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ORIGINAL ARTICLE

Mood improving Potential of a Vitamin Trace Element Composition – A randomized, double blind, placebo controlled clinical study with healthy volunteers

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Key words: **Multivitamin trace element composition; Serotonin metabolism; Kynurenine; Tryptophan; Vitamin B3 (Niacin); B6 (Pyridoxin); Vitamin D; Chromium; Zinc; Copper**

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Abstract

OBJECTIVES: Neurotransmitters regulate mood, attention vigilance and other clinical symptoms linked with depression. Various medications ameliorate symptoms of depression and mood disorders by interference with the serotonic metabolism. Serotonin metabolism depends on nutritional cofactors such as pyridoxin together with essential mineral and trace elements. Both, inflammation and metabolic conditions seem to affect the bioavailability of serotonin crucially. We hypothesized that serotonin supply depends on relevant gastrointestinal precursor absorption and on the availability of nutritive antiinflammatory cofactors.

METHODS: We performed a randomized placebo controlled clinical trial in healthy participants to study the bioavailabilty of ingredients of the multivitamin and trace element LaVita® in a prospective randomized placebo controlled double blind trial to establish the mood ameliorating potential. Serotonin and its precursor tryptophan were measured in dry blood samples. Serum parameters like chromium and zinc, as well as vitamin D, vitamin B3 and B6 were determined before intake, and after 3 months and 6 months consumption of the test substances.

RESULTS: After 3 months a slight increase of tryptophan (p=0.059) and a significant increase of serum serotonin (p<0.013) was observed in the verum group. After 6 months the verum group showed a highly significant mean increase in niacin (p<0.001) and the cofactors of serotonin metabolism pyridoxin (p=0.03), chromium p<0.01), and zinc (p<0.001). Serotonin levels droped after 6 months indicating a low risk for overdosing.

CONCLUSION: We conclude that a continuous supply with ingredients from the natural source compound LaVita® may contribute to mood improving neurotransmitter activity.

INTRODUCTION

Depression and anxiety are frequently seen in a doctors office. The various clinical phases and the rate of depression are associated with the expression and metabolism of neurotransmitters like serotonin. The proteinogenic aminoacid tryptophan serves as a precursor for the synthesis of serotonin as well as for kynurenine (Sainio *et al.* 1996). Depressive states are typically associated with disturbed pathways of serotonin, in particular in the tryptophan metabolic pathway. A meta-analysis including 24 studies from the literature involving a total of 744 patients and 793 controls provided convincing evidence for reduced plasma tryptophan (Trp) levels in patients with depression particularly if unmedicated (Ogawa *et al.* 2014).

SEROTONIN

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter primarily synthesized from tryptophan, found in the gastrointestinal tract (GI tract), blood platelets, and the central nervous system. Approximately 90% of the human body's total serotonin occurs in the enterochromafin cells of the GI tract, where it regulates peristaltic movements (Berger *et al.* 2009). Serotonin secreted from the enterochromaffin cells eventually finds its way out of tissues into the blood. Tryptophan and its metabolite 5-hydroxytryptophan (5-HTP), crosses the blood-brain barrier. If supplemented, it was hypothesized that both substances are effective serotonergic agents (Schaechter & Wurtman 1990).

Most clinically effective antidepressants increase the extracellular levels of 5-HT. Thus, it has been hypothesized that antidepressant responses result from the reversal of endogenous 5-HT deficiency. Consequently, psychopathological or biological markers that predict the specific increase of 5-HT neurotransmission, are potential substances for the treatment or prevention of mood disorders (Young 2007; Homan *et al.* 2015).

Brain serotonin levels were corresponding with the precursor molecule Trp (Mohajeri *et al.* 2015). Repeated administration of Trp increased agreeable behavior and reduced quarrelsome behavior in irritable people, who were also considered to be at risk for depression (Hogenelst *et al.* 2015). Correspondingly, lowering Trp levels triggered a drop in brain serotonin (Bell *et al.* 2001). Disturbances of the Trp metabolism are a major etiological factor of depression (Lapin & Oxenkrug 1969).

There is circumferential evidence that major depression is also associated with mild pro-inflammatory state. Both physiological and psychological stress can induce increased production of pro-inflammatory mediators, reactive oxygen species (ROS) and hypothalamo–hypophyseal–adrenal axis disturbances. While both pro-inflammatory mediators and ROS could activate the tryptophan breakdown and kynurenine pathway with a shift toward the neurotoxic arm, chronic hypercortisolism could also enhance tryptophan breakdown and induce neurodegenerative changes. The imbalanced kynurenine metabolism in terms of neuroprotective and neurotoxic effects was demonstrated in major depression, and in drug-induced neuropsychiatric side effects, such as interferon-treated depression. The changes in periphery have an impact on central changes. While some of the currently available antidepressants could reverse the pro-inflammatory state of the depressed patients, these medications could not efficiently improve those metabolic and neurochemical changes within the period that could induce clinical improvement (Myint *et al.* 2012).

Serotonin degration is predominantly controlled by activation of two enzymes [Tryptophan 2,3-dioxygenase (TPO) and Indolamine 2,3-dioxygenase (IDO)], both act as the rate-limiting step for the kynurenine metabolic pathway (Capuron *et al.* 2011).

TPO is located primarily in the liver, and is induced by tryptophan and metabolic steroids, with high specificity for it´s substrate. IDO is induced with less substrate specificity by pro-inflammatory cytokine expression (IFN) from various immune response and inflammatory stimuli (Yoshida *et al.* 1981; Ozkan *et al.* 2014).

KYNURENINE

It is established that proinflammatory cytokines released by activated T-cells and other leucocytes induce reactive oxygen and nitrogen species production in macrophages and neutrophils causing oxidative stress (Nathan *et al.* 1983; Wichers *et al.* 2005). This may contribute to an increase of kynurenine and respectively a decrease of 5-HT production due to altered metabolic pathways of tryptophan (Rubin 1967; Forrest *et al.* 2004; Anderson & Maes 2015).

The redox-sensitive and IDO-mediated tryptophan metabolism underpins the role of nutrition in mood quality and depression. Apparently neurotransmitter supply is closely linked to sufficient antioxidative nutrient supply (Himmerich & Erbguth 2014; Gostner *et al.* 2015).

This hypothesis was supported by clinical and experimental data refering to age-associated cognitive impairment and dementia caused by vitamin deficiencies (Seppala *et al.* 2014). In accordance patients with depression had significantly lower levels of antioxidant vitamins in comparison to healthy controls in another study. After dietary supplementation of vitamins for a period of 6 weeks, a significant reduction in anxiety and depression scores was observed (Gautam *et al.* 2012)

VITAMIN B_3, NIACIN

Trp is a precursor of Vit B3 (Niacin) through the kynurenine/quinolinic acid pathway. Studies in adults have shown that only an average of 3.3% of administered Trp converts to niacin compounds (Sainio *et al.* 1996). On a molar basis 1 mg of niacin requires approximately 60 mg dietary Trp supply. In case of niacin deficiency tryptophan is the preferred source for the synthesis of niacin, compared to serotonin. Accordingly diets low in niacin lead to decreased plasma tryptophan levels, and reduce the NAD+ concentration in erythrocytes (Fu *et al.* 1989).Niacin deficiency can lead to depression, insomnia and anxiety and if left untreated, niacin deficiency can cause dementia and pellagra, a serious disease that resembles schizophrenia. Niacin has therefore been described as an antidepressant agent (Prakash *et al.* 2008).

VITAMIN B_6, PYRIDOXIN

Tryptophan-hydroxylase is the rate-determining enzym of serotonin metabolism. Under physiological conditions this enzyme is only half saturated (Young & Gautier 1981). High substrate availability (tryp) stimulates the activity of this enzym (Heuther *et al.* 1992) which contributes to an increased 5-hydroxytryptophan (5-HTP) formation along the blood brain barrier. The clinical oberservation of low vit B6 levels in depressive patients has been atttributed to its coenzym function of Tryptophan-hydroxylase (Hvas *et al.* 2004).

VITAMIN D

Also vitamin D (Vit D) seems to have an impact on Tryptophan hydroxylase 2 (TPH2) expression in the brain by stimmulating gene transcription (Patrick & Ames 2014; Patrick & Ames 2015). Administration of 1,25-dihydroxyvitamin D 50 ng/kg/day or 100 ng/kg/day over 6 weeks enhanced Vit D receptor protein level in the brain of rats without affecting serum calcium and phosphate status. Calcitriol treatment promoted TPH2 expression without changing serotonin status however with increased serotonin metabolites (Jiang *et al.* 2014). Similar results were produced in human TPH2 experiments implying that Vit D affects human brain serotonin concentrations, which may be relevant for psychiatric disorders (Kaneko *et al.* 2015).

CHROMIUM

The discretly controlled serotonin metabolism requires specific trace elements like chromium, which directly enhances tryptophan metabolism and serotonergic activity (Attenburrow *et al.* 2002). It promotes and increases tryptophan transport across the blood brain barrier into the brain, thereby altering the 5-HT synthesis fernstrom (Fernstrom & Wurtman 1971) and improving anxiety and depression (Mlyniec *et al.* 2014a). In a small double-blind crossover study the supplementation of chromium was superior to placebo in ameliorating mood-symptoms (Brownley *et al.* 2013a). In the treatment of binge eating disorder the findings suggest a dose response with larger effects of chromium in the high dose compared to moderate dose group (Brownley *et al.* 2013b). In patients with refractory mood disorders chromium ameliorated symptoms and functioning, while side-effects were rare and mild (McLeod & Golden 2000). In a double-blind, randomized placebo controlled multicenter study, 113 adult outpatients with atypical depression the Chromium group showed significant improvements compared with the placebo group (Docherty *et al.* 2005). In a small placebo-controlled, double-blind, pilot study in 15 patients with major depressive disorder (atypical type) patients received 600 microgramm for 8 weeks and the majority of patients (N=12) responded to the chromium therapy (Davidson *et al.* 2003).

ZINC AND COPPER

Zinc also is a key element of many proteins and a limiting co-factor of many enzymes involved in brain function and serotonin mediated antidepressiv effects. A zinc deficient diet for three or six weeks caused depressive and anxiety-like behaviors in laboratory animals (Tassabehji *et al.* 2008). The symptoms induced by a zinc deficient diet could be reversed by typical antidepressants (Mlyniec *et al.* 2013). Citalopram – a classical antidepressing, pharmacological agent – significantly increased the zinc level in blood serum (Nowak *et al.* 2004) and repeated administration of zinc increased a pool of synaptic zinc in the hippocampus (Szewczyk *et al.* 2006). A lower serum zinc concentration accompanied ante-partum and post-partum depressive symptoms (Wojcik *et al.* 2006). Zinc supplementation significantly reduced depressive scores after 6 and 12 weeks of treatment when compared to placebo (Nowak *et al.* 2003). Zinc is among the effective antidepressing agents that can be combined with classical antidepressant pharmacologic substances and reduce unwanted side-effects (Mlyniec *et al.* 2014a). Supplementation of zinc over a period of six months increased the serum-zinc concentration and decreased symptoms of depression and anxiety in school age children (DiGirolamo *et al.* 2010). Evidence from several areas of neuroscience has led to the notion that copper and zinc modulate neuronal excitability (Aedo *et al.* 2007). Elevated copper and depressed zinc have been associated with hyperactivity, attention deficit disorders, behavior disorders, and depression. (Faber *et al.* 2009; Mlyniec *et al.* 2015). Antidepressive treatment had also an relevant impact on copper/zinc ratio of patients (Mlyniec *et al.* 2014b).

In view of this complex metabolic pathways of serotonin, we investigated the potential of a multivitamin and trace element composition in a healthy population

under randomised placebo controlled conditions. We focussed on mood limiting neurotransmitter metabolism by analysing serotonin/kynruenine ratio and tryptophan levels, as well as the bioavailability of key vitamines and trace elements contained in the test substance for depression and mood improvement.

MATERIAL AND METHODS

Study design

The study design was as described before (Muss *et al.* 2015a). Briefly, 159 healthy volunteers were recruited according to protocol by medical physicians. Volunteers with medical conditions and treatments which could possibly interfere with the endpoints were not admitted. The trial was a randomized, prospective, double-blind, placebo-controlled study, complying with GCP research guidelines (Good Clinical Practice). We determined blood parameters and subjective symtoms at 3 time points, at participation start (M0), after 3 months (M3), and after 6 months (M6). The test substances were verum (LaVita®) – a vitamin and trace element compound produced from fruits and vegetable fortified with minerals and trace elements. It contained secondary plant constituents, enzymes, amino acids, minerals, trace elements, vitamins, and semi-vitamins such as L-carnitine and coenzyme Q10, and omega-3 fatty acids. Verum and placebo, as well as the analyses of specific biochemical parameters were performed with standardized laboratory methods as described before (Muss *et al.* 2015b).

For each volunteer the biochemical data from three visits (M0, M3, and M6) were entered to a data base by two different teams independently. The entries were subtracted from each other. A result different from "0" visualized obvious transcription errors, which were corrected. The completeness of the data set was controlled vis a vis the monitors documentation. Missing laboratory protocolls were the subject of querries to complete the file. Then the data set was declared complete, and statistically analysed.

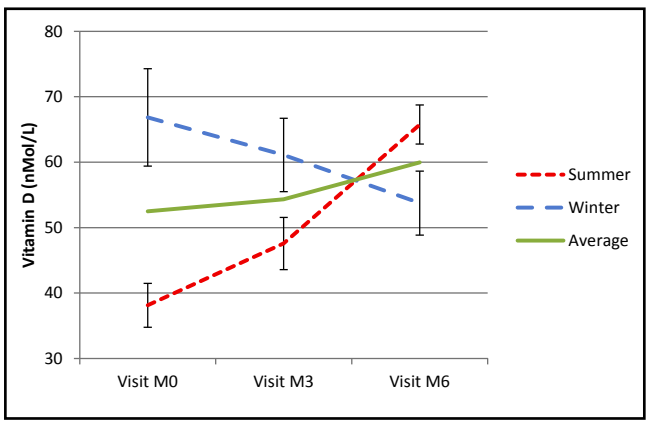

Fig. 3. Seasonal impact of Vit D supplementation. In the group from March to September (summergroup) the increase of serum Vit D was highly significant (-----). In the wintergroup (- - - -) from October to March the Vit D serum levels decreased despite supplementation. Although the average values increased slightly, the decline in the winter group (- - - -) indicates that the Vit D concentration in the trial substance, could and should be increased, at least during the winter season.

Parameters

The blood and serum parameters were determined by certified medical laboratories. We chose to investigate serum, tryptophan and kynurenine levels in dry blood technique which allows the specimen material to be analyzed in a fixed state (Wagner *et al.* 2014) or alternatively in urine. The result tables list two results for the same parameter if two analytical methods were involved.

When analysing Vit B3 the serum parameters of 2 participants were extremly high (up to 900 g/L), these outliers were not included into the statistical analyses.

The Vitamin D blood samples were analysed from 30 Participants who received verum. The first group (N=15) started the six month period in spring, and the second group (N=15) started in autum to take the substance during the winter months. When we analysed the groups separately we found remarkable seasonal effects (Figure 3).

Statistical analysis

The time for the intervention and laboratory sample collection spanned all 12 months of the year and all seasons. We used the softwarepackage IBM-SPSS (Version 22) to compare the data from the verum- and placebo-group, aswell the parameters at start (M0) after 3 months (M3) and 6 months (M6). The data for the timepoints M0, M3, and M6 were analysed for differences by the students T-test for independend groups, or for paired samples as appropriate. Furthermore we analysed the group's variances, setting the starting levels (M0) as covariable (ANCOVA). The parameter changes during the first and second period of three months and the changes over the complete participation time (six months) were computed per participant and group and reported in the result tables.

RESULTS

Participants

The randomization allocated 117 recruited participants to the verum group, of which 46 were male (mean age: 40±16.5 years ± Std); 71 were female (mean age: 44±14.1 years ± Std). In the placebo group 11 males were 48±15.4 years old; 32 female participants were 45±15.6 years old. The overal drop out rate was 10.7%, from the 159 starting participants 142 finished both terms of 3 months. The drop out rate after 6 months was 7.1% in the verum group and 16.3% in the placebo group. The participants provided feedback during the monitors telephone interviews, did not reveal any indications of adverse side effects, neither in the placebo,

nor verum group. No participant complained about allergic reactions.

Group comparison (Verum – Placebo)

At participation start (timepoint M0) the analysis of laboratory parameters did not reveal significant differences between the verum and placebo group. Specifically, we observed no significant differences in serum levels of tryptophan and serotonin (Table 3).

After three months the Vit B3 ANCOVA with the baseline variables as covariable revealed significantly higher serum levels in the verum group (Table 4). Serotonin excretion in urine had increased (Figure 2), but as for the other parameters the difference to placebo was not significant (Table 5).

Dynamics of parameters changes

To estimate the natural dynamics of some parameters we analysed the parameter changes in the placebo group over six months. None of the changes reached statistical significance (Table 6).

Table 7 lists the means and variability of parameters in the verum group. During the first three month term several parameters increased significantly to drop during the second three month term (Table 8). After six months nine out of 18 parameters remained significantly increased. Specifically the Vits B3 (niacin) and B6 (pyridoxine) raised significantly after 3 and 6 months (Table 8). The significant rise of kynurenine after 3 months (p=0.041) discontinued during the trial, effecting the tryptophan/kynurenine ratio to a significant drop (p=0.010) after six months. Blood serotonin showed a significant rise after 3 months (p=0.013) and dropped again during the second three month term (p=0.011).

Trace elements essential in the serotonin pathway raised significantly (Table 8). Zinc levels grew slowly (Figure 4), the increase was statistically significant during the second 3-month term (Table 8). Chromium increased significantly during the first three month

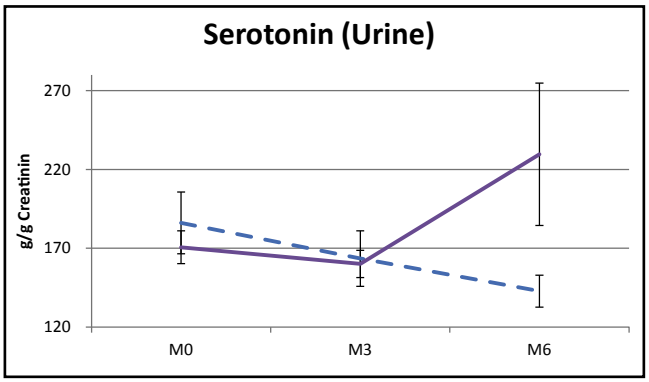

Fig. 2. Serotonin (Urine) at basline (M0), after three months (M3) and six months (M6), only after 6 months the excretion in the verum group is high. After 3 months reservoirs of serotonin and metabolites may be filled, before the renal excretion increases. verum (—), placebo (----).

Tab. 2. Laboratory parameters analysed in this study.

Parameter	Abbrev	Method of analysis	Uni	Range
Vitamin B_3	VIT B3	LCMS	g/l	8.0–52
Vitamin B_6	VITB6E	HPLC	ng/ml	4.1–43.7
Vitamin D_3	VITD25	ELISA	nmol/l	62.5–170
Kynurenine	KYNURDBS	ELISA	ng/ml	300–400
Tryptophan	TRYPDBSEX_DBS	ELISA	mg/dl	8–14
Tryptophan	TRYPTOAS	LCMS	mg/dl	1.2–1.8
Ratio Kyn/Trpt	TRYPTKYNURENQRECH	Computational	–	25–35
Serotonin (Blood)	SeroDBSEX		ng/ml	>140
Serotonin (Urine)	SeroQU		g/gCrea	140–230
Chrom (Blood)	CRHBEX	ICP - MS	g/l	<3.0
Zinc (Blood)	ZinkHB	ICP - MS	mg/l	7.30–7.70
Zinc (RBC)	ZinkHK	Computational	mg/l	7.30–7.70
Copper (Blood)	CUHB	ICP - MS	mg/l	1.10–1.20
Copper (RBC)	CUHK	ICP - MS	mg/l	1.10–1.20
Ratio Cu/Zn (RBC)	QCuZnHK	Computational	–	0.135–0.165

Blood – Whole blood, RBC - Red Blood Cells

Tab. 3. Homogeneity of groups, laboratory parameters at participation start (before the regular intake).

		Placebo			Verum			ANOVA
Visit 1 (M0)	**LabCode**	**Mean**	**SEM**	**N**	**Mean**	**SEM**	**N**	***p*-value**
Vit B3	B1vVITB3_200	11.78	1.83	39	9.41	0.92	111	0.212
Tryptophan (Urine)	B1vTRYPTOAS	0.98	0.03	40	0.95	0.02	75	0.526
Serotonin (Urine)	B1vSEROQU	186.04	19.59	36	170.58	10.41	80	0.449

Placebo and verum group were compared by the students T-Test for independent variables; no parameter differed significantly between the groups. Hence the randomization procedure for the participant assignment produced two fairly comparable groups.

Tab. 4. Group differences between verum and placebo after 3 months of regular intake, ANCOVA taking the values at intake start (M0) as covariables.

		Placebo			Verum			ANCOVA
Visit 2 (M3)	**LabCode**	**Mean**	**SEM**	**N**	**Mean**	**SEM**	**N**	***p*-value**
Vit B3	B2vVITB3_200	10.95	1.28	37	20.45	2.14	110	**0.004**
Tryptophan (Urine)	B2vTRYPTOAS	0.99	0.03	39	0.99	0.03	73	0.516
Serotonin (Urine)	B2vSEROQU	163.43	17.62	39	160.06	8.63	77	0.972

Tab. 5. Group differences between verum and placebo after 6 months regular intake; ANCOVA taking the values at intake start (M0) as covariables.

		Placebo			Verum			ANCOVA
Visit 3 (M6)		**Mean**	**SEM**	**N**	**Mean**	**SEM**	**N**	***p*-value**
Vit B3	B3vVITB3_200	17.49	3.43	35	17.24	1.96	109	0.777
Tryptophan (Urine)	B3vTRYPTOAS	1.02	0.04	35	0.97	0.02	77	0.314
Serotonin (Urine)	B3vSEROQU	142.76	10.16	32	229.62	45.15	74	0.218

Tab. 6. Parameter course in the placebo group. The laboratory parameter differences between participation start (visit 1, M0), after 3 months (visit 2, M3) or 6 months (visit 3, M6) were computed, and analysed for each term within the group by means of the paired student t-test.

Placebo	**From visit 1–2, Three month term 1**				**From visit 2–3, Three month term 2**				**From start to end, 6 months**			
Parameter	**meanDiff**	**SEM**	**N**	***p*-value**	**meanDiff**	**SEM**	**N**	***p*-value**	**meanDiff**	**SEM**	**N**	***p*-value**
Vitamin B_3	–1.22	1.84	36	0.513	5.44	3.05	34	0.084	4.91	3.12	35	0.125
Tryptophan	0.005	0.026	39	0.838	0.045	0.035	34	0.204	0.055	0.032	35	0.097
Serotonin	–20.18	15.93	35	0.214	–3.08	10.95	32	0.780	–30.42	15.51	29	0.060

In column *p*-value bold figures indicate statistically significant parameter changes ($p<0.05$) during the term. In the placbo group not a single parameter changed significantly.

term and remained high throughout the complete observation time (Figure 5).

In case of the Vit D analysis we observed a strong seasonal influence, therefore a subanalysis focusssed on seasonal effects of serum Vit D (Figure 3).

DISCUSSION

Because nutritients can contribute to a higher level of mental function (Davison & Kaplan 2012) our results underline the preventive potential of the studied multivitamin mineral and trace element composition. The regular intake had a detectable influence on serotonin metabolism, it altered the kynurenine serotonin rate in healthy subjects. Subjects can benefit from these bioavailable nutrients improving tryptophan levels, which serve as precursor for sufficient serotonin synthesis in the brain.

Serum Trp levels can distinguish moderate and severe depression from healthy control with good sensitivity and specificity (Liu *et al.* 2015). Humans do not possess the enzymatic machinery to synthesize it from simpler molecules. Dietary tryptophan not used for protein synthesis is degraded in the liver through a series of metabolic steps collectively known as the kynurenine pathway. In the nervous system and gut, tryptophan is a substrate for the synthesis of serotonin. In the

Tab. 7. Means and standard error of means (SEM) of determined verum parameters at baseline (visit 1, M0), after three months (visit 2, M3), and after 6 months (visit 3, M6).

Verum	at visit 1 M0			at visit 2 M3			at visit 3 M6		
Parameter	**Mean**	**SEM**	**N**	**Mean**	**SEM**	**N**	**Mean**	**SEM**	**N**
Vit B6	28.74	4.28	30	70.58	6.90	30	61.37	8.86	29
Vit D3	52.49	4.81	30	54.34	3.61	30	59.96	3.06	29
Kynureine	291.70	17.41	30	361.70	24.79	30	266.00	37.86	15
Tryptophan (Blood)	7.49	0.84	30	8.69	0.81	30	11.51	0.48	15
Ratio Trp/Kyn	47.72	3.77	30	59.91	7.91	30	22.72	2.58	15
Serotonin (Blood)	109.53	7.82	30	127.60	9.33	30	107.98	8.42	29
Selen (Blood)	93.97	4.95	30	108.80	7.65	30	100.24	6.26	29
Chromium (Blood)	0.60	0.09	30	0.93	0.07	30	0.94	0.06	29
Copper (Blood)	1.21	0.05	30	1.19	0.05	30	1.16	0.05	30
Copper (RBC)	1.21	0.05	30	1.19	0.05	30	1.17	0.05	30
Zinc (Blood)	6.43	0.17	30	6.60	0.18	30	7.06	0.19	29
Zinc (RBC)	6.34	0.15	30	6.46	0.16	30	6.82	0.15	29
Ratio Cu/Zn	0.19	0.01	30	0.19	0.01	30	0.18	0.01	29

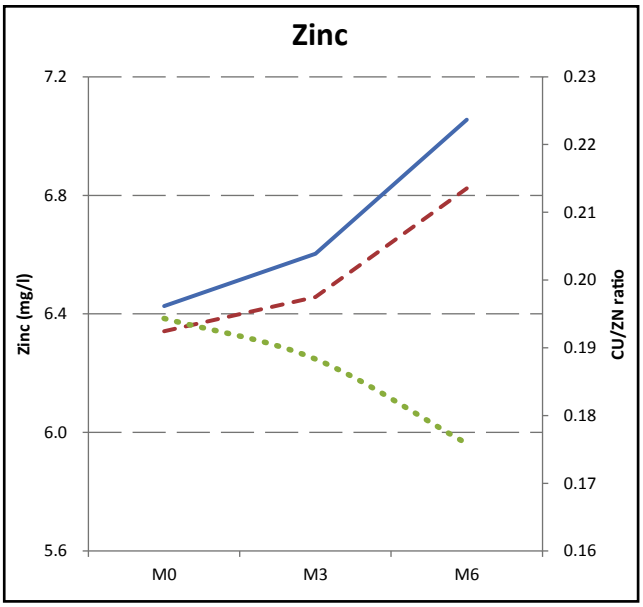

Fig. 4. Zinc increases in red blood cells (—) and whole blood (---), Copper/Zinc ratio (…) decreases.

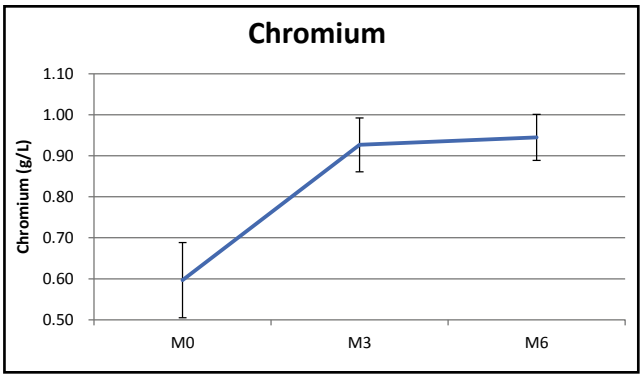

Fig. 5. Chromium increases during the first three month term and remains elevated throughout the complete intake phase.

pineal gland it is the key molecule for for the synthesis of melatonin (Figure 1).

Because of a change of laboratory analysis methods during the trial for tryptophan, serotonin, and kynureine not all laboratory parameters were convertible. However, tryptophan serum levels raised in the control group, but just failed to reach significance after 3 months. We attribute this change to the well discribed mechanism of trypophan metabolism. The metabolic balance of tryptophan is mainly under the control of kynurenine degration pathway.

Niacin is commonly considered a natural antidepressant, as it can reduce anxiety and depression (Prakash *et al.* 2008). In our trial the increase of niacin and the significant rise of kynurenine are in favor of our hypothesis that subjects of the verum group benefited from tryptophan supply and thereby were able to increase their niacin level significantly after 3 and 6 months.

From recent therapeutic observations, both the serotonin (5-HT) and kynurenine pathways of tryptophan metabolism may be of particular importance to improve our understanding of depression (Kim *et al.* 2015).

The best prove of efficacy was seen in the significant rise of blood serotonin levels after 3 months in our verum group. This tendency discontinued after 6 months which underpins the safety the serotonin supply. No overdosing tendency was discovered after a longer period of verum consumption.

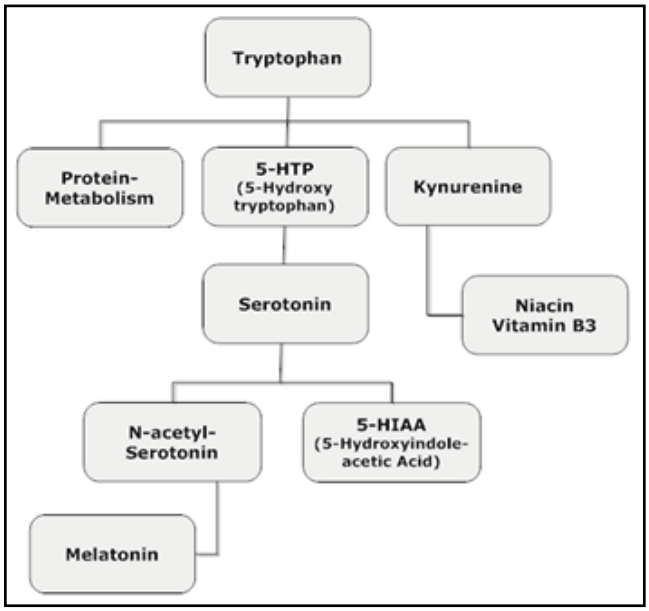

Fig. 1. Serotonin metabolic pathway.

Tab. 1. Ingredients, 10 ml is the recommended daily dose.

Ingredients	per 10 ml
ß Carotene	4000 µg
Vitamin B1	3 mg
Vitamin B2	2,5 mg
Viamine B3 (Niacine)	40 mg
Viamine B5	8 mg
Vitamin B6	4 mg
Vitamin B9 (Folic Acid)	400 µg
Vitamin B12	5 µg
Vitamin C	300 mg
Vitamin D	5 µg
Vitamin E	30 mg
Vitamin K	30 µg
Vitamin H (Biotin)	70 µg
Coenzym Q10 (Qu10)	5 mg
Chromium	15 µg
Copper	25 mg
Iodine	25 µg
Iron	4 mg
Magnesium	30 mg
Mangan	1 mg
Molybdenium	30 µg
Selenium	35 µg
Zinc	5 mg
L-carnitine	30 mg
Tryptophane	not determined
Omega 3 fatty acids	30 mg

Diets poor in Trp are well described to induce depression as this essential amino acid is not naturally abundant even in protein-rich foods. Trp-rich diet is important in patients susceptible to depression. CNS serotonin synthesis can therefore be controlled by proper intake of tryptophan-rich diet together with supportive cofactors such as pyridoxine (Shabbir *et al.* 2013). We anticipate this effect despite an unknown content of Trp as our verum group received 4 mg Vit B6 in 10ml LaVita (Table 1) over 6 months constantly and showed corresponding effects on their serotonin extinction in urine (Figure 2). Vit B6 levels raised significantly after 3 (p<0.001) and 6 months (p=0.03) respectively in comparison to output measurements in our verum group with an increasing impact on blood serotonin after 3 months (p=0.013).

Our main conclusion is also corroborated by the significant rise of trace elements relevant for the serotonin synthesis. Zinc serves as a limiting co-factor of enzymes involved in brain function plays a significant role in serotonin mediated antidepressiv effects (Tassabehji *et al.* 2008) and chromium promotes and increases tryptophan transport across the blood brain barrier into the brain, thereby altering the 5-HT synthesis (Fernstrom & Wurtman 1971) and therefore can be an effective supplement in the treatment of anxiety and depression (Mlyniec *et al.* 2014a).

Both, chromium and zinc showed a steady increase in blood levels whereas zinc/copper ratio dropped after 3 and 6 months of verum consumption in our trial. Others have described copper and zinc as modultators of neuronal excitability (Aedo *et al.* 2007) and high copper levels were associated with depression scores in other clinical observations (Faber *et al.* 2009; Chang *et al.* 2014; Mlyniec *et al.* 2015).

Also Vit D3 seems to have an impact on serotonin metabolism. However, in our trial due to the fact that our verum group received only 5 µg cholecalciferol (Vit D) per intake, we received only marginal non significant improvements of Vit D3 blood levels after 3 and 6 months of supplementation (p=0.637 and p=0.197). Obviously, the Vit D dose was here too low to increase blood levels significantly during the winter season (Figure 3). Aggregation of published data concerning Vit D revealed that almost 3000 IU per day (i.t. 75mg) is needed to achieve higher serum 25-hydroxyvitamin D (25(OH)) concentrations such as 50 nmol/L or more in 97.5% of healthy individuals. For normal weight, overweight and obese subjects the dosage was even proposed 75–175 mg Vit D respectively (Veugelers *et al.* 2015). In the light of these recommendations, the low dose Vit D concentration allows longterm supplementation. Our data reflect the seasonal influence on the Vit D metabolism. Since the recruitment of one

Tab. 8. Parameter changes (MeanDiff) in verum group. The laboratory parameter differences between participation start (visit 1, M0), after 3 months (visit 2, M3) or 6 months (visit 3, M6) were computed, and analysed for each term within the group by means of the paired student t-test.

	From visit 1–2, Three month term 1				From visit 2–3, Three month term 2				Start to End, 6 months			
	MeanDiff	**SEM**	**N**	***p*-value**	**MeanDiff**	**SEM**	**N**	***p*-value**	**MeanDiff**	**SEM**	**N**	***p*-value**
Vit B3	11.25	2.12	109	**0.000**	–3.56	2.83	108	0.210	8.50	2.11	108	**0.000**
Tryptophan (Urine)	0.06	0.03	67	0.059	–0.02	0.02	71	0.246	0.03	0.02	70	0.249
Serotonin (Urine)	–10.85	12.03	76	0.370	66.70	48.25	69	0.171	62.00	48.57	72	0.206
Vit B6	41.84	7.98	30	**0.000**	–10.93	8.84	29	0.226	32.18	9.92	29	**0.003**
Vit D3	1.85	3.87	30	0.637	4.99	3.24	29	0.134	7.51	5.69	29	0.197
Kynureine	70.00	32.70	30	**0.041**	–3.87	48.43	15	0.937	–67.40	41.35	15	0.125
Tryptophan (Blood)	1.20	0.64	30	0.072	–1.14	0.85	15	0.201	1.46	1.36	15	0.301
Ratio Trp/Kyn	12.19	7.48	30	0.114	1.40	3.19	15	0.667	–17.86	5.97	15	**0.010**
Serotonin (Blood)	18.07	6.83	30	**0.013**	–23.71	8.73	29	**0.011**	–4.84	8.09	29	0.554
Chromium (Blood)	0.33	0.14	30	**0.029**	0.03	0.08	29	0.707	0.33	0.12	29	**0.010**
Copper (Blood)	–0.02	0.02	30	0.399	–0.01	0.02	29	0.567	–0.03	0.02	29	0.148
Copper (RBC)	–0.02	0.02	30	0.496	–0.01	0.02	29	0.598	–0.03	0.02	29	0.229
Zinc (Blood)	0.18	0.11	30	0.113	0.48	0.07	29	**0.000**	0.63	0.12	29	**0.000**
Zinc (RBC)	0.12	0.09	30	0.187	0.41	0.06	29	**0.000**	0.49	0.09	29	**0.000**
Ratio Cu/Zn	–0.01	0.00	30	0.158	–0.01	0.00	29	**0.006**	–0.02	0.00	29	**0.000**

In column *p*-values bold figures indicate statistically significant parameter changes like ($p<0.05$) during the term.

group took place in March (summergroup) and one in October (wintergroup) Vit D levels of our verum group were under different seasonal impact (Figure 3). This explains why despite the average values increased slightly, we had a decline in the winter group. We conclude that Vit D supplementation should be increased at least during the winter season.

Some laboratory measurements did not reveal a linear increase of serum parameters corresponding to the bioavailable ingredients in our trial. This may be attributed to a redistribution of the substances to tissues storages where they are not measurable via blood samples (Muss *et al.* 2015a). Our hypothesis is supported by other reports about blood measurements not reflecting central nervous system vitamin function or severity of affective syndromes in other trials (Bell *et al.* 1991).

Over all our results are in line with other clinical oberservations proving the beneficial effect of bioavailable antioxidants and specific nutrients in the prevention of mental disorders. As such, a cross-sectional survey using 3-day food records in 97 adults with bipolar or major depressive disorder showed positive effects of combined food and supplement intake (Davison & Kaplan 2011).

CONCLUSION

Our findings indicate that the investigated multivitamin and trace element composition LaVita® has a certain potential to protect from subclinical neuroinflammation and contributes to the amelioration of mild depression improving mood quality in early clinical stages. As a conclusion, we recommend an adequate supply of minerals and vitamins (antioxidants) by a balanced diet and ingredients from a natural source to protect against and to improve mood disorders.

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