Essential micronutrients to improve nutritional supply levels in healthy volunteers

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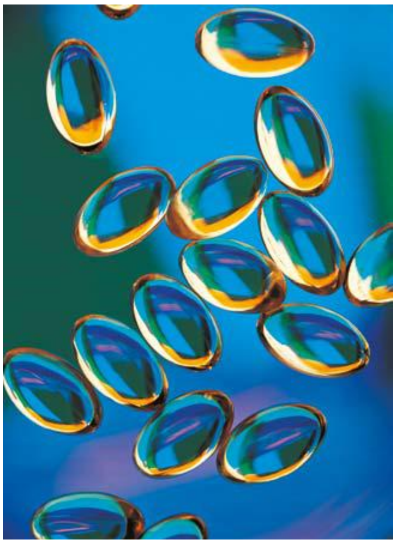
Abstract

The benefit of micronutrient supplementation is the subject of controversial debate in the scientific community. However, there are indications that various diseases are promoted under conditions with small micronutrient deficiencies.
A randomised, placebo-controlled, double-blind study was carried out over a period of 3 months in order to examine the effect of a nutritional supplement comprising a wide range of coordinated micronutrients in 48 healthy volunteers. The aim was to receive information about both the extracellular and the intracellular nutritional status. The results show that this kind of supplementation supports the nutritional supply even in healthy individuals and can therefore positively affect individual metabolism parameters.

Key words

Micronutrient supplement; micronutrient supply; antioxidants; homocysteine; omega-3 index

Balanced and well-rounded nutrition is the prerequisite for meeting daily nutrient requirements and thus to preserve health and performance. In addition to nutrient intake, endogenous factors, such as individual metabolism, genetics, lifestyle as well as various other environmental factors also affect the nutritional status [1,2].
The standard procedure to determine nutritional supply is the analysis of different micronutrients in blood serum or plasma. The results, however, represent only a snapshot of a system that is at once very dynamic and at the same time strongly regulated in important processes. Intracellular determination of important micronutrients may provide an additional new insight into nutrient supply. Mouth mucosa membrane cells (buccal mucosa cells) and erythrocytes are discussed in the literature as compartments suitable for such examinations [3,4].
The goal of the pilot study presented here was to examine the effect of a regular, long-term, extensive nutritional supplement with micronutrients at physiological dosage in healthy volunteers. The effects were detected by a selection of biomarkers characterising both micronutrients exogenously supplied by the nutritional supplement and their bioavailability and metabolism intermediate products or sum parameters.



Nutritional supplement study: Can selected micronutrients be measurably introduced to the physiological network?

Methods

Study groups and study design

24 female and 24 male healthy volunteers participated in this monocentric, randomised placebo-controlled, double-blind study in parallel design – 46 completed the study (Tab. 1). The study was reviewed and positively advised by the Stuttgart Ethics Committee. The consent of the volunteers was obtained prior to study inclusion.
After study inclusion, volunteers were randomly assigned to one of the two groups “Verum” and “Placebo” at a ratio of 3:1. Selected parameters of the intra- and the extracellular system were analysed at the beginning, after 4 and after 12 weeks.

Tab. 1: Demographic data of the study groups.

	Placebo group	Verum group
Number of volunteers	11 (6 women, 5 men)	35 (17 women, 18 men)
Age [years]	43.9 ± 2.6	41.7 ± 1.2
BMI [kg/m²]	26.5 ± 0.9	24.0 ± 0.6

Study preparations

The "Verum" was an extensive, coordinated supplementation containing physiological concen-trations of vitamins, minerals, trace elements, dietary fibres and other bioactive nutrients including the compounds listed in Tab. 2 amongst others. The combination was taken dissolved in water daily, mornings and evenings, over a period of 12 weeks. This daily dose fall far short of the "Tolerable Upper Intake Level" (the quantity of micronutrients which can be taken up over a long period by a healthy human without any concerns) of the Scientific Committee on Food of the European Commission. The volunteers assigned to the placebo group received preparations without active compounds.

Sample material

Sampling was carried out in the morning on an empty stomach after standardised dinner. Plasma and serum was obtained from blood samples and erythrocytes were isolated. Furthermore, mouth mucosa cells were brushed off the inner cheek. These samples were used to determine parameters on antioxidative status as well as on folic acid, homocysteine and omega-3 fatty acids.

Statistics

The study results were evaluated using Graph Pad Prism Version 3.0. In order to detect changes within the study groups, analysis of variance using repeated measure-ments (ANOVA) was carried out. Other testing methods included the t-test to examine the differences between the study groups after 3 months. A significance level of p=0.05 was used. Data are given as mean ± standard error (SEM).

Results

Antioxidative capacity and oxidative stress

The concentration of antioxidants (vitamin C and vitamin E) in the buccal mucosa cells of both groups was comparable prior to the beginning of supplementation. The vitamin C concentration increased in both groups over the course of the study, but there was no statistically significant difference between the groups. In contrast, the vitamin E concentration increased significantly by 24% (p<0.001) in the verum group (Fig. 1). On average, the concentration in the placebo group decreased from 24.6 to 21.3 pmol/µg DNA. Thus, the results show that both groups differ significantly (p<0.01) from each other.

The results of the analyses of the oxidative stress (TOS) and antioxidative capacity (TAS) sum parameters in plasma show that the determination of total oxidative stress could be reduced significantly (Δ TOS = -101 µmol/l, p<0.001) after taking the micronutrient combination. In contrast, the changes in the placebo group were not statistically significant. Corresponding to this, the oxidative stress increased in the verum group but decreased in the placebo group (Fig. 2).

Folate and homocysteine

At the beginning of the examination, the folate concentration in serum was 7.7 ± 0.6 ng/ml in the verum group and 9.0 ± 1.3 ng/ml in the placebo group. After 3 months of supplementation, a significant

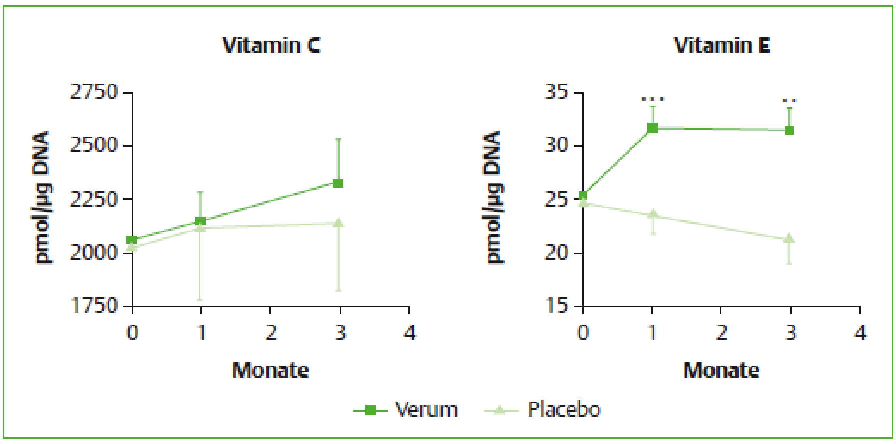


Fig. 1: Vitamin C and vitamin E concentrations in mouth mucosa cells. Mean ± standard error; *** p<0.001. ** p<0.01.

Tab. 2: Selection of essential micronutrients from the study preparations

Mikronährstoffe	Tagesdosis
Vitamin C	150 mg
Vitamin E (Tocopherol-Äquivalent)	106 mg
Vitamin B_6	3 mg
Folsäure	300 µg
Vitamin B_{12}	1,5 µg
Omega-3-Fettsäuren	268 mg

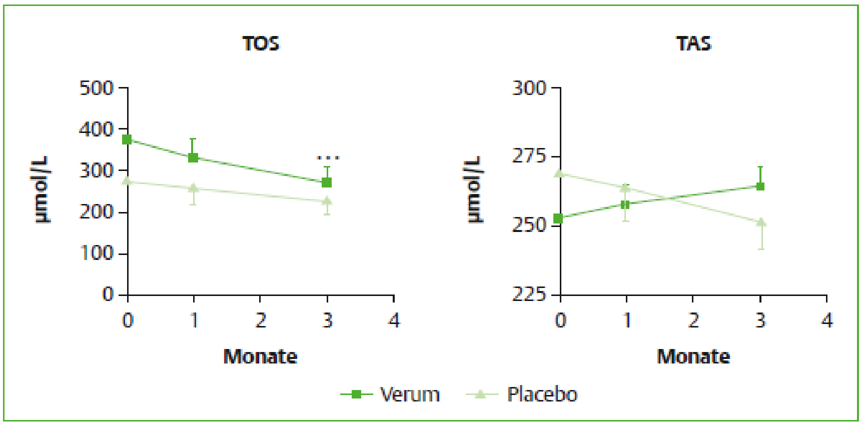


Fig. 2: TOS and TAS concentrations in plasma. Mean ± standard error; *** p<0.001.

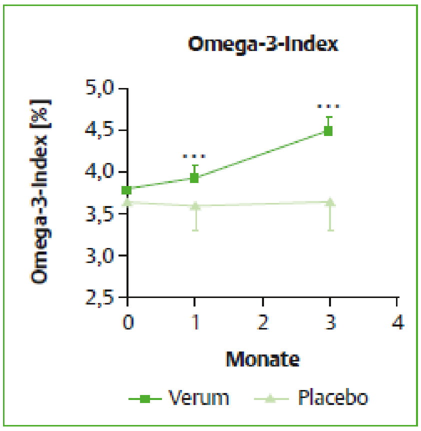


Fig. 3: Omega-3 index [%]. Mean ± standard error; *** p<0.001.

average increase in concentration by 4.3 ng/ml ($p<0.001$) could be observed. At the same time, the folate concentration in the placebo group decreased by 1.7 ng/ml. The differences between the groups in the changes of the nutritional supply are statistically significant ($p<0.001$).

The homocysteine concentration decreased by an average of 0.9 µmol/l from 8.7 ± 0.3 µmol/l to 7.8 ± 0.3 µmol/l ($p<0.001$) in the verum group, whereas the homocysteine level in the placebo group increased slightly from 9.1 ± 0.8 µmol/l to 9.4 ± 0.9 µmol/l.

Omega-3 fatty acids

The omega-3 index increased from 3.8% to 4.5% ($p<0.001$) in the verum group and remained stable at 3.6% in the placebo group. The change of the omega-3 index differs significantly in a comparison between groups ($p<0.0001$) (Fig. 3).

Discussion

A balanced diet, possible in western countries because of the variety of available food, can normally provide all the nutrients needed to ensure optimal health and performance. Several nutrition surveys, however, have shown that especially the intake of dietary fibre, folate, calcium, vitamin D and iron is insufficient in the European population [5-7]. This study assessed the nutrient intake in 48 healthy volunteers using a three-day nutrient protocol at the beginning and at the end of the study. The results confirmed the nutrition surveys, in which the study groups did not reach the recommended intake levels of the DGE, especially for dietary fibres, folic acid, and vitamin D but also for vitamin E.

The bioavailability of micronutrients depends to a large extent on dose, formulation and mutual interactions of the individual nutrients. In the study presented here, the benefit of intake of a broad spectrum of different micronutrients was analysed for selected parameters in a healthy collective over a period of 3 months. The effect was examined both by intracellular analysis of buccal mucosa cells and erythrocytes and extracellular analysis blood plasma and serum. The volunteers were encouraged not to change their nutritional habits during the course of the study. Compliance was ascertained by a nutrition log. Because minor physiological fluctuations are expected, the placebo group as the control was chosen to be smaller.

Why intracellular measurements?

Data on intracellular concentration provide information about the actual local nutrient supply. Furthermore, in contrast to concentration levels in blood, this concentration is subjected to less short-term fluctuation, for instance by recent food intake. Absorption of micronutrients by buccal mucosa cells was already shown by Peng et al. [8]. However, to our knowledge determination of vitamin C in buccal mucosa cells has not yet been described in literature. Due to water solubility and limited intracellular vitamin C storage capacity, cells may only reflect the absorption in a limited way. A tendency for increased vitamin C concentration was observed in the verum group.

In contrast, vitamin E is a fat-soluble substance which accu-mulates in cell membranes. In this study, a significant increase in the intracellular vitamin E concentration (over the course of time and between the verum and placebo groups) could be shown.

Supplementation with antioxidants is described in blood by the sum parameters oxidative stress (reduction of TOS) and anti-oxidative status (increase of TAS). The measuring principle is based on the detection of lipid peroxides. A reduced antioxidative status or an increased concentration of free radicals can cause oxidative stress, which in turn can lead to damage to proteins, lipids or DNA [9]. It is suspected that a surplus of free oxygen species is involved in the aetiology of various diseases such as cancer, neurodegenerative and cardiovascular disorders as well as age-related macular degeneration and influences the general aging process [9-14].

Folic acid is a vitamin with widespread deficiency in all age groups [15]. New data indicate that folic acid as well as vitamin B_{12} and riboflavin can have a protective effect against several diseases such as cardiovascular disorders and cognitive impairments [19, 20]. Thus, folic acid was chosen as the analysis parameter from among the B vitamins. A significant improvement could, as expected, be achieved by supplementation.

In order to further describe the metabolic effects of the B vitamins, homocysteine concentration was recorded as an intermediate product of methionine metabolism. Vitamins B_6, B_{12} and folic acid are essential for homocysteine metabolisation [16, 17]. It is undisputed that supplementation of the vitamins B_6, B_{12} and folic acid can decrease homocysteine levels [18]. These data could be confirmed within our study, in which homocysteine level decreased by an average of 10% in the verum group.

New biomarker omega-3 index

The omega-3 index (see Info) is being discussed as a new biomarker for the determination of omega-3 fatty acid intake in current publications [21]. Furthermore, this marker serves as a risk factor for cardiovascular diseases [21, 22]. In the present study, the omega-3 index in most volunteers was below 4% at the beginning; according to Harris and von Schacky, this value is associated with increased coronary risk [21]. In relation to the baseline value, the omega-3 index increased significantly by 18% ($p<0.001$) in the verum group. In contrast, no increase could be observed in the placebo group.

Conclusions

Overall, all parameters included in the analysis showed that a measurable intake of micronutrients supplemented in physiological doses occurs even in healthy volunteers at both the extra- and intracellular level. The present study proves that an overall strengthening of the metabolic network is possible. Particularly worthy of mention is that both the intracellular and the extracellular supply of nutrients interact like a network. Thus, this kind of supplementation as nutritional supplement can optimise the supply situation.

Omega-3 index

The Omega-3 index is the percentage of the long-chain omega-3 fatty acids EPA and DHA in the total amount of fatty acids in red blood cells. It represents the EPA and DHA status of a person in the same way that HbA1c reflects the glucose metabolism of diabetics and indicates the risk for sudden cardiac death. The parameter is attributed to William S. Harris, University of South Dakota, USA and Prof. Dr. Clemens von Schacky, Ludwig Maximilians-University Munich. They propose the following risk areas: high risk< 4 %; medium risk 4–8 %; low risk > 8 % (21).

When is one well supplied? In a Spanish study, an average daily intake of 0.9 g EPA + DHA resulted in an omega-3 index of 7.1 % [23]. This cannot always be achieved with the 2 recommended fish meals per week.

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Further literature is available in online version www.thieme-connect.de/ejournals.

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