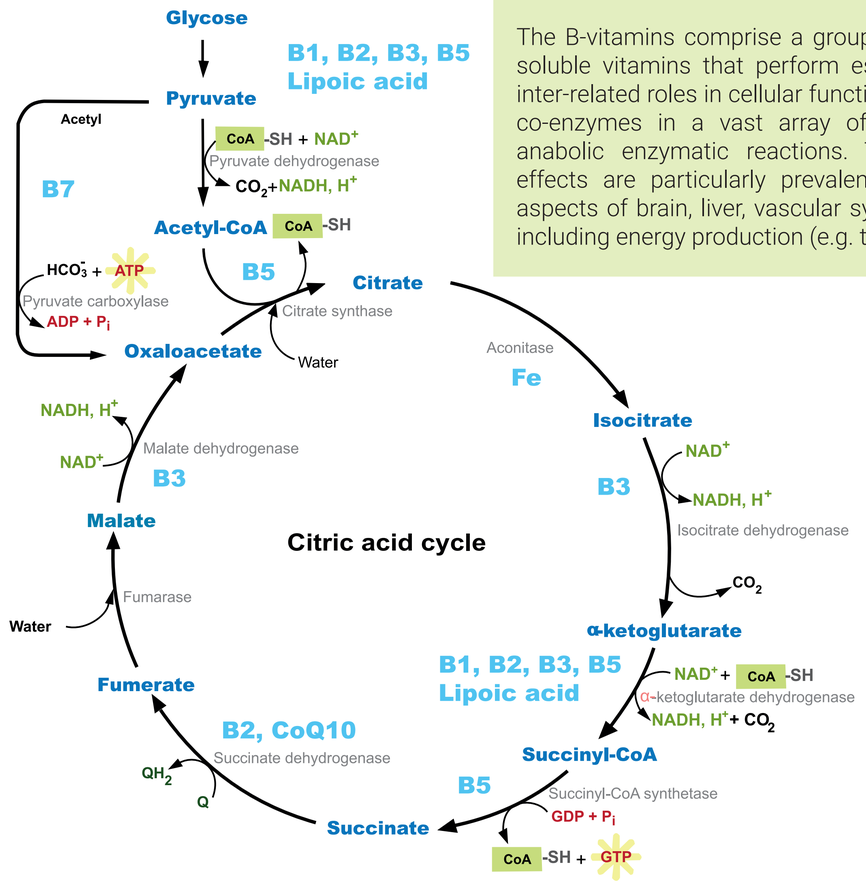
B Vitamins Reference Ranges Determination Using HPLC-MS/MS and Retrospective Statistical Analysis

Sadykov Arseny, Mamedov Ilgar, Zolkina Irina, Aushkap Sergey, Sevko Darya, Yurasov Vasiliy.
Scientific laboratory «Chromolab», Moscow, Russia.

Introduction

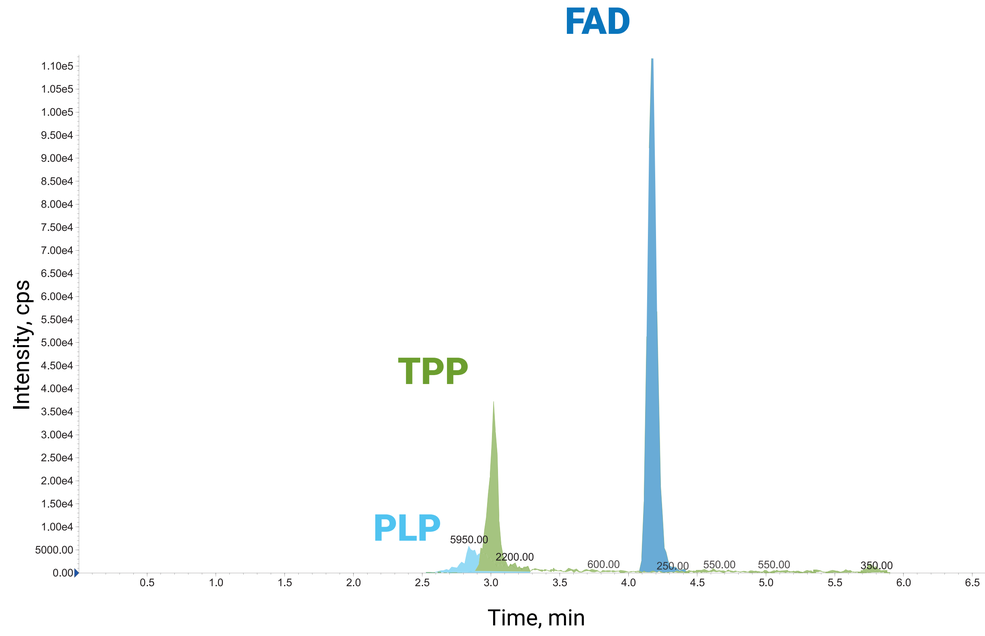
Determination of the correct reference intervals (RIs) is the basis for precise interpretation of the results in routine laboratory testing. Ideally, each laboratory should have its own RIs, however their determination is prohibitively expensive for many laboratories. One of the compromise solutions is a retrospective mathematical analysis of routine tests for RIs calculation. The objectives of the study were to establish reference ranges for B vitamins complex in whole blood and plasma in the Caucasian population. The advantages and disadvantages of several statistical methods were considered and clear criteria for choosing a particular approach were developed.



The B-vitamins comprise a group of eight water soluble vitamins that perform essential, closely inter-related roles in cellular functioning, acting as co-enzymes in a vast array of catabolic and anabolic enzymatic reactions. Their collective effects are particularly prevalent to numerous aspects of brain, liver, vascular system functions including energy production (e.g. the Krebs cycle).

Materials and methods

In this study HPLC/MS/MS was used for the concentration determination of vitamins in whole blood and plasma. Bhattacharya, Hoffmann and Mixed Gaussian Models methods were compared for retrospective data analysis and reference ranges establishment.

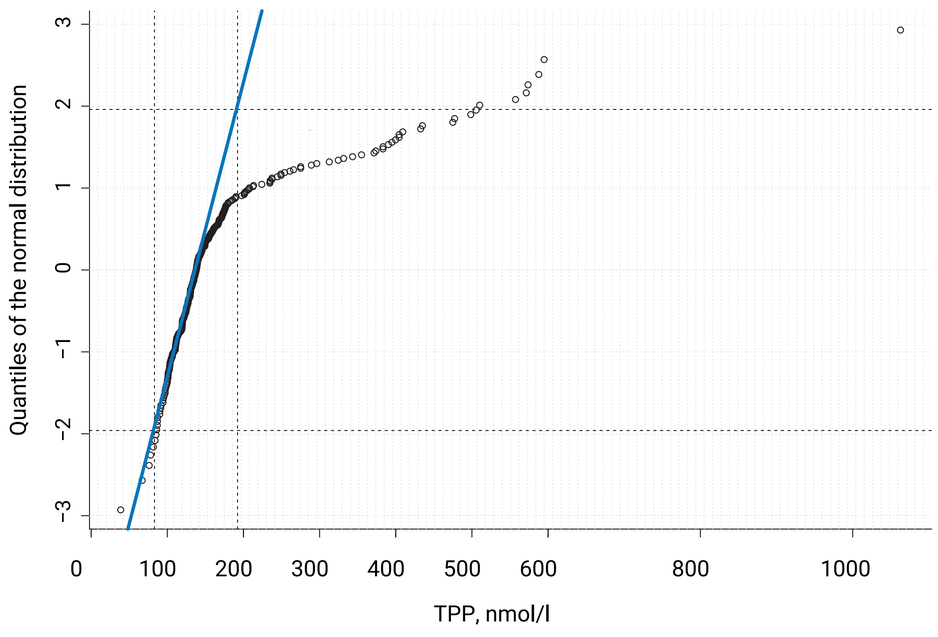


Results

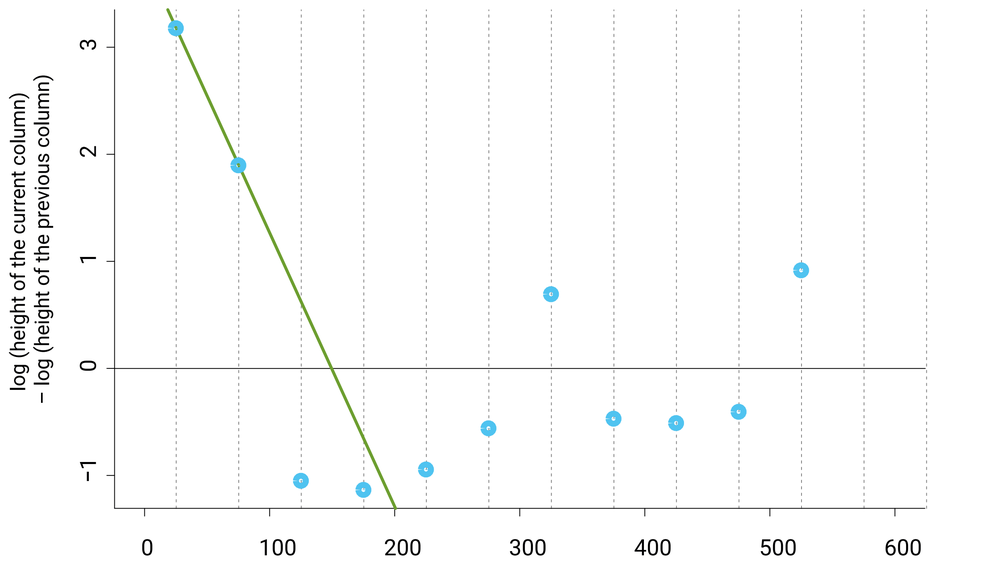
664 patients were analyzed during the study and RIs for Caucasian population were established.

Plasma RI, nmol/l	Whole blood RI, nmol/l
vitamin B2 (FAD) 56-97	vitamin B1 (TPP) 82 – 239
vitamin B2 (riboflavin) 4-43	vitamin B2 (FAD) 116 – 393
vitamin B3 (niacin) 13-161	vitamin B6 (PLP) 3,5 – 80
vitamin B5 (PA) 54,5 – 604,4	
vitamin B6 (PLP) 11,3- 302	
vitamin B7 (biotin) 0,025-5,647	

Determination of reference intervals using Hoffmann method



Determination of reference intervals using Bhattacharya method



TPP concentration. Column height corresponds to the number of results in the column width range

Conclusions

At the moment there is no universal method for reference intervals determination. Depending on the data structure, each method has its own advantages and disadvantages. The Hoffman method is considered to be the most optimal in most cases presumably due to the effective excluding the results of patients who take B vitamins as dietary supplements.

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