Journal of Vitamins and Minerals

Huber J, et al. J Vitam Miner 4: 107.

www.doi.org/10.29011/JVM-107.100007

www.gavinpublishers.com

OPEN ACCESS

GAVIN PUBLISHERS

Research Article

Oxidative Stress, Cell Protection, Anti-Aging: How Efficient are Plant-based Vitamin Complexes? Results of a Randomized Double-Blind Study

Johannes Huber[1], Bodo Kuklinski[2], Meinrad Lindschinger[3], Kathrin Mikkeleit[4], Julia Horn[4], Kerstin Schrotter[4], Dietmar Rösler[4], Gertrude Markolin[4*], Barbara Kocher[4], Norbert Fuchs[4]

[1]Medical University of Vienna, Spitalgasse 23, 1090 Vienna, Austria

[2]Diagnostic and Therapy Center for Environmental Medical Illnesses, 18055 Rostock, Germany

[3]Institute of Nutritional and Metabolic Diseases, Outpatient Clinic Laßnitzhöhe, 8301 Laßnitzhöhe, Austria

[4]Institute for Nutritional Medicine (INUMED GmbH), 5585 Unternberg, Austria

[*]**Corresponding author:** Gertrude Markolin, Institute for Nutritional Medicine, Moosham 29, 5585 Unternberg, Austria

Citation: Huber J, Kuklinski B, Lindschinger M, Mikkeleit K, Horn J, et al. (2023) Oxidative Stress, Cell Protection, Anti-Aging: How Efficient are Plant-based Vitamin Complexes? Results of a Randomized Double-Blind Study. J Vitam Miner 4: 107. DOI: 10.29011/JVM-107.100007

Received Date: 20 February, 2023; **Accepted Date:** 01 March, 2023; **Published Date:** 07 March, 2023

Abstract

Objective: Saturated blood levels of B vitamins have beneficial effects on various cellular processes by preventing oxidative stress, which is associated with accelerated aging processes and with the development of several chronic diseases. The aim of the present study was to determine the impact of the daily intake of a B vitamin complex to the antioxidative status in healthy adults. **Methods:** In a randomized, double-blind clinical trial a natural vitamin preparation (N) was compared to a synthetical (S) one. 30 subjects were allocated to either group N or group S. Blood was drawn from the subjects prior to the supplementation phase (T1), after the six-week supplementation phase (T2), and after a subsequent two-week wash-out phase (T3). The blood samples were analyzed for their total antioxidant capacity (TAC), total oxidant capacity (TOC), total polyphenol microtiter (PPm) and for the endogenous peroxidase activity (EPA). **Results:** In both groups TAC was enhanced after T2 (N: +26%; S: +6%), but reached significance only in group N ($p < 0.05$). TOC was reduced in both groups at T2 and decreased even further after T3. EPA increased at both, T2 (N: +29%; S: +41%), and T3 (N: +80%; S: +68%). A PPm decrease occurred in both groups. **Conclusions:** These results demonstrate that B vitamin additives might be helpful to decrease oxidative stress. Natural vitamins yielded a modestly stronger effect compared to manufactured ones.

Keywords: Vitamin B-complex; Total antioxidative capacity; Total oxidant capacity; Peroxidase activity; ROS

Introduction

The eight vitamins of the B complex have central biochemical functions in metabolic processing of the alimentary calorie suppliers carbohydrates, fats and proteins. B vitamins including riboflavin, niacin, pantothenic acid, pyridoxine first have to be activated through linkage to (adenosyl)phosphates before they can play their physiological role as enzymatic cofactors [1]. Pantothenic acid, for instance, is a structural component of coenzyme A, the key molecule that enables the introduction of all energy suppliers into the mitochondrial circulation in the first place [2]. Biotin also plays an essential metabolic role in this recruitment process [2]. Folic acid and cobalamin in turn control almost all enzymatic single carbon transfers (in particular methyl and formyl groups) and are therefore indispensable for our reparative and regenerative metabolism [3]. Pyridoxine, as coenzyme of transferases and decarboxylases among other things, regulates the metabolization of amino and keto acids and the biosynthesis of numerous neurotransmitters [4].

Riboflavin and niacin have a special biochemical status in the group of B vitamins. In the form of their biologically activated coenzymes $FADH_2$/FAD, $FMNH_2$/FMN (Flavin Adenine Dinucleotide, Flavin MonoNucleotide) and NAD(P)H+H+/NAD+ (Nicotinamide Adenine Dinucleotide), they execute the electron and hydride transfer in the cytosol, in the mitochondrial metabolism of the citrate cycle and in the respiratory chain. In cooperation with transfer enzymes (NADH dehydrogenases, reductases, iron-sulphur complexes, ubiquinone, cytochrome, copper-, sulphur- and haem-complexes), they enable the electronic processing of our alimentary calorie carriers. On the energetic level, the acquired electron carriers are oxidized with the respiratory oxygen into the universal "energy currency" ATP. With their standard redox potentials of -190 mV (flavoenzymes) to -320 mV (NAD(P)H), riboflavin and niacin are at the top levels of the transfer cascade of the biological electron donors [5].

Biological electron donors play an important role not only within the scope of mitochondrial energy production. Especially reduced flavoproteins and NAD(P)H with their strong redox potentials are among the strongest anti-oxidants in the human metabolism. Pro-oxidative processes are essential energy-producing (respiratory chain) and immunological processes (oxidative burst due to phagocytosis). On the other hand, this is precisely why the presence and function of anti-oxidants is essential in order to protect healthy cell and tissue compartments (membrane fatty acids, enzymes, DNA) from Reactive Oxygen Species (ROS) and / or Reactive Nitrogen Species (RNS) [6]. Biological anti-oxidant systems protect the organism from oxidative and nitrosative stress and thus play an important positive role in slowing down aging processes [7], in improving stress tolerance [8], in stabilizing the heart [9], circulation [10], and immune system [11].

The plant metabolism displays many analogies to the human biochemistry. The plant organism is also at the mercy of permanent pro- and anti-oxidative processes. Unlike the human organism, however, the plant is capable of covering its demand for organic oxidoreductive biomolecules through own synthesis. It can be assumed that in the course of millions of years plants have undergone an evolutionary development such that they orient their oxidoreductive balance in accordance with fundamental biochemical and ecological principles.

Plants display special biochemical features primarily in periods of growth and germination. Germinating grain and plant seeds activate their own vitamin synthesis with increasing intensity of their metabolism, in order to satisfy the needs of their increasing metabolic turnover. With growing metabolic activity, however, the oxidative stress is on the rise as well, resulting in an enhanced biosynthesis of plant antioxidants. Studies with germinating quinoa sprouts, for instance, demonstrated a higher anti-oxidative capacity by comparison with non-germinated quinoa [12]. They offer a higher total phenolic content and total flavonoids compared with seed extracts, which is correlated with its high antioxidant capacity [13]. These changes are due to a variety of biochemical processes that cause a different composition of primary and secondary metabolites, which in turn leads to an altered production of phenolic compounds [14].

The aim of this study was to objectify whether these germinating quinoa seeds also demonstrated an anti-oxidative influence on the human organism. For this purpose, the so-called "Total Anti-Oxidative Capacity (TAC)", "Total Oxidative Capacity (TOC)", "Endogenous Peroxidase Activity (EPA)" and the "peroxide concentration (total organic carbon TOC)" in the serum of study subjects was quantified. The measurement of TAC in biological liquids provides a flat-rate value for the capacity of the studied system to resist a standardized radical-induced provocation. Both biomarkers describe and quantify the tolerance against oxidative stress or the background exposure to pro-oxidative factors. Since the studied quinoa sprouts contained standardized concentrations of B vitamins, the anti-oxidative impact of these plant vitamins compared with the impact of an equivalent synthetic vitamin B complex was surveyed.

Methods

Study design and subjects

A randomized, double-blind pilot study was conducted in thirty healthy subjects at the Institute of Nutritional and Metabolic Diseases in the Outpatient Clinic Laßnitzhöhe. The primary

outcome of the study was to quantify and to assess the influence of natural and synthetic vitamin B complexes on changes in participant's blood levels of total antioxidant capacity, polyphenol content, peroxidase activity, and peroxide load. The study population consisted of 22 women and 8 men between 18 and 64 years of age. Exclusion criteria were low compliance (<80% of vitamin B complexes), pregnancy or lactation, cholesterol >240 mg/dl, supplementation of vitamins, trace elements or fatty acids, vegan diet, consumption of more than one beer (0.5 l) per day, impaired renal and/or hepatic liver function, knowledge of chronic diseases including cardiovascular disease, cancer, psychosis, diabetes mellitus, and autoimmune diseases including chronic infections or participation in any other clinical trials within the last three months prior to study start.

36 subjects were enrolled in the study and assessed for eligibility. Six persons had to be excluded from the trial due to poor compliance to the inclusion criteria. 30 subjects were considered suitable. A three-week upstream run-in phase was conducted prior to the real test phase. In this period participants subsisted according to a standardized diet plan, which corresponded to the criteria of a light wholefood to minimize nutritive influences on baseline measurements and were encouraged to take regular exercise. After this run-in phase, test persons were randomly allocated into equally sized groups by using the validated randomization software R package blockrand from Greg Snow (randomization for block random clinical trials. R package version 1.3. Published 2013-01-18 [15]. All persons involved in the monitoring and documentation of the study were blinded including study participants, trial personnel and data manager until the database was closed. Patient number was assigned by the order of participant enrolment.

15 test persons were allocated to group N (natural vitamins) and received a botanical B vitamin complex. The verum product is available as a food ingredient with the trade name PANMOL® B-Complex. It is also marketed as a nutritional supplement (PANMOL® B-Complex; vis vitalis gmbh, Salzburg, Austria). Three capsules (one daily dose) contained 2100 mg pulverized quinoa sprouts and have to be taken in the morning before breakfast with sufficient water (approximately ¼ liter). To 15 additional subjects, integrated to group S (synthetic vitamins), a synthetic vitamin B complex was provided. Concentrations of single B vitamins in both groups were identical due to adaption of the quantitative composition of the synthetic B vitamin complex to the botanical PANMOL® B-COMPLEX. Composition of the B vitamin complexes and origin of the synthetic B vitamin test substances are listed in table 1.

	Synthetic vitamin B component	**Origin of the synthetic vitamin B component**	**daily dose [mg]**	***RDI in %**
vitamin B1	thiamine hydrochloride	DSM Nutritional Products Europe, Basel, Switzerland	2.93	266
vitamin B2	riboflavin	DSM Nutritional Products Europe, Basel, Switzerland	3.98	284
vitamin B3	nicotinamide	DSM Nutritional Products Europe, Switzerland	29.85	187
vitamin B5	calcium-D-pantothenate	Productos Químicos Gonmisol SA, Barcelona, Spain	10.95	183
vitamin B6	pyridoxine hydrochloride	DSM Nutritional Products Europe, Basel, Switzerland	3.38	241
vitamin B7	biotin premix 1%	Rieser GmbH, Mattersburg, Austria	0.108	216
vitamin B9	folic acid premix 1%	Rieser GmbH, Mattersburg, Austria	0.69	345
vitamin B12	cyanocobalamin	DSM Nutritional Products Europe, Basel, Switzerland	0.00885	354
*RDI (Reference Daily Intake) = Reference quantity for daily intake according to EU-guidelines 1169/2011.				

Table 1: Origin and content of the vitamins in the synthetical preparation.

Natural B vitamins were obtained from germinating quinoa sprouts (ecotype "Real", grown in Bolivia). Quinoa seeds were grown in a vitamin-enriched medium and then allowed to germinate. During this germination process, the seeds absorb the vitamins and incorporate them into their cells and into their plant metabolism where the vitamins were partially converted into their biologically active forms. After 96 h the sprouts were washed, dried, pulverized and encapsulated. More information about the patented manufacturing process of the natural vitamin B complex is described elsewhere [16].

Shape and package of study supplement were identical for both test groups. Vitamin preparations were administered in white coloured hydroxypropyl methylcellulose capsules of size 0. Before pooling, all raw materials were monitored and temperature, as well as relative humidity, were noted. Batches were mixed according to SOP-05-005 (Sama, Drais, Prodima AC-LI500S mixer) in the Department of Galenics by use of a Prodima ACMJ 50 or Rhönrad mixer.

The total supplementation period constituted six weeks (=42 days) with a daily intake of three capsules every morning consumed with 250 ml of water. The study participants were instructed regarding dosage and application and test substances were applied by the study participants themselves. All subjects completed the study except one person from group N, who had to be excluded after developing a flush upon the first intake of the product.

Clinical surveys were performed after the three-week run-in phase at baseline (T1) before starting the supplementation, at week six after the supplementation period (T2), and at week eight after a two-week washout period (T3). Blood samples (max. 20 ml) were drawn from the subject's antecubital vein.

Measurement methods

Total antioxidant capacity (TAC®)

Total antioxidant capacity measurement was performed using a colorimetric assay from LDN (Labor Diagnostic Nord, Nordhorn, Germany). For this purpose, the peroxide/peroxidase reaction with the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB) served as response for the Reactive Oxygen Species (ROS) content of the subject`s blood samples. Peroxide/peroxidase reaction was operated at 4°C and stopped after 20 minutes. Absorbance was measured at 450 nm and reference wavelength constituted 620 nm. A linear standard curve using Horse Radish Peroxidase (HRP) and TMB in different dilutions (0-1 mM) was used for quantification.

Total oxidant content (TOC®)

Total peroxide determination was executed with a commercially available colorimetric test system from LDN (Labor Diagnostik Nord, Nordhorn, Germany). The reaction of horseradish peroxidase with serum or plasma peroxides leads to a blue-green substrate cation (TMB). After 20 minutes the adding of a stop reaction solution leads to a colour change to yellow which can be measured at wavelength 450 nm (reference wavelength: 620 nm). For sample quantification a linear standard curve by means of Horse Radish Peroxidase (HRP) and TMB in different dilutions (0-1 mM) was used.

Endogenous Peroxidase Activity (EPA)

Endogenous peroxidase activity was determined according to the method of Tatzber, et al. using a colorimetric test system from LDN (Laboratory Diagnostics North, Nordhorn, Germany) feasible for the determination of the endogenous peroxidase activity. Serum peroxidase activity was quantified by comparison with a standard curve using 10 µl 2.5 and 25 mU/ml HRPA, respectively [17].

Polyphenol Microtiter (PPm®)

The total polyphenol content of the serum samples was determined according to the manufacturer's instructions by means of an adapted Folin-Ciocalteu colorimetric microtiter test kit from Omnignostica GmbH & CoKG (Höfein/D., Austria). This method was described elsewhere [15] and refers to the reaction of polyphenols with transition metals which generates a dark-coloured complex. This aggregate was measured at 766 nm. Sample quantification was performed by means of a standard curve with serial dilutions of gallic acid.

		Group N	Group S
	timepoints	**MV ± SD**	**MV ± SD**
	T1	1.15 ± 0.43	1.45 ± 0.23
TAC [mmol/l]	T2	1.45 ± 0.38	1.54 ± 0.22
	T3	1.2 ± 0.22	1.31 ± 0.29
	T1	100.00 ± 46.17	111.17 ± 96.01
TOC [µM/l]	T2	97.36 ± 39.69	99.58 ± 31.84
	T3	79.27 ± 25.86	71.25 ± 25.69
	T1	9.88 ± 0.34	9.57 ± 0.34
PPm [mmol/l]	T2	9.49 ± 0.35	9.46 ± 0.41
	T3	9.73 ± 0.29	9.54 ± 0.53
	T1	3.37 ± 1.74	3.07 ± 1.09
EPA [U/l]	T2	4.36 ± 0.83	4.34 ± 0.55
	T3	6.05 ± 1.82	5.15 ± 1.24
T1: baseline values before the supplementation period, T2: after a supplementation period of six weeks, T3: after the two-week washout phase (eight weeks after T1).			

Table 2: Mean (MV) and standard deviation (SD) for the total antioxidant capacity (TAC), peroxide load (TOC), polyphenols (PPm) and endogenous peroxidase activity (EPA). Values are shown for both test groups for all time points.

Statistical Analysis

The selected sample size of 30 participants with a postulated dropout rate of 20% provides information about the antioxidant impact on the human organism.

Data sets of metric variables were proven for normal distribution using Kolmogorov-Smirnov test with Lilliefors significance correction (α=10%). Group comparisons of normally distributed data sets were performed using t-test for independent samples (test for variance homogeneity: Levene's test, α=5%). Calculations of data sets for continuous variables without normal distribution used Mann-Whitney U test.

The conciseness of the course of biomarker blood levels within groups was documented by two-sided 95% confidence intervals of differences between two trials. The use of the term "significant" reflects a local p value <0.05. Analyses were performed using software R, version 3.4.1 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 36 participants enrolled to the study between May 8, 2017 and October 3, 2017. From these, 30 (83.3%) were eligible for inclusion in the trial, who were randomized into the equally sized treatment arms. One participant withdrew from the study due to an adverse reaction after taking the supplement for the first time. All other subjects completed the study.

The primary outcome of the study was to investigate the total antioxidative capacity (TAC), the total peroxide content and the peroxidase activity after supplementation of either synthetic or plant-derived B vitamins of the participant's blood samples and to evaluate the antioxidative impact to the human organism.

Total Antioxidative Capacity (TAC)

At the end of the supplementation period (T2), a significant increase in antioxidant levels was observed in group N (N: +26%), while in group S (S: +6%) no significant difference compared to baseline values was notable ($p<0.05$) (Figure 1 / Table 2). After the wash-out period (T3), the antioxidant levels in both groups showed similar levels in both groups close to the baseline conditions (T1).

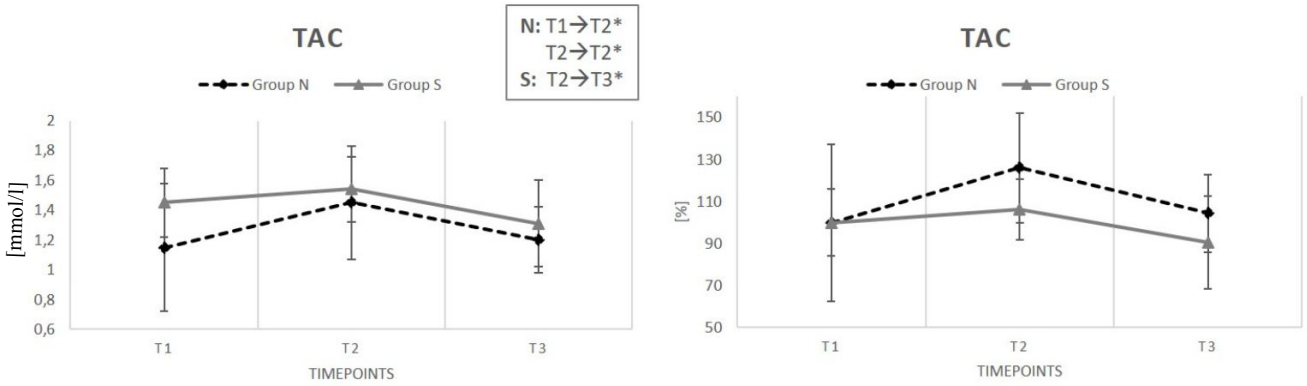

Figure 1: Changes in serum Total Antioxidant Capacity (TAC) with supplementation of natural (N) and synthetic (S) B vitamins. Left: TAC content is shown in mmol/l. Right: Baseline values were set to 100% in order to illuminate the trend. Results are illustrated for group N (natural vitamins; dashed line) and for group S (synthetical vitamins; continuous line). Data are presented as mean ± standard deviation (group N: n = 14; group S: n = 15). Significant differences ($p < 0.05$) were indicated as (∗). T1 = baseline (immediately before the first intake of the test preparations); T2 = six weeks (end of supplementation); T3 = eight weeks (washout period).

Total Oxidant Capacity (TOC)

Baseline conditions for TOC were related in both groups. B vitamin supplementation contributed slightly to the reduction of total serum peroxide content at time point T2 versus T1, both in group N and in group S (Figure 2 / Table 2). After the washout period (T3), TOC continued to be reduced in both groups which implicated that the significance level ($p < 0.05$) was reached.

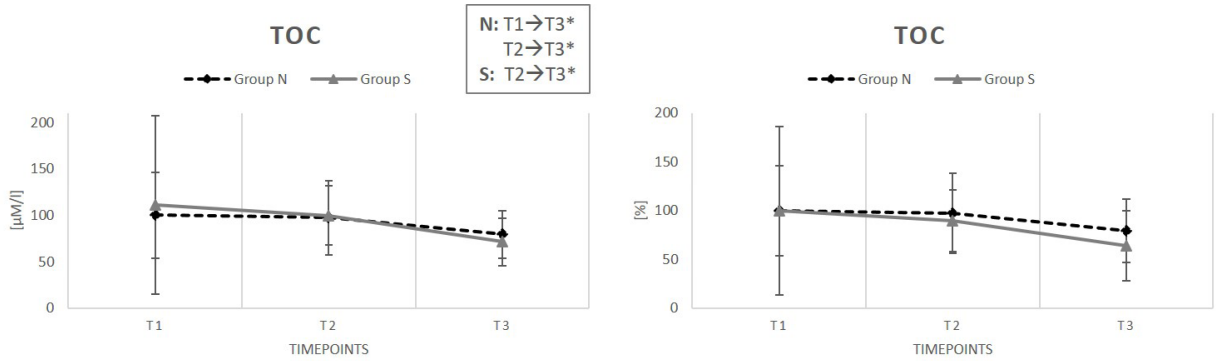

Figure 2: Changes in serum Total Oxygen Activity (TOC) in the follow-up for both subgroups supplemented with natural (N; dashed line) or synthetic (S; continuous line) B vitamins. Left: TOC is diagramed in µM/l. Right: Initial values were set to 100%. Results are shown for group N (natural vitamins) and for group S (synthetical vitamins). Data are presented as mean ± standard deviation (group N: n = 14; group S: n = 15). Significant differences ($p < 0.05$) were indicated as (*). T1 = baseline (immediately before the first intake of the test preparations); T2 = six weeks (end of supplementation); T3 = eight weeks (washout period).

Endogenous Peroxidase Activity (EPA)

The proportion of baseline factors was similar across the study groups in EPA. At the end of the first supplementation period (T2), endogenous peroxidase activity (EPA) was increased in both groups (N: +29%; S: +41%), with no significant difference in group N (Figure 3 / Table 2). However, there was a significant difference in group S. After the washout period (T3), EPA increased significantly in both groups compared to baseline levels (N: +80%; S: +68%). Polyphenol Microtiter (PPm).

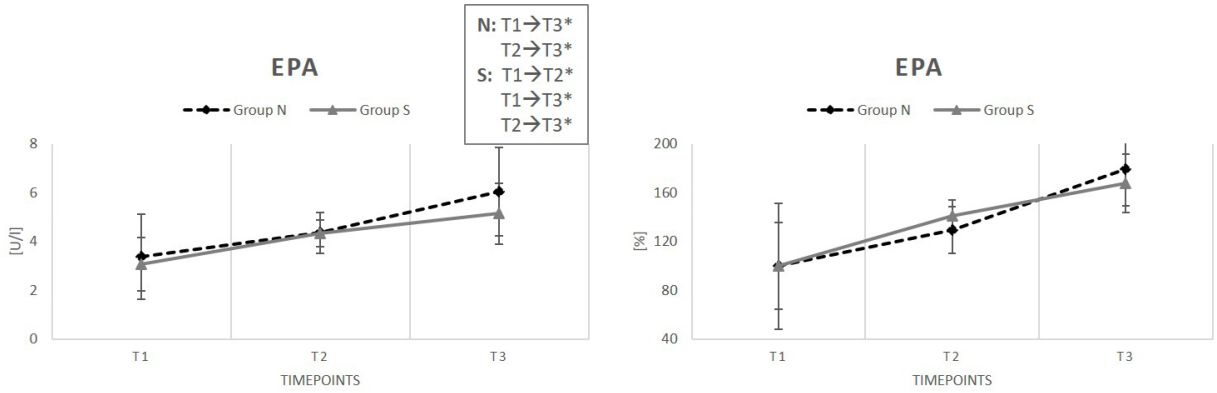

Figure 3: Changes in Endogenous Peroxidase Activity (EPA) with supplementation of natural (N) and synthetic (S) B vitamins. Left: EPA content is shown in U/l. Right: Baseline values are set to 100%. Results are shown for group N (natural vitamins; dashed line) and for group S (synthetical vitamins; continuous line). Data are presented as mean ± standard deviation (group N: n = 14; group S: n = 15). Significant differences ($p < 0.05$) were indicated as (∗). T1 = baseline (immediately before the first intake of the test preparations); T2 = six weeks (end of supplementation); T3 = eight weeks (washout period).

Polyphenol Microtiter (PPm)

In the blood sera of participants in group N significant changes in the PPm levels could be monitored after the supplementation phase (T2). The PPm value of this group was reduced significantly (from 9.88 mmol/l to 9.49 mmol/l) while in group S the reduction of the polyphenol content (from 9.57 mmol/l to 9.46 mmol/l) did not reach significance ($p < 0.05$) (Figure 4 / Table 2). At the end of the washout phase (T3) a significant increase in serum PPm's in group N was evident.

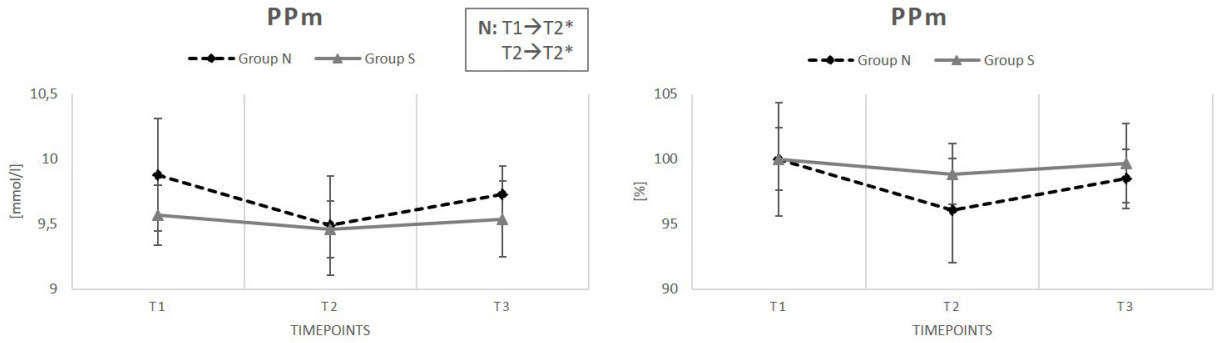

Figure 4: Serum total Polyphenol Microtiter (PPm) under supplementation of natural (N) and synthetic (S) B vitamins. Left: TOC is diagramed in mmol/l. Right: Initial values were set to 100% to illustrate the trend. Results are shown for group N (natural vitamins; dashed line) and for group S (synthetical vitamins; continuous line). Data are presented as mean ± standard deviation (group N: n = 14; group S: n = 15). Significant differences ($p <0.05$) were indicated as (∗). T1 = baseline (immediately before the first intake of the test preparations); T2 = six weeks (end of supplementation); T3 = eight weeks (washout period).

Discussion

Oxidative Stress and Reactive Oxygen Species

Oxidative stress comes along with an imbalance of the pro-oxidant and the antioxidant state within the human organism. The consequence of pro-oxidative burden is an excess of endogenous Reactive Oxygen Species (ROS) like free radicals and peroxides. Although, in limited amounts, ROS such as hydrogen peroxide (H_2O_2), superoxide anion (O_2-) and hydroxyl radical (HO.) are essential for cell homeostasis and redox signaling, a surplus of endogenous Reactive Oxygen Species (ROS) and Reactive Nitrogen Species

(RNS) might lead to a break of chemical bonds which results in oxidative damage of biomolecules like biomembranes, DNA, RNA, proteins, enzymes, lipids and polyphenols and finally provokes cell death [8].

Molecules destroyed by ROS activate inflammatory processes and implicate the generation of free radicals. Another contemporary aspect, appearing prevalently in obese persons, is the over-consumption of oxygens, which generates free radicals in the mitochondrial respiratory chain that is coupled with oxidative phosphorylation in mitochondria [18]. An excessive ROS production contributes to an abnormal cell proliferation, apoptosis and uncontrolled cell growth, which promotes the onset of several chronic diseases like cancer [19], diabetes [20], cardiovascular disease [21], and atherogenic processes [18].

Aging

Oxidative stress plays a major role in the development of aging. Aging is characterized by a progressive decline in cellular functions associated with the accumulation of DNA damage, which also evokes mitochondrial dysfunction. NAD+ availability has been suggested as a possible cause: A decrease in NAD+ levels is associated with an altered metabolism that ultimately accelerates aging processes [5]. With age, free radicals accumulate and lead to progressive oxidative damage, which in turn substantially influences aging processes. An appropriate antioxidant precaution, for instance, an optimal intake of antioxidant nutrients, may help to reduce free radical damage, can lead to an improved quality by minimizing the risk of age-related diseases and can even positively influence the life span [22]. So, the prevention and reduction of the ROS production may help to protect the human cell and may decelerate aging processes. Therefore, antioxidants are key factors to preserve health of cell and tissue compartments from the impact of oxygen damage.

For the evaluation of the endogenous pro-oxidant and the antioxidant state and continuative for the prevention of diseases, state biomarkers of oxidative stress are necessary. One approach to assess the oxidant level is the measurement of the TAC and/or the TOC from blood samples.

Total Anti- and Pro-Oxidant Capacity (TAC/TOC)

Not only an increased burden of oxidants, but also a decrease in the cellular antioxidant capacity plays an influential role to oxidative stress [23]. The Total Antioxidative Capacity (TAC) characterizes the cumulative capability of compounds in a test system to scavenge reactive species while the Total Oxidative Capacity (TOC) reflects the pro-oxidative state of a sample by measuring the total peroxide content. In the organism, there are numerous effective substances, which entirely establish an effective anti- and pro-oxidant network. The determination of the concentration of only a few of these components in elected compartments does not reflect the overall antioxidant situation. Therefore, for a holistic overview, it is reasonable to determine the TAC and the TOC.

In the present study, the endogenous TAC increased during the supplement period while TOC declined, also after the wash out period. This effect was even stronger in group N where subjects achieved supplements of natural B vitamins. Only in this group the increase of TAC was significant after T2. Likewise, in group N TOC-decline at T3 was significant when compared with T2 and also with T1. In group S, the decrease in TOC was only significant when T3 was compared to T2.

EPA

The determination of the endogenous peroxidase activity provides an additional parameter that helps to obtain a general overview of the subject`s pro-oxidative / antioxidative status. In both test groups EPA is increased at T2 and T3. Peroxidases are a group of enzymes that catalyze the reduction of peroxides (ROOH) including hydrogen peroxide (H_2O_2). They act as preventive antioxidants to remove the noxious peroxides. An elevated EPA could reflect two different things: on the one hand an increased peroxide activity can indicate an excessive oxidative stress due to a higher substrate accessibility. On the other hand, a diminished substrate level can be the consequence of an enhanced peroxide conversion rate: more peroxides can be detoxified per time. This consideration coincides with the result of an elevated TAC- and a reduced TOC-value. However, why is the peroxide activity enhanced? Maybe the B vitamin supplementation backfills the store with necessary substances which impacts the whole metabolism including peroxidase function. The endogenous peroxidases themselves do not require any coenzymes in form of vitamins for their function, so the causal link why the enzyme conversation rate is enhanced is missing. Investigations of the kinetic properties of peroxides depending on the B vitamin level would be interesting.

PPm

Polyphenols are plant-derived, secondary metabolites possessing an aromatic benzene ring that is substituted by at least two hydroxyl groups, including their functional derivatives [24]. Polyphenols operate as potent antioxidants by stabilizing free radicals, scavenging ROS by donating hydrogen atoms or single electrons or by chelating the pro-oxidant metal ions. Accordingly, these exogenous antioxidants work synergistically with endogenous antioxidants like Superoxide Dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione (GSH) to reduce free radical cytotoxicity [25]. Furthermore, polyphenols inhibit and modulate gene expression from ROS-activating enzymes like cyclooxygenases or lipoxygenases [26]. Due to all these effects, a protection against various chronic

diseases is provided. Polyphenols gained much attention by the fact of positively affecting diseases like cancer [27], diabetes mellitus [28, 29], atherosclerosis [30] and cardiovascular diseases [28, 31, 32].

The measurement of the PPm serves as a marker for the determination of the oxidative capacity of a system. The polyphenol measurement according to Folin-Ciocalteu is rapid, simple and well reproducible and therefore probably the most popular test for the assessment of the antioxidant capacity by measuring the reducing capacity of a sample. Hence, it was used for this purpose. In this study, the PPm declined in the temporal context to the B vitamin supplementation accompanied by a subsequent increase after the wash out phase.

The decrease of PPm after the supplementation period was unexpected depicting a diminished oxidative capacity. However, an over-interpretation of the results is not appropriate, as PPm is dependent on the consumption of phenol-containing foods or beverages. The rapid metabolism of these substances also leads to great data [23]. Deviations in the PPm are inevitable. Moreover, possibly polyphenols are consumed while they fulfil their task of protecting B vitamins [33]. That means a decrease of the polyphenol content is expected in a vitamin B-rich environment. With regard to the findings in this study, the decrease of PPm during vitamin B supplementation could reflect their biochemical role as herbal antioxidant missing links in connection with an intensified antioxidant metabolism. Generally, conclusions drawn regarding PPm results should be considered with caution, as the measurements, performed in vitro using Folin-Ciocalteu reagent lacks in specificity. For instance, Folin-Ciocalteu reagent also interacts with non-phenolic substances such as ascorbic acid, uric acid, and cysteine and thus impairs the validity of the results [34,35]. Therefore, PPm- determination via Folin-Ciocalteu is well suited as a supplement to the assessment of an oxidative state and serves as an extension for the validation of the TAC/TOC measurement. For a detailed polyphenol analysis HPLC measurements are recommendable.

Vitamins

B vitamins are present in numerous metabolic and regulatory processes acting as cofactors for enzymes involved in the energy metabolism, DNA and protein synthesis and repair, homocysteine methylation and, in particular, in various cortical processes [2, 36]. They are key intermediates of pathways representing essential cofactors like thiamine pyrophosphate from vitamin B1, flavin mononucleotide /flavin adenine dinucleotide (FMN/FAD) from vitamin B2, coenzyme A from vitamin B5, pyroxidal phosphate (vitamin B6), biotin-adenine mono-phosphate (biotin-AMP) from vitamin B7, tetrahydrofolate from vitamin B9 and cobalamin [37]. Neural inflammation and oxidative stress were associated with insufficient concentrations of such vitamins.

Previous studies emphasized the effect of single vitamins to the oxygen status [38-40]. Other studies focussed on an impact of high-dose vitamin B supplementation on reducing oxidative stress and inflammation [37, 41]. Both concepts suggest that oxidative triggering of the metabolism is significantly reduced by B vitamin supplementation with beneficial consequences for myelination, cellular metabolism, and energy. Indeed, these insights are crucial, though, by using a single vitamin approach, the interdependence and complex effect of the entire group of B vitamins is neglected. B vitamins have a manifold and complex function in the human metabolism. Thus, a holistic approach, including a multi-B vitamin complex, seems reasonable. Another point is that physiologically adjusted vitamin complexes efficiently support the oxidoreductive system compared to high doses of singular vitamins. An improvement of cognitive effects in association with B vitamin complex supplementation could already be noted at physiological concentrations [42, 43].

The aim of the present study was to investigate the effect of B vitamins on the total oxidative state. Furthermore, it was evaluated whether the impact of natural vitamin complexes differs from synthetic ones. These findings provide preliminary evidence that B vitamin supplementation at near-physiological concentrations already exhibit an ostensive antioxidant capability for all three, TAC, TOC and EPA and these low doses are sufficient to cause an antioxidant effect. Insofar this observation is valuable since a low-content intake of B vitamins offers a better prevention from adverse effects due to over dosage [44].

In this trial, the antioxidative effect caused by plant-derived B vitamins was compared to synthetical vitamins. In both groups, an increase of the TAC and EPA paired with a decrease in TOC and PPm could be observed. This oxidative impact was even stronger by consuming natural, plant-derived B vitamins compared to the supplement of synthetic ones, because in group N increase in TAC reached significance. So, what is the difference between natural and synthetical vitamins? Natural vitamins occur in a variety of related forms known as vitamers. These numerous derivates of the pure forms may contribute to the complex metabolic network pathways. Plants are immobile. Hence, they have to maintain the balance of their oxido-reductive equilibrium by the self-synthesis of antioxidant molecules. Plant-derived substances, including vitamins, are adapted to their role as counterbalancing the oxido-reductive state [45].

Kutan et al. could show that vitamins from natural sources like pomegranate extract, green tea extract, with a high content of polyphenols and ascorbic acid showed major antagonizing effects on oxidative stress and lipid peroxidation in patients with Type 2 diabetes mellitus and might be beneficial in preventing cardiovascular diseases [28]. But this benefit is not exclusively expressed in terms of vitamins. Considering the effect of

hormones for therapy of postmenopausal disorders in women, the use of bioidentical hormones including estrone, 17β-estradiol, and progesterone offers a favorable adverse effect profile over synthetically produced hormones by contemporaneously being equally effective in managing menopausal symptoms. For instance, bioidentical hormones led to a decrease of the incidence of mastocarcinoma while the use of synthetic hormones resulted an elevated breast cancer occurrence [46].

Generally, plants with an elevated antioxidant capacity are more tolerant toward stress and a high stress resistance is associated with an efficient ROS removal system. In this study, germinating quinoa sprouts (Chenopodium quinoa Willd.) were used for the production of the natural B vitamin preparation. They were grown in a vitamin-enriched medium, and, therefore, are high in these essential substances. For usual, germinating seeds have a higher demand on vitamins and micronutrients. Hence, during germination, the young plant contains higher amounts of vitamins and minerals [47]. In addition to the elevated metabolic activity, oxidative stress increases. This again leads to an increased biosynthesis of plant antioxidants [12].

Since this trial was a pilot study, which by definition involves only a restricted number of participants, the main weakness of the study is the low number of subjects. This may impact some trends between the groups to reach significance. To manifest the present results, we recommend a subsequent clinical trial with an adequate number of participants.

The present study demonstrates clearly that these germinating quinoa sprouts indeed render antioxidative effects to the human metabolism and are able to ameliorate the total antioxidative capacity. The oxidative impact was even more distinct with the supplementation of natural vitamins compared to the intake of their synthetic counterparts.

Conclusion

This clinical double-blind pilot study illuminates the impact of short-time B-vitamin supplementation in low-dose concentrations to the human antioxidant status. Natural or synthetically produced B vitamins were allocated to a randomized group of 30 healthy adults. Concentrations close to physiological conditions (about 2.5 times of the daily recommended intake) supplied for a period of six weeks showed a clear impact to measurable biomarkers and to lead to a reduction in oxidative stress. The total antioxidant capacity was increased while simultaneously the total oxidative capacity was reduced. The content of serum polyphenols is diminished during the supplementation period and increased again after washout. This may be due to their biochemical role as an herbal antioxidant: The polyphenol consumption may be increased as a response to an intensified antioxidant metabolism. Endogenous peroxidase activity was elevated during supplementation and even after the two-week wash-out phase.

The remarkable increase in serum antioxidant status for both, enzymatic and non-enzymatic antioxidants, could be observed in both test groups at the end of supplementation. However, in group N, who received a plant-derived vitamin-B complex, the effect was even stronger, because measured data, which defined the oxidative state, reached significance. These natural vitamin-B complexes derived from germinating quinoa sprouts. Particularly in this early stage of life, where the plant metabolism must deliver products for growth and development, it is naturally rich in nutrients and minerals. Additionally, plant-based vitamins do not only occur in a unique, chemically defined form, but offer a broad range of various derivate which might have positive effects to the human metabolism.

This study demonstrates that the daily intake of B vitamins can be effective in reducing oxidative stress even at low doses. This might also prevent inflammation processes through increasing oxidative metabolism, and may promote myelination, cellular metabolism, and energy storage [37].

In summary, these findings highlight the importance of essential B group vitamins -as a holistic complex- in the maintenance of a balanced pro-oxidant and antioxidant state and B vitamin supplementation represents a promising strategy in the prevention and alleviation of diseases.

Ethics and Dissemination

Ethics approval was received from the local ethics committee on 22nd of March 2017 (EK 29-271 ex 16/17). The study was performed in accordance with the Declaration of Helsinki.

Trial registration number: NCT03444155. URL: http://www.clinicaltrials.gov

Acknowledgments

Study design and data collection and -analysis was executed by the INUMED GmbH team guided by Mag. Norbert Fuchs. The authors gratefully acknowledge the scientific advice and support of Doz. Dr. sc. med. Bodo Kuklinski.

References

1. Peterson CT, Rodionov DA, Osterman AL, Peterson SN (2020) B vitamins and their role in immune regulation and cancer. Nutrients 12: 3380.
2. Miller AL (2003) The methionine-homocysteine cycle and its effects on cognitive diseases. Altern Med Rev 8: 7-19.
3. Penberthy WT, Sadri M, Zempleni J (2020) Biotin. In: Present Knowledge in Nutrition. Elsevier; 1: 289-303.
4. Calderón-Ospina CA, Nava-Mesa MO (2020) B Vitamins in the nervous system: Current knowledge of the biochemical modes of

action and synergies of thiamine, pyridoxine, and cobalamin. CNS Neurosci Ther 26: 5-13.

5. Hrubša M, Siatka T, Nejmanová I, Vopršalová M, Kujovská Krčmová L, et al. (2022) Biological Properties of Vitamins of the B-Complex, Part 1: Vitamins B_1, B_2, B_3, and B_5. Nutrients 14: 484.

6. Pisoschi AM, Pop A (2015) The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem 97: 55-74.

7. Simioni C, Zauli G, Martelli AM, Vitale M, Sacchetti G, et al. (2018) Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. Oncotarget 9: 17181-1798.

8. Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7: 405-410.

9. Calenic B, Miricescu D, Greabu M, Kuznetsov AV, Troppmair J, et al. (2015) Oxidative stress and volatile organic compounds: interplay in pulmonary, cardio-vascular, digestive tract systems and cancer. Open Chemistry 13: 1-11.

10. Faizal P, Satheeshan B, Adarsh AK, Shilpa R, Roshni P, et al. (2013) Antioxidant status and oxidative stress in the circulation of younger and elderly human subjects. Indian J Clin Biochem 28: 426-428.

11. Laddha NC, Dwivedi M, Mansuri MS, Gani AR, Ansarullah M, et al. (2013) Vitiligo: interplay between oxidative stress and immune system. Exp Dermatol 22: 245-250.

12. Pitzschke A, Fraundorfer A, Guggemos M, Fuchs N (2015) Antioxidative responses during germination in quinoa grown in vitamin B-rich medium. Food Sci Nutr 3: 242-251.

13. Enciso-Roca EC, Aguilar-Felices EJ, Tinco-Jayo JA, Arroyo-Acevedo JL, Herrera-Calderon O (2021) Biomolecules with Antioxidant Capacity from the Seeds and Sprouts of 20 Varieties of *Chenopodium quinoa* Willd.(Quinoa). Plants (Basel) 10: 2417.

14. Quassinti L, Gianfranceschi G, Lupidi G, Miano A, Bramucci M (2016) Antioxidant and Pro-Oxidant Activities of Savoy Cabbage (*Brassica Oleracea* L. *Var. Sabauda)* Sprout Extracts. J Food Biochem 40: 542-549.

15. Snow G (2019) Blockrand: randomization for block random clinical trials. R package version 13.

16. Fuchs N, Loidl R, Sadeghi B (2007) Process for the preparation of a plant enriched with B vitamins. Patent EP 1 867 728 B8.

17. Tatzber F, Griebenow S, Wonisch W, Winkler R (2003) Dual method for the determination of peroxidase activity and total peroxides-iodide leads to a significant increase of peroxidase activity in human sera. Anal Biochem 316: 147-153.

18. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, Esquivel-Soto J, Morales-González A, et al. (2011) Inflammation, oxidative stress, and obesity. Int J Mol Sci 12: 3117-3132.

19. Ames BN, Shigenaga MK, Gold LS (1993) DNA lesions, inducible DNA repair, and cell division: three key factors in mutagenesis and carcinogenesis. Environ Health Perspect 101: 35-44.

20. Kaneto H, Katakami N, Matsuhisa M, Matsuoka TA (2010) Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis. Mediators Inflamm 2010: 453892.

21. Sugamura K, Keaney Jr JF (2011) Reactive oxygen species in cardiovascular disease. Free Radic Biol Med 51: 978-992.

22. Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 4: 118-126.

23. Domej W, Oettl K, Renner W (2014) Oxidative stress and free radicals in COPD--implications and relevance for treatment. Int J Chron Obstruct Pulmon Dis 9: 1207-1224.

24. Lin D, Xiao M, Zhao J, Li Z, Xing B, et al. (2016) An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules 21: 1374.

25. Salla S, Sunkara R, Ogutu S, Walker LT, Verghese M (2016) Antioxidant activity of papaya seed extracts against H2O2 induced oxidative stress in HepG2 cells. LWT - Food Science and Technology 66: 293-297.

26. Kim HP, Son KH, Chang HW, Kang SS (2004) Anti-inflammatory plant flavonoids and cellular action mechanisms. J Pharmacol Sci 96: 229-245.

27. Tabrez S, Priyadarshini M, Urooj M, Shakil S, Ashraf GM et al. (2013) Cancer chemoprevention by polyphenols and their potential application as nanomedicine. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 31: 67-98.

28. Fenercioglu AK, Saler T, Genc E, Sabuncu H, Altuntas Y (2010) The effects of polyphenol-containing antioxidants on oxidative stress and lipid peroxidation in Type 2 diabetes mellitus without complications. J Endocrin Invest 33: 118-124.

29. Santos RMM, Lima DRA (2016) Coffee consumption, obesity and type 2 diabetes: A mini-review. Eur J Nutr 55: 1345-1358.

30. Ishida N, Iizuka M, Kataoka K, Okazaki M, Shiraishi K, et al. (2018) Improvement of blood lipid profiles by Goishi tea polyphenols in a randomised, double-blind, placebo-controlled clinical study. Int J Food Sci Nutr 69: 598-607.

31. Jumar A, Schmieder RE (2016) Cocoa flavanol cardiovascular effects beyond blood pressure reduction. J Clin Hypertens (Greenwich) 18: 352-358.

32. Pang J, Zhang Z, Zheng TZ, Bassig BA, Mao C, et al. (2016) Green tea consumption and risk of cardiovascular and ischemic related diseases: A meta-analysis. Int J Cardiol 202: 967-974.

33. Lindschinger M, Tatzber F, Schimetta W, Schmid I, Lindschinger B, et al. (2020) Bioavailability of natural versus synthetic B vitamins and their effects on metabolic processes. MMW Fortschr Med 162: 17-27.

34. Huang D, Ou B, Prior RL (2005) The chemistry behind antioxidant capacity assays. J Agric Food Chem 53: 1841-1856.

35. Prior RL, Wu X, Schaich K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 53: 4290-4302.

36. Blom HJ, Smulders Y (2011) Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. J Inherit Metab Dis 34: 75-81.

37. Ford TC, Downey LA, Simpson T, McPhee G, Oliver C (2018) The effect of a high-dose vitamin B multivitamin supplement on the relationship between brain metabolism and blood biomarkers of oxidative stress: a randomized control trial. Nutrients 10: 1860.

38. Matsumoto AK, Michelin AP, Semeão L de O, Sepúlveda-Loyola W, Pedrão JV de L, et al. (2021) A Randomized Trial of Short-term

Treatment with Folic Acid to Reduce the Oxidative Stress of Patients with Chronic Kidney Disease. Curr Drug Metab 22: 1139-1150.

39. Spinneker A, Sola R, Lemmen V, Castillo MJ, Pietrzik K, et al. (2007) Vitamin B6 status, deficiency and its consequences-an overview. Nutr Hosp 22: 7-24.

40. van de Lagemaat EE, de Groot LC, van den Heuvel EGHM (2019) Vitamin B_{12} in relation to oxidative stress: a systematic review. Nutrients 11: 482.

41. Scholey A, Bauer I, Neale C, Savage K, Camfield D, et al. (2013) Acute effects of different multivitamin mineral preparations with and without guaraná on mood, cognitive performance and functional brain activation. Nutrients 5: 3589-3604.

42. Schoenthaler SJ, Bier ID, Young K, Nichols D, Jansenns S (2000) The effect of vitamin-mineral supplementation on the intelligence of American schoolchildren: a randomized, double-blind placebo-controlled trial. J Altern Complement Med 6: 19-29.

43. Chawla J, Kvarnberg D (2014) Hydrosoluble vitamins. Handb Clin Neurol 120: 891-914.

44. Asensi-Fabado MA, Munné-Bosch S (2010) Vitamins in plants: occurrence, biosynthesis and antioxidant function. Trends Plant Sci 15: 582-592.

45. Conaway E (2011) Bioidentical hormones: an evidence-based review for primary care providers. J Am Osteopath Assoc 111: 153-164.

46. Bohn L, Meyer AS, Rasmussen S (2008) Phytate: impact on environment and human nutrition. A challenge for molecular breeding. J Zhejiang Univ Sci B 9: 165-191.

47. Hotz C, Gibson RS (2007) Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. J Nutr 137: 1097-1100.