

***Advantages of Blood Spot Specimens
for Amino Acid Assessment***

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Summary

Assessing plasma amino acid status in an individual has proven to be pivotal in the treatment of disease. When interpreting plasma and blood spot data, one must consider the differences between the two sample types. Certain amino acid levels such as aspartate, glutamate, and taurine appear significantly higher in blood spot samples. This difference may be due to the presence of active amino acid transporters in red blood cell membranes. Furthermore, unlike plasma, short term dietary changes may be less evident in blood cells, which comprise approximately 40% of the blood spot sample. We conclude, therefore, that a blood spot amino acid test may provide information not available in plasma studies and possibly correlates more closely with long term amino acid status.

Stabilization of glutamine, glutamic acid and arginine

Plasma glutamine has been shown to be highly susceptible to degradation in plasma specimens not immediately stored at -20 degrees F or below. Apparently the action of glutaminase causes hydrolysis of the gamma-amide bond to produce glutamic acid and ammonia. The rising levels of these two can cause an increase in arginine due to activation of the urea cycle. The net result is that plasma amino acid profiles may show low glutamine and high glutamic acid with mildly elevated arginine concentrations due to warming of specimens prior to or during transit to the laboratory. The immediate, thorough drying required for transport of a blood spot specimen inactivates enzyme activities, removing the glutaminase action on glutamine.

Presentation of long term, intracellular effects

Plasma has been shown to be superior to urine for assessment of essential amino acid status because of lower influence from recent (previous day) dietary intake. By the same argument, even more long term and intracellular effects are revealed by blood spot amino acid concentrations. The whole blood that is initially gathered on filter paper contains approximately 40% red blood cells by volume. When the specimen is dried, the cells release their free amino acid contents that are later extracted, along with those from plasma, during processing of the specimen in the laboratory. Thus, the measured concentrations represent the average of 40% intracellular and 60% plasma levels.

For some amino acids the concentrations inside the cells and in plasma are approximately the same. For these analytes we find blood spot data correlating with plasma data as shown in Figure 1 for tyrosine.

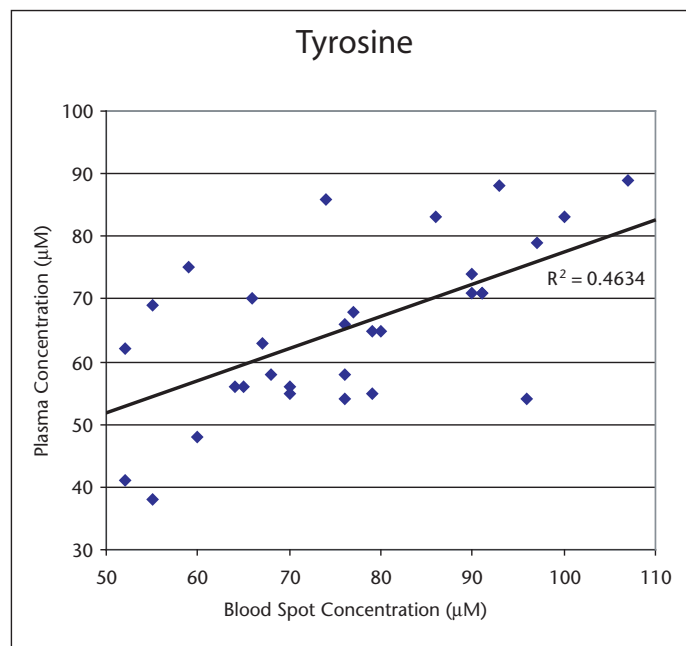


Figure 1. Correlation of blood spot and plasma tyrosine

Tyrosine concentrations are approximately equivalent in plasma and blood spot specimens from the same individual as shown in this study. Plasma and blood spot specimens were taken at the same time from a group of healthy volunteers. There is a moderately strong, positive correlation between the two tyrosine results.

We find some surprising differences between plasma and blood spot levels of other amino acids because of the ability of red blood cells* to maintain a positive concentration gradient. Glutamic acid and especially aspartic acid concentrations are much higher inside the erythrocyte than in plasma (Table 1). These differences indicate an active pumping mechanism to maintain the positive concentration gradient inside the cells. Taurine concentrations are much higher in erythrocytes, possibly to protect against oxidative damage.

Table 1. Comparison of plasma and blood spot concentrations

Amino Acid	Blood Spot/Plasma Ratio
Aspartic acid	23.6
Glutamic acid	4.6
Taurine	4.8

Although fasting blood spot methionine range approximates that for plasma, individuals with very high methionine intake

* Similar active transport mechanisms may be found in lymphocytes, but the contribution to blood spot levels is much less that for erythrocytes.

can show 100% increases in blood spot concentration while plasma levels are only slightly elevated. Figure 2 illustrates this rapid cellular absorption in two healthy volunteers who consumed a gram of methionine prior to the sample collection. Phenylalanine and valine also show this tendency, although less dramatically than methionine.

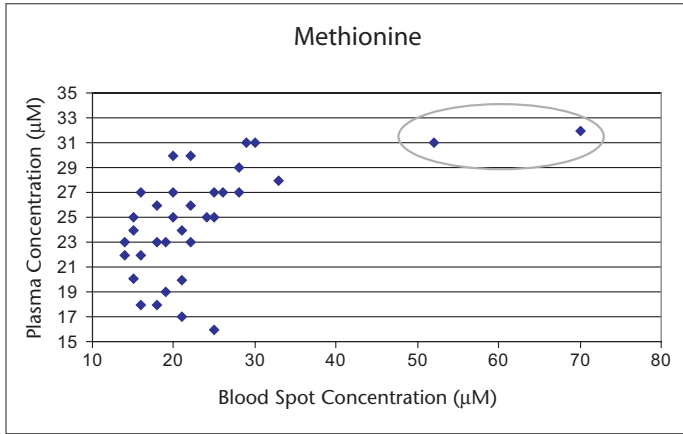


Figure 2. Sensitivity of blood spot analysis to methionine intake

Plasma and blood spot specimens were taken at the same time from a group of healthy volunteers. The two individuals shown inside the ellipse consumed methionine prior to sample collection.

Reason for lack of correlation between plasma and blood spot results

Since the blood spot specimen is composed of approximately 40% RBC and 60% plasma, the rate of change for the two partitions may differ in the course of a patient becoming

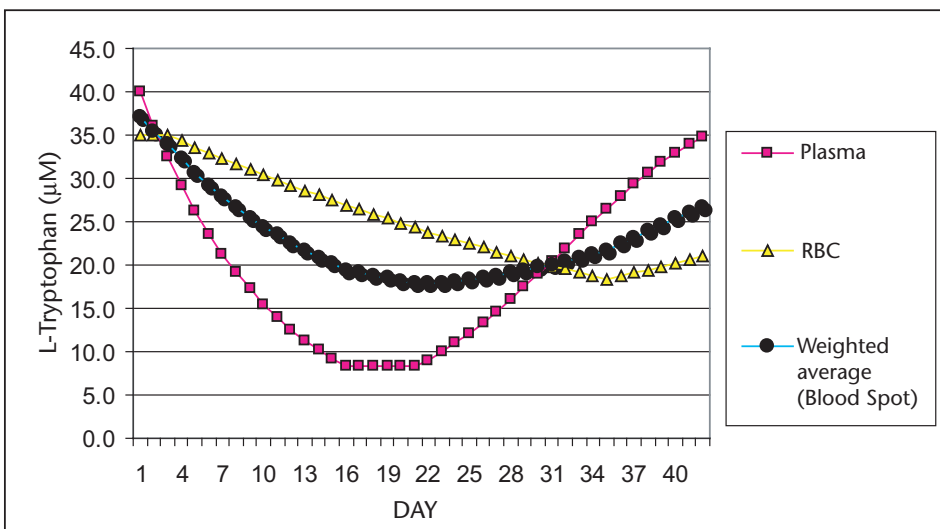


Figure 3. Theoretical experimental results from amino acid depletion trial

Plasma tryptophan is represented by the filled squares, RBC by the filled triangles and the weighted average is shown by the solid black circles. On days 0-16 the subject is fed a tryptophan-free diet. Then on day 18, dietary tryptophan is reintroduced at levels that cause rapid tissue repletion. The weighted average is computed to represent an erythrocyte and plasma at 40 and 60% contributions, respectively. The boxed areas show the region in which the result falls into the abnormally low range for each type of data.

depleted and then replete in amino acids. Figure 3 illustrates a hypothetical experiment in which an individual is made deficient in L-tryptophan. Low tryptophan is more quickly detected in plasma than in RBC because of the slow rate of erythrocyte turnover. The weighted average corresponds to the result that should be obtained from measuring tryptophan in a blood spot specimen. Plasma concentrations fall quickly into the abnormal low region, followed days later by RBC values falling. The calculated blood spot results would correspond to plasma or red cell concentrations only following relatively long periods of stable dietary intake.

The clinically significant amino acid deplete state would be better represented by the blood spot data than plasma because the depleted intracellular state is revealed.

Conclusion

Sensitive modern instrumentation that allows measurement of a 20-amino acid profile on a blood spot specimen has advanced routine clinical evaluation of amino acid status. The drying of blood spot specimens affords the advantage of stopping enzyme activities that can act upon glutamine during transport of plasma specimens. Blood spot specimens reveal clinically significant essential amino acid deficiency and they allow more sensitive detection of physiological changes of non-essential amino acids. The effect that dietary intake and metabolic demand have on amino acids occurs at different time scales in plasma and blood spot data, therefore preventing a direct comparison between tests.