had fold-changes (FC) in the same direction in both matrices. Four of the remaining five metabolites, had similar fold-changes in both matrices but were not significant ($p>0.10$) in plasma, with a single metabolite not matching either the directionality of change or significance. Beyond the same directionality of change, **Figure 3** shows the similarity in fold changes of 31 common Fed/Fasted markers in the two matrices, indicating good agreement between them. Lastly, the results from this experiment were also compared to another data set collected previously at Metabolon, which showed that 40 of the top 50 metabolites were shared across analyses.

Detection of other metabolic signatures in DBS cards

Beyond the specific Fed/Fasted signatures known from plasma that are reliably detected in DBS cards, we have also noted from these and additional experiments that other known metabolic signatures are also maintained when using DBS cards. Using Z-scores calculated for each sample type, two common markers of diabetes, glucose

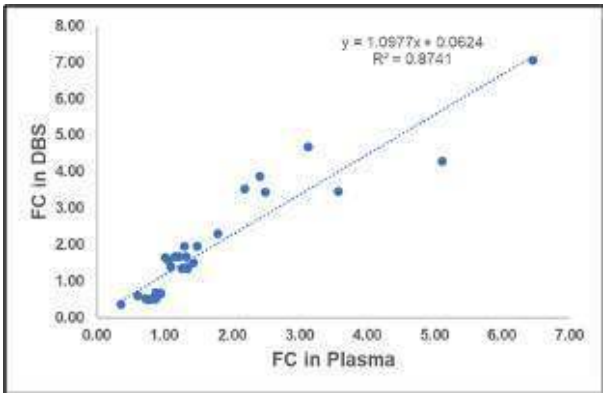

Figure 3 - Fold changes for 31 markers of Fed/Fasted status in plasma vs. DBS (correlation = 0.93).

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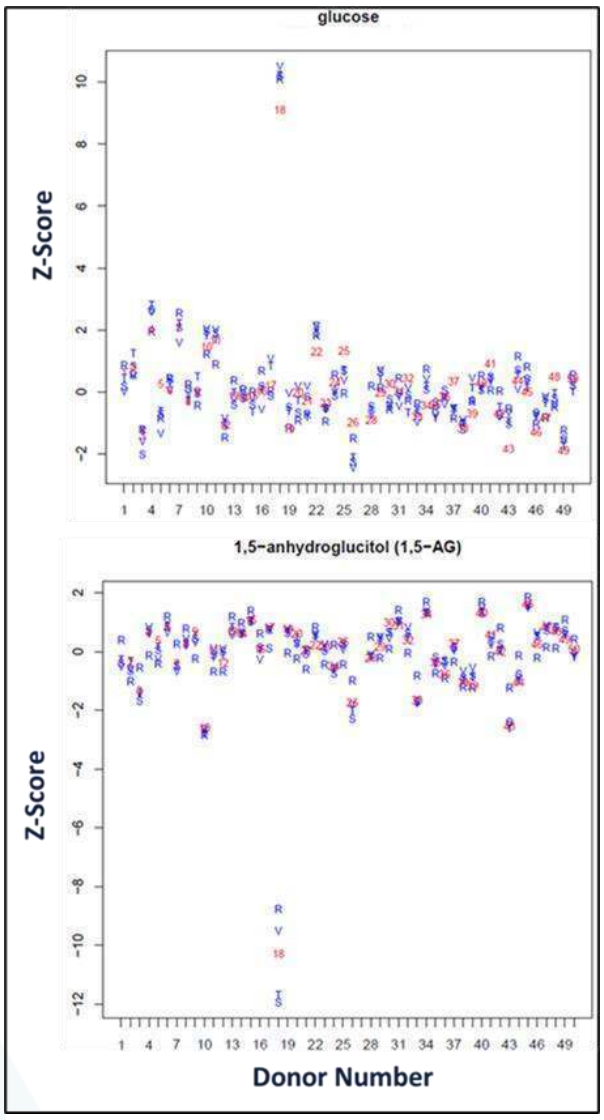

Figure 4 – Z-score analysis of two common diabetes biomarkers in plasma (4 analyses) and DBS cards. Donor 18 self-reported a diabetic status that was evident using both matrices.

and 1,5-anhydroglucitol (1,5-AG), show similar patterns for one donor whose metadata indicated ongoing diabetes (**Figure 4**). Another in-house study that utilized 22 donors preparing DBS cards daily over the course of 4 weeks also revealed that the precision over time of measurements from DBS cards can capture metabolic signatures within individuals. For example, one signature that was noted in this cohort was the presence of elevated levels of Hydroxy-CMPF (**Figure 5**) in donors who self-reported taking a fish-oil supplement, similar to as described previously (5). Interestingly, it was also noted that in one donor with generally lower levels, two days of samples showed elevated levels of the biochemical (see green circles in the No boxplot in Figure 5). Further investigation revealed that the donor had in fact taken a supplement that was not initially disclosed. Another signature showed one individual who underwent a 4-day water-only fast during the collection period. As can be seen in **Figure 6**, the ketone body 3-hydroxybutyrate (BHBA) and the stress marker ophthalmate both increase in that donor's samples as a result of the change in diet, as expected from fasting (6, 7). Increases in branched chain amino acid metabolites were also noted, another described effect of fasting (8). Other metabolic signatures detected included those associated with aerobic exercise, pesticide exposure, and steroid hormone metabolism.

Conclusions

Plasma has been thoroughly studied and well-characterized using Metabolon's global untargeted Precision Metabolomics™ platform. Given the low temperature conditions that are recommended for plasma storage (9), it is not surprising that researchers want to know whether DBS cards are a viable alternative, given the less stringent requirements for their production and storage. It is important to understand that *DBS cards and*

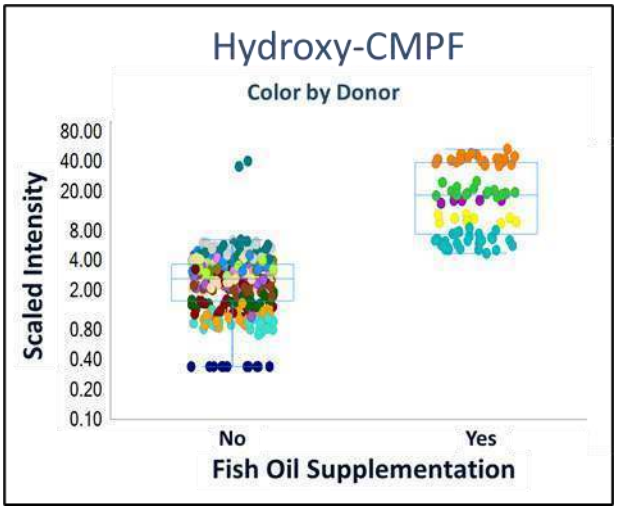

Figure 5 – Elevated levels of Hydroxy-CMPF, a surrogate for dietary fish oil supplementation, detected in 5 Donors with self-reported usage. Note 2 outliers in the No group that were later revealed to come from initially unreported intake.

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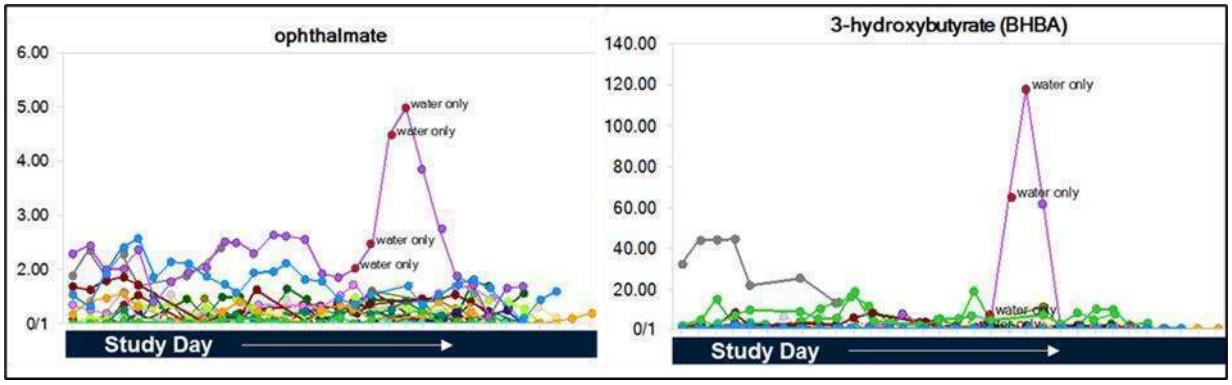

Figure 6 – Levels of the stress marker ophthalmate and ketone body 3-hydroxybutyrate (BHBA) during a 4-day water-only fast show the precision and maintenance of metabolic signatures within individuals over time using DBS cards.

plasma are different matrices, and direct comparison between them is not recommended. However, even with these inherent differences, the data presented *supports the conclusion that metabolomic profiles are similar between plasma and DBS*, and that *known metabolic signatures detected plasma are maintained in DBS samples*. DBS is a complex matrix type, consisting of a combination of dried whole blood and the filter paper (or cards) it is applied to at the time of collection. Metabolon's validation of the Whatman™ 903 DBS card on our global Precision Metabolomics™ platform showed 85% of biochemicals detected in both sample matrices with similar analyte coverage of all Super pathways. This validated solution provides researchers with an alternative sample type when access to, or appropriate storage and handling of, blood plasma and/or serum is limited or not possible.

References

1. Lim, M.D., *Dried Blood Spots for Global Health Diagnostics and Surveillance: Opportunities and Challenges.* Am J Trop Med Hyg, 2018. **99**(2): p. 256-265.
2. Timmerman, P., et al., *EBF recommendation on the validation of bioanalytical methods for dried blood spots.* Bioanalysis, 2011. **3**(14): p. 1567-75.
3. *Dried Blood Spots: Applications and Techniques*, W. Li and M.S. Lee, Editors. 2014, John Wiley & Sons, Inc. p. 376.
4. Evans, A.M., et al., *High Resolution Mass Spectrometry Improves Data Quantity and Quality as Compared to Unit Mass Resolution Mass Spectrometry in High-Throughput Profiling Metabolomics.* Metabolomics, 2014. **4**(2): p. 132.
5. Xu, L., et al., *Furan fatty acids - Beneficial or harmful to health?* Prog Lipid Res, 2017. **68**: p. 119-137.
6. Achanta, L.B. and C.D. Rae, *β-Hydroxybutyrate in the Brain: One Molecule, Multiple Mechanisms.* Neurochem Res, 2017. **42**(1): p. 35-49.
7. Dello, S.A.W.G., et al., *Systematic review of ophthalmate as a novel biomarker of hepatic glutathione depletion.* Clinical Nutrition, 2013. **32**(3): p. 325-330.
8. Batushansky, A., et al., *GC-MS metabolic profiling reveals fructose-2,6-bisphosphate regulates branched chain amino acid metabolism in the heart during fasting.* Metabolomics, 2019. **15**(2): p. 18.
9. González-Domínguez, R., et al., *Recommendations and Best Practices for Standardizing the Pre-Analytical Processing of Blood and Urine Samples in Metabolomics.* Metabolites, 2020. **10**(6).

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