B-Vitamins: Vital parameters for research and routine medical labs

Novel tool enables quantitative analysis of bioactive B-vitamins

B-Vitamins – action figures for our health

The water-soluble family of B-vitamins consists of chemically distinct compounds which are often present in the same food source. Their task is to promote cell metabolism such as cell growth and division – as a matter of fact, most biochemical processes rely on B-vitamins as coenzymes. Often, several family members act together to promote a particular physiological process. Not surprisingly, these compounds are essential from the first second of life until old age. B-vitamins are essential for healthy skin as well as for an intact immune and nervous system. Moreover, they play a protective role in stress related diseases such as depression or coronary artery diseases.

Supply of B-Vitamins are subject to change

The half-life of B-vitamins in our body differs between individual molecules. Although some vitamins are synthesized by gut bacteria, consistent daily intake is important and ample supply is usually secured by a varied diet. In certain phases of life however, e.g. during embryogenesis, childhood, pregnancy or old age, the organism is susceptible to B-vitamin deficiency due to a higher need or lower supply of these essential compounds. In addition, B-vitamin balance can be disturbed by life style factors such as malnutrition, drug abuse, chronic stress, or high performance sport (see Fig. 1).

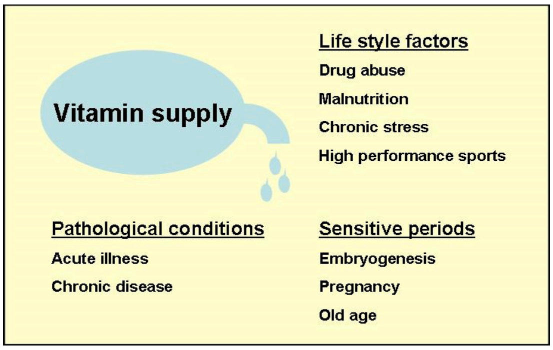

Fig. 1: Influencing factors of vitamin supply

Lack of B-Vitamins is causal and symptomatic for many diseases

Due to their vital metabolic role most B-vitamin deficits are associated with a variety of acute and chronic diseases and are attributed either to pathogenesis or to symptoms of a particular illness. There are many different factors which can cause a shortage, most of them specific to a particular family member and its metabolism. However, a general Achilles' heel of B-vitamin supply is the intestinal tract where most vitamins are extracted from the diet or synthesized by gut bacteria. Therefore, B-vitamin status analysis is especially useful in diagnosis and therapy of patients with chronic intestinal diseases.

The nervous system in particular is affected by a lack of B-vitamins. Physicians treating any medical condition that involves an impairment of mental, cognitive or psychological health should pay attention to the status of B-vitamins. Despite this general role, most B-vitamin family members exert characteristic effects.

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Vitamin B6 (Pyridoxin): indicator of heart diseases

Vitamin B6 is a cofactor in more than a hundred enzyme reactions. One of its functions is to perform transamination, a key step in both breaking down and producing amino acids in the body. Vitamin B6 is also necessary for the synthesis of neurotransmitters as well as haemoglobin in red blood cells and plays a central role in fat metabolism. Finally, vitamin B6 regulates the degradation of homocystein – a cardiovascular risk factor- to cystein. Vitamin B6 deficiency is therefore considered as an indicator for myocardial infarction, peripheral vascular diseases and atherosclerosis, especially in connection with the regulation of homocystein metabolism. Determination of the vitamin B6 status is indicated in patients with chronic inflammatory bowel disease (IBD), alcohol abuse, homocysteinuria or pregnancy.

Vit B12 is low in vegetarians

Vitamin B12 is essential for neuronal and erythrocyte development and is not available from plant sources. Physicians treating vegetarians should therefore keep an eye on the vitamin B12 status of their patients. Another group with potentially low vitamin B12 level are individuals with intestinal resorption disorders. In addition, take up can be reduced up to 50% in elderly, causing weariness or cognitive impairments. Furthermore, vitamin B12 can prevent major birth defects such as neural tube defects. Vitamin B12 should therefore be monitored along with folic acid in women aiming for a pregnancy.

Folic acid is vital for foetal development

Folic acid (vitamin B9) plays a central role in cell division. It is essential for the formation of red blood cells, for the establishment of the bone marrow and for healthy nerve activity. It is hence crucial for foetal development, most importantly in preventing neural tube defects. A lack of folic acid can lead to premature birth and severe abnormalities. Therefore, folic acid should be a routine laboratory parameter of pregnancy monitoring programmes.
In addition, folic acid improves memory and cognitive capacity in elderly. Among the B vitamins, short supply of folic acid is the most widespread vitamin deficiency in Europe and North America – the result of poor nutritional habits with little fresh fruits and vegetables. Characteristic deficiency syndromes include weariness, irritability, concentration problems, appetite loss, mucous membrane inflammation, anaemia and neurological problems.
Folic acid deficiency is the most common cause of hyperhomocysteinaemia, an important risk factor in arteriosclerosis. Therefore, the determination of folic acid should be an integral part of a cardiovascular risk analysis.

Niacin – metabolic wizard

Niacin, the third in the vitamin B nomenclature, is an essential component of the universal coenzymes NAD^+ and $NADP^+$ and as such required in as many as 200 enzymatic electronic transfer reactions. Since almost every metabolic pathway uses either NAD^+ or $NADP^+$, it is not surprising to find signs and symptoms of niacin deficiency in severe metabolic disorders. The worst of these is pellagra which is characterized by the four D's, representing dermatitis, diarrhoea, dementia and death. In addition to this essential physiological role, niacin has been identified as a cholesterol lowering drug which increases HDL cholesterol and reduces LDL cholesterol and triglycerides.

Pantothenic acid: novel parameter for neuroscience

Pantothenic acid (vitamin B5) is an integral part of coenzyme A (CoA). CoA plays a key role in biosynthesis and metabolism of lipids and the catabolic disposition of carbohydrates and ketogenic amino acids. In addition, vitamin B5 is involved in the formation of the neurotransmitter acetylcholine. A metabolic antagonist of pantothenic acid, pantoyl-GABA is widely used in Japan as an antidementia drug for treating Alzheimers's disease.

Biotin for beauty

Biotin or vitamin B7 is a growth factor present in every living cell and plays an indispensable role as a coenzyme in numerous carboxylation reactions. Biotin is well known as "skin and hair vitamin", promoting growth and vitality of blood cells and sebaceous glands in skin, hair and nails. Biotin deficiency is extremely rare since gut bacteria synthesize an excess of the daily requirement. Interest in the role of biotin in human nutrition and therapy originates from reports of various biotin-responsive syndromes, such as malnutrition or the short-gut syndrome.

Vitamin B2 (riboflavin) improves energy production

Vitamin B2 (riboflavin) is a component of flavin-nucleotides (e.g. FAD) and as such acts as an essential coenzyme in many oxidation-reduction reactions, most importantly in the respiratory chain. Due to this central role in cellular energy production, riboflavin is critical for the metabolism. In addition, vitamin B2 serves as a scavenger of free radicals. Riboflavin shortage is usually associated with insufficient consumption of milk and other animal products but can also result from intestinal diseases.

Practical and meaningful B-vitamin analysis: Squaring the circle?

Given the high relevance of B-vitamins in clinical praxis, there is a need for hands-on detection methods. Up to date, standard techniques such as HPLC or LC/MS are used for the analysis (see table 1). However, chromatography lacks the sensitivity to detect traces of certain vitamins, such as vitamin B12, folic acid or biotin. In addition, this technique is expensive due to necessary hardware, special laboratory equipment and trained personnel. Especially the establishment of lab automates for vitamin analysis usually requires a contract with the supplier.

ELISA, another routine analysis tool, is more practical than HPLC but lacks the required precision. The problem roots in the adaptation of the assay to each sample matrix, which includes the risk of interferences with other matrix components such as serum or blood.

Vitamin	Prevalent method for analysis
Vitamin B1 (Thiamine)	HPLC
Vitamin B2 (Riboflavine)	HPLC
Vitamin B6 (Pyridoxine)	HPLC
Niacin (Vitamin B3)	LC/MS
Pantothenic acid (Vitamin B5)	LC/MS
Folic acid	Automate
Vitamin B12 (Cyanocobalamin)	Automate
Biotin (Vitamin B7)	ELISA

Table 1: Standard techniques for the analysis of B-vitamins

In addition to these general technical drawbacks, none of the current methods is able to determine the concentration of biologically active vitamin B compounds, which is crucial for the exact diagnosis and therapy monitoring of a disease. Given the multitude of metabolic variants such as precursor and degradation molecules, the acquisition of meaningful data is a great challenge.

Determination of biologically active B-vitamins: utilizing bacteria to close the gap

Microbiological methods for vitamin determination have been established over the past 30 years in the area of food analysis but the traditional technique is time-consuming and the handling cumbersome.

A novel method has emerged which applies the old principle of microbiological analytics – determining biologically active vitamins through bacterial growth – to a miniaturised and optimised process. The ID-Vit® technology determines B-vitamins turbidimetrically in human or animal samples using simple microtiter plate assays. This microbiological method has the exceptional advantage of specifying only vitamin compounds which can be metabolized by bacteria and are therefore physiologically relevant.

The assay is based on a defined number of lyophilized microorganisms which are immobilized on microtiter plate wells and grow only in the presence of the analyte (a certain vitamin). All that needs to be done to revitalise the bacteria is to add exact volumes of vitamin deficient nutrient medium, sample, or vitamin standard solution to the wells. Bacterial growth is proportional to the vitamin concentration present in the solutions. Best results are achieved with an incubation step of 48 hours which makes the assay an ideal "happy weekend test". A dose response curve of absorbance (OD at 620-630 nm, ELISA reader) vs. concentration is generated using the values obtained from the standard. The vitamin concentration in the samples can be directly obtained from this curve.

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The straightforward handling of the ID-Vit® assays is appealing to medical lab personnel and enables accurate results by a simple, direct measurement of samples in microtiter plates. The system can be adapted to automated systems, permitting high throughput and electronic evaluation of test results. Compared to other vitamin analysis techniques, this novel method is more accurate in measuring relevant vitamins and at the same time more cost- and user-friendly.

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