Neuroendocrinology Letters Volume 36 No. 1 2015
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

Neuroprotective impact of a vitamin trace element composition – a randomized, double blind, placebo controlled clinical trial with healthy volunteers

Claus Muss [1], Wilhelm Mosgoeller [2], Thomas Endler [3]

1 Internationale Gesellschaft für angewandte Präventionsmedizin I-GAP, Währingerstrasse 63, A-1090 Wien, Austria. Associated Professor Public Health Department St. Elisabeth University, Bratislava, Slovakia
2 Inst. f. Krebsforschung, KIM-1, Medizinische Universität A-1090 Wien, Austria
3 Labor Endler, Währingerstrasse 63, A-1090 Wien, Austria

Correspondence to: Assoc. Prof. Claus Muss, MD., PhD.
International Research Group for applied Preventive Medicine (I-GAP)
Währingerstrasse 63, A-1090 Wien.
TEL: +49 170 5343 167; E-MAIL: profmuss@gmail.com

Submitted: 2015-02-01 *Accepted: 2015-02-18* *Published online: 2015-00-00*

Key words: **Vitamin B1; Vitamin B6 (Pyridoxin); Vitamin B9 (Folic acid); Vitamin B12 (Cobalamin); Vitamin E (Tocopherol); Coenzym°Q10 (Ubiquinone); mitochondrial dysfunction; neurodegeneration; blood brain barrier; homocysteine; lipidperoxidation; superoxid dismutase activity**

Neuroendocrinol Lett 2015; **36**(1):101–110 **PMID:** ----- NEL360115AXX © 2015 Neuroendocrinology Letters • **www.nel.edu**

Abstract

OBJECTIVES: Neurotoxic metabolites and oxidative and nitrosative stress reactions play a crucial role in the pathways leading to neuronal cell death and neurodegeneration. The bioavailability of the many antioxidant ingredients a vitamin and trace element composition was investigated, to reveal the neuroprotective (preventive) potential of the composition.

METHODS: We recruited 159 healthy volunteers, assigned them randomly and double blind to a placebo and verum group. Physicians excluded volunteers with severe chronic diseases or interfeering medications. 142 participants finished the six month trial. Laboratory parameters were determined 1) before participation, and 2) after three and 3) six months. We confirmed the bioavailability of ingredients, and determined metabolic parameters associated with the integrity of the blood brain barrier, mitochondrial deficiency (Q 10), neurodegeneration (homocystein), and antioxidative capacity (e.g. lipidperoxidation), and superoxid-dismutase activity.

RESULTS: Starting from baseleine, after three months neuroprotective ingredients increased within their physiological borders, folic acid ($p<0.003$), pyridoxin ($p<0.001$), cobalamin ($p=0.001$), and the fat soluble vitamin tocopherol ($p<0.001$). In parallel, homocytein decreased after 3 and 6 months ($p<0.001$, and $p<0.025$, respectively). Other paramters like zinc reacted slower, significant changes were observed only after 6 months.

CONCLUSION: The observed metabolic changes and alteration of the oxidative status after 3 and six month of regular intake underlines the compositions' potential to ameliorate neurodegenerative processes. We conclude that the subsitution of vitamins and traceelements with natural source in a proper manner may be effective for neuroprotection in healthy population.

O R I G I N A L A R T I C L E

INTRODUCTION

The increase of life expectance in industrial countries is paralleled by increasing prevalence of various neurodegenerative diseases. To date, no causal therapy is known. Several pathomechanisms are debated.

Mitochondrial dysfunction in nerve cells is a commonly discussed aetiologic factor for many forms of neurodegeneration. Mitochondria play a pivotal role in cellular bioenergetics and cell-survival. Prolonged oxidative stress and the resultant hypoperfusion in the brain tissues stimulate the expression of nitric oxide synthase (NOS) enzymes. This further drives the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which collectively contributes to the dysfunction of the blood-brain barrier (Aliev *et al.* 2014).

In animal studies the activity of the mitochondrial enzyme activity of Mn-superoxide dismutase (SOD) plays an important role regulating oxidative stress in the mitochondria, resulting in high levels of lipid peroxidation, and low levels of ATPase and cytochrome C oxidase activity in the infected cerebral mitochondria (Choi *et al.* 1998). A positive impact of oxidative and nitrosative stress reduction and morphological findings in the cortex indicated that particularly at the onset of progressive neurodegeneration, compounds with antioxidative properties may be effective in slowing down brain injury (Gasparova *et al.* 2012; Gasparova *et al.* 2014).

In a rat model of paraquat-induced neurodegeneration, coenzym Q10 supplementation halted the progression of neurodegeneration (Muthukumaran *et al.* 2014). A neuroprotective effect was also observed after kainate injection when coenzym Q10 pretreatment significantly attenuated severity and incidence rate of the higher seizure severity during status epilepticus and spontaneous seizure phases (Baluchnejadmojarad & Roghani 2013). Again in a rat model the combination of resveratrol, omega-3 fatty acids, and coenzym Q10 ameliorated a cisplatin-induced peripheral neuropathy (Bhadri *et al.* 2013).

The role of cholesterol homeostasis in the neurodegenerative process has been debated (Anchisi *et al.* 2012). The levels of high-density lipoprotein (HDL) cholesterol were positively correlated with cognitive impairment of subjects and increased triglycerides associated with bilateral grey matter loss (Gonzalez-Escamilla *et al.* 2014).

Homocystein markedly increased the vulnerability of hippocampal neurons to excitotoxic and oxidative injury in cell culture and in vivo, suggesting a mechanism by which HCY may contribute to the pathogenesis of neurodegeneration (Maler *et al.* 2003): Under in vitro conditions D,L-HCY caused a time and dose-dependent gliotoxic effect (Kruman *et al.* 2000).

There is a still ongoing controversy about adaequate supply of antioxidants in the population by normal diet (Guallar *et al.* 2013; Bjelakovic *et al.* 2014; Caldwell *et al.* 2014). Antioxidant treatment has been suggested as remedy for Alzheimers disease (Gilgun-Sherki *et al.* 2003). Oxidative stress may deteriorate the blood brain barrier (Enciu *et al.* 2013). Some evidence suggests that dietary supplementation with folate and other homocystein lowering vitamins reduce the risk for neurodegeneration (Mattson *et al.* 2002). Folic acid deficiency and HCY weaken the DNA repair in neurons, sensitizing them to oxidative damage induced by neurotoxic proteins (Kruman *et al.* 2002). Accordingly, epidemiological studies show a positive, dose-dependent relationship between mild-to-moderate increases in plasma total HCY concentrations and the risk of neurodegenerative diseases (Herrmann & Obeid 2011).

The test substance LaVita® is a vitamin-trace-element-composition (ViteC). In view of the ingredients and the oxygenradical absorbance capacity we hypothesized whether or not the regular intake generates a neuroprotective potential to justify regular intake as preventive strategy.

MATERIAL AND METHODS

The trial was announced in regional newspapers. 159 healthy volunteers were recruited according to predefined inclusion and exclusion criteria by a medical physician.

We aimed to recruit healthy vounteers in a steady state life condition. The exclusion criteria eliminated participants with known risk factors for study bias. Volunteers acute disease and/or medical treatments which could interfere with our endpoints were not admitted. More specifically, we excluded persons with:

- age below 18 and over 90 years
- acute disease; hospitalization in the last 4 weeks
- recovering from surgery (surgery in the last 12 weeks)
- holiday or other larger travel (availability, change of living environment)
- diabetes or severe metabolic disease, fructose intolerance, to reduce interference with metabolic conditions
- drug or alcohol abuse to reduce risk of low compliance
- oncologic treatment in the last 3 months
- inflammatory bowel disease (e.g. Colits), signs of malabsorption
- disease with remissions and relapses (Arthritis, Multiple sclerosis, etc.) to exclude endpoint variance related to disease associated conditions
- dementia, diagnosed and medically treated neurodegeneration?
- receiving cortison treatment or any orther acute medical intervention (antibiotics) to exclude intereference and study bias due to medical condition treatment
- participation at another trial, to exclude interference with other trials.

Copyright © 2015 Neuroendocrinology Letters ISSN 0172–780X • www.nel.edu

Trial design

The trial was a randomized, prospective, double-blind, placebo-controlled study. The protocol was designed by a research-consulting firm (SCIgenia, Vienna, Austria, www.scigenia.com). It complies with international research standards like GCP (Good Clinical Practice).

Figure 1 displays the trial specific activities per participant. The endpoints were biochemical parameters, determined at 3 time points, at participation start (M0), after 3 months (M3), and after 6 months (M6).

After three months (timepoint M3) the volunteers were appointed to visit the study centre. Blood samples were drawn for biochemical analyses. The participants received the study substance for the remaining three months.

After six months (timepoint M6) the volunteers were appointed again to the study centre for the exit visit, and they provided their third blood sample for biochemical analyses.

To increase the participants' compliance and to detect possible organisational shortcommings, between the appointments the participants were contacted by phone and interviewed about their experiences with the study. The interview was mainly about organisational issues, like their schedule and appointments for trial specific laboratory visits. Any feedback, including reports on adverse reactions, was documented. Suggestions of the participants which could improve the trial were discussed within the study team.

Test substances

Verum

The verum test substance was the multivitamin-trace element composition LaVita® produced from fruits and vegetable fortified with minerals and trace elements (19 fruits, 11 vegetables, 26 herbs, 5 vegetable oils, 7 juices, partially fermented with lactic acid). The ViteC contained secondary plant constituents, enzymes, amino acids, minerals, trace elements, vitamins, and semi-vitamins such as L-carnitine and Coenzyme Q10 (ubichinone), and Omega-3 fatty acids (Table 1).

Placebo

The placebo was produced of apple juice concentrate (30%), orange juice concentrate (40%), red beet concentrate (10%); it contained 20% fructose and distilled water. It matched the verum in colour, taste and consistency. The daily dose was the same as for the verum. The participants were instructed as with the verum: to dilute 10 ml test substance in a glas of drinking water twice a day.

Endpoints

To determine the bioavailability of selected ViteC ingredients and and to monitor parameters associated with neurodegeneration, we determined serum levels, antioxidatitive, and metabolic effects at three time points (Figure 1).

Specific biochemical parameters

The parameter analyses were performed in the accredited medical laboratories Endler (Vienna, Austria) and Biovis (Limburg, Germany) with standard medical laboratory methods (Table 2).

Data acquisition and statistical analysis

For every volunteer the biochemical data from three visits (start, month 3, month 6 were transferrred to a data base by two different teams independently (double entry method). After the entries were subtracted from each other any result different from "0" pointed out obvious transcription errors, these were corrected. The completeness of the data base was controlled vis a vis the monitors' documentation. Missing laboratory protocolls were the subject of querries to complete the data base.

Tab. 1. Verum ingredients.

Ingredients	Conc.
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
Calcium	7 mg
Chromium	15 µg
Copper	25 mg
Iodine	25 µg
Iron	4 mg
Magnesium	30 mg
Mangan	1 mg
Molybdenium	30 µg
Potassium	65 mg
Selenium	35 µg
Zinc	5 mg
L-carnitine	30 mg
Tryptophane	not determined
Omega 3 fatty acidy	30 mg

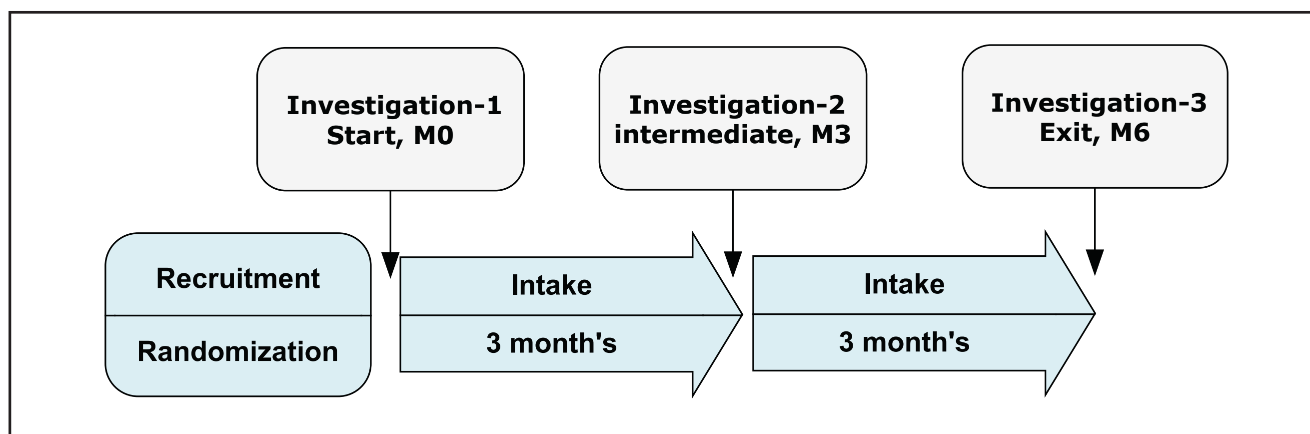

Fig. 1. Sequence of trial specific activities.

Tab. 2. Laboratory parameters used in this study; method of analyses, SI-units, and normal range.

Parameter	Abbrev. Lab. ID.	Method, analysis	Unit	Range M/F
Vitamin B1	VITB1EB	HPLC	µg/l	35–99
Vitamin B6	VITB6E	HPLC	ng/ml	4.1–43.7
Folsäure Speicherstatus	FOLEBMOD, AltFolsex	Competitive Chemoluminescence	ng/ml	280–800
Vitamin B12	ALT_VITB12CO	Electrochemiluminescence Immunoassay, ECLIA	pg/ml	193–982
Biotin	BIOTIN	ELISA	ng/l	>200
Vitamin E	VITE	ELISA	mg/l	5–20
Coenzym Q10	Q10	HPLC	mg/l	0.88–1.43
Cholesterol	CHOLB	Encymatic colour reaction	mg/dl	<200
HDL-Cholesterin	HDLB	Enzymatic colour reaction	mg/dl	M>55.F>65
Chol/HDL Ratio	CHOLQ	Computational	–	4–4.5
Homocystein	HOMO	Encyme recycling essay	µmol/l	M:8–12;F:6–10
Superoxid-Dismutase	SUPERDIS	ELISA	ng/ml	3.9–39.8
Lipidperoxidation	LIPIDPEROX	Photometry	mol/l	<200
Kynurin	KYNURDBS	ELISA	ng/ml	300–400
Tryptophan (Trp)	TRYPTOAS	LCMS	mg/dl	1.2–1.8
Tryptophan	TRYPDBSEX_DBS	ELISA	mg/dl	8–14
Kyn/Tryp-Quotient	TRYPTKYNURENQRECH	Computational	–	25–35
Nitrotyrosin	NITROYROSINEP	ELISA	nmol/l	<55

We used the softwarepackage IBM-SPSS (Version 22) to compare the data from the verum-, and placebo-group. The differences between groups at the time-points M0, M3, and M6 were analysed by means of the students T-test. The parameter changes during the first and second periode of three months and the changes over the complete participation time (six months) were computed for each participant and analysed with the paired students T-test.

In some cases (indicated in the text) – to remove the effects of different parameter levels at participation start which modify the relationship of the categorical independents to the interval dependent, i.e. to consider the rather large contrast between parameter variability at participation start and expectable effect size in a healthy population, we performed one-way analysis of covariance (ANCOVA), considering the baseline values (at start, timepoint M0) as covariable.

RESULTS

Participants

The age and gender distribution within the groups are given in table Table 3. The drop out rates per group after 3 or 6 month (midterm or participation end, respectively) are shown in Table 4.

 Copyright © 2015 Neuroendocrinology Letters ISSN 0172–780X • www.nel.edu

The participants feedback during the monitor telephone interviews did not reveal any occurence of adverse side effects, neither in the placebo, nor verum group. No participant complained about allergic or similar reactions.

Table 5 shows that before participation start (timepoint M0) and before the regular intake of the study substances, the randomisation after recruitment produced compareable groups. Specifically we observed no significant differences in serum levels of selected ingredients, HCY , or parameters of lipid metabolism, or oxidative status or any other parameters related to neuronal health.

Group comparison

After three months the comparison between placebo or verum intake revealed subtle differences between

Tab. 3. Age and group distribution of recruited volunteers.

	Placebo			Verum		
	Mean	Std	N	Mean	Std	N
M	48	15.4	11	40	16.5	46
F	45	15.6	32	44	14.1	7o

Tab. 4. Participants drop out rates in the gr.

	Placebo		Verum		Total
	Male	Female	Male	Female	N
B1, Start, M0	11	32	46	70	159
B2, Middle, M3	10	30	44	67	151
B3, End, M6	10	26	41	65	142
Drop out rate (%)	16.3		8.6%		10.7%

Tab. 5. Homogeneity of groups. Laboratory parameters at participation start (before the regular intake) of the Placebo and verum group were compared by the students T-Test revealed no parameter differed significantly between the groups. Hence the randomization procedure for the participant assignment produced two comparable groups.

Visit 1 (M0)	Placebo			Verum			
Start	Mean	SEM	N	Mean	SEM	N	*p*-value
Vitamin B12	532.14	50.23	40	445.34	18.36	112	0.111
Coenzym Q10	1.28	0.16	40	1.10	0.05	112	0.258
Cholesterol	203.56	6.29	43	202.16	3.73	116	0.847
Chol/HDL ratio	2.98	0.16	43	3.17	0.13	116	0.420
Homocystein (HCY)	12.42	0.63	43	11.62	0.32	116	0.221
a-Oxidant., FORD	1.27	0.07	25	1.25	0.06	54	0.773
a.-Ox.Cap., FORT	296.44	25.49	25	308.00	12.68	54	0.651
Lipidperoxidation	291.05	60.82	40	282.27	33.43	110	0.895
Nirotryrosine	26.02	5.08	40	29.57	3.51	106	0.586
SOD	3.68	0.18	40	3.90	0.38	112	0.737

Tab. 6. Group difference after 3 months regular intake (ANCOVA, based on pretreatment data), After verum intake Vit B12 and the anti-oxidative capacity (FORT) was significantly increased. Lipidperoxidation and Superoxid-Dismutase was lowered in the verum group. The lowering of Homocsytein in the verum group was highly significant.

Visit 2 (M3)	Placebo			Verum			
Pm	Mean	STD	N	Mean	STD	N	*p*-value
Vitamin B12	528.21	293.88	39	535.99	214.24	112	**0.001**
Coenzym Q10	1.53	1.10	39	1.51	0.82	112	0.365
Cholesterol	200.30	35.23	40	201.84	37.37	112	0.679
Chol/HDL ratio	3.10	1.01	40	3.23	1.30	112	0.888
Homocystein (HCY)	11.77	5.47	39	9.40	2.60	112	**0.001**
Antioxidants	1.35	0.49	25	1.24	0.41	53	0.284
Anti-Ox. Cap. FORT	328.76	136.29	25	289.79	89.37	53	**0.049**
Lipidperoxidation.	220.28	351.54	39	157.31	239.73	109	0.214
Nirotryrosine	34.29	45.48	40	36.96	38.76	105	0.894
SOD	5.24	7.35	40	3.81	1.51	112	**0.050**

the verum and control group. The metabolic parameter homocystein was significantly lower in the verum group. Table 6 lists the analyses for the ingredients vitamin B12 and coenzym Q10, as well as metabolic parameters reflecting the oxidative status or lipid metabolism.

After six month the comparison between placebo or verum intake revealed significant differences for Coenzyme Q10, and Superoxiddismutase (SOD). The HCY serum levels in the verum group continued to significantly stay below the placebo group level. Table 7 lists the serum levels for Vitamin B12 and coenzym Q10 , and serum parameters reflecting the oxidative status or lipid metabolism.

Bioavailability

To monitor whether or not the ingredients of the test-substance are resorbed and/or accumulate to effective concentrations, or even reach toxic concentrations we measured selected parameters corrresponding to ingredients throughout the whole participation period. Table 8 summarises the serum concentrations at start, after 3, and 6 months of participation, of the Vitmamins B1, B6, B9, B12 Vitamin H, and Viamin E. In addition coenzym Q10 , Copper, Mangan, and Zink was monitored.

The serum vitamin levels were most elevated after the first three months. After 6 months the majority of measures were below the peak, but – with one exception – significantly above the baseline level at M0. Coenzyme Q10 and Zinc continued increasing until the end of the term (Table 8).

Effect course

In the placebo group – during the first three month (between M0 and M3) – one parameter change revealed statistical significance (p<0.05). The analyses for the the second period and the complete trial term revealed two changes each, that appeared significant by chance (Table 9).

Tab. 7. Group difference after 6 months regular intake by means of analysis of variance and covariance of pretreatment data. After 6 months the increase of Coenzyme Q10 and the decrease of homocysteine and SOD was significant.

Visit 3, (M6)	Placebo			Verum			
Pm	Mean	STD	N	Mean	STD	N	*p*-value
Vitamin B12	557.17	361.55	36	512.32	203.71	107	0.484
Coenzym Q10	1.24	0.53	36	1.55	0.67	107	**0.001**
Cholesterol	197.94	38.68	36	202.58	38.22	107	0.592
Chol/HDL ratio	2.94	1.12	36	3.07	1.14	107	0.820
Homocystein (HCY)	12.08	6.04	36	9.95	2.69	107	**0.025**
a-Oxyd. FORD	1.41	0.32	22	1.30	0.29	52	0.188
a-Ox.Cap., FORT	348.23	127.49	22	370.69	98.46	52	0.709
Lipidperoxidation	227.14	267.28	35	211.36	296.60	105	0.549
Nirotryrosine	25.04	46.49	36	26.47	34.61	101	0.745
SOD	6.03	5.31	36	4.04	2.43	106	**0.003**

Tab. 8. Bioavailability of selected ingredients, starting from M0 (Start); with exception of copper and mangan the parameters go up after 3 months and remain above start level after 6 months.

	Visit1(M0)			Visit2 (M3)			Course	Visit3 (M6)			Course
Start	Mean	SEM	N	Mean	SEM	N	M0–M3	Mean	SEM	N	M0–M6
VitB1	37.86	2.40	14	72.55	4.07	15	**0.000**	43.39	2.15	29	**0.007**
VitB6	28.74	4.28	30	70.58	6.90	30	**0.000**	61.37	8.86	29	**0.003**
Fol.Acid	348.20	19.21	30	404.83	19.50	30	**0.003**	337.10	15.68	29	0.496
VitB12	445.34	18.36	112	535.99	20.24	112	**0.000**	512.32	19.69	107	**0.000**
Biotin	206.52	29.32	31	433.16	37.97	30	**0.000**	317.03	37.29	29	**0.038**
VitE	12.24	0.52	30	15.67	0.91	30	**0.000**	14.53	0.94	29	**0.009**
Coenz.Q10	1.10	0.05	112	1.51	0.08	112	**0.000**	1.55	0.06	107	**0.000**
Copper	1.21	0.05	30	1.19	0.05	30	0.399	1.16	0.05	30	0.148
Mangan	8.51	0.42	30	8.70	0.40	30	0.554	8.49	0.36	29	0.814
Zinc	6.43	0.17	30	6.60	0.18	30	0.113	7.06	0.19	29	**0.000**

Copyright © 2015 Neuroendocrinology Letters ISSN 0172–780X • **www.nel.edu**

Tab. 9. Parameter change in placebo group, The difference of laboratory parameters between participation start (M0), after 3 months (M3) or 6 months (M6) was computed, and analysed within the placebo group by means of the paired student t-test. Occasionally the test indicated significance which most likely reflects a statistical type 1 error (by chance result, false positive).

Pm Changes	from start to three months				3 – 6 months				Start to End, 6 months			
Placebo	**meanDiff**	**SEM**	**N**	***p*-value**	**meanDiff**	**SEM**	**N**	***p*-value**	**meanDiff**	**SEM**	**N**	***p*-value**
Vitamin B12	+10.43	21.30	39	0.627	+10.73	38.70	36	0.783	+29.00	45.72	36	0.530
Coenzym Q10	+0.29	0.14	39	**0.043**	−0.33	0.18	36	0.071	−0.01	0.16	36	0.953
Cholesterol	−1.65	4.24	40	0.699	−0.22	4.16	36	0.958	−1.28	3.83	36	0.741
Chol/HDL ratio	+0.10	0.12	40	0.401	−0.14	0.14	36	0.324	+0.03	0.12	36	0.822
Homocystein (HCY)	−0.72	0.51	39	0.166	+0.44	0.38	36	0.249	−0.50	0.57	36	0.385
a-Oxid. FORD	+0.08	0.14	25	0.558	+0.02	0.13	23	0.864	+0.08	0.09	22	0.415
a-Ox.Cap., FORT	32.32	21.35	25	0.143	+43.35	12.03	23	**0.002**	+64.32	18.39	22	**0.002**
Lipidperoxidation	−77.54	40.61	39	0.064	+63.40	33.04	35	0.063	−19.43	25.80	35	0.457
Nirotryrosine	+8.28	3.64	40	**0.029**	−9.02	3.37	36	**0.011**	+0.31	3.98	36	0.939
SOD	+1.56	1.09	40	0.160	+1.26	1.48	36	0.399	+2.32	0.88	36	**0.013**

Tab. 10. Parameter change in verum group, The difference of laboratory parameters between participation start (M0), after 3 months (M3) or 6 months (M6) was computed, and analysed within the group by means of the paired student t-test. In Column P bold figures indicate statistically sinificant parameter changes. After the first 3 months period of regular intake the changes in 10 parameters were statistically significant. While with most vitamins the serum levels increased relatively fast, to drop again in the second period, serum levels of coenzym Q10 , and Zink increased steadily over the six months observation time.

Changes	1. period (3 months)				2. period (3 months)				CoompewkteXXX (6 months)			
Verum	**MeanDiff**	**SEM**	**N**	***p*-value**	**MeanDiff**	**SEM**	**N**	***p*-value**	**MeanDiff**	**SEM**	**N**	***p*-value**
VitaminB1	+34.58	4.37	14	**0.000**	−21.21	3.11	14	**0.000**	+12.59	3.89	13	**0.007**
VitaminB6	+41.84	7.98	30	**0.000**	−10.93	8.84	29	0.226	+32.18	9.92	29	**0.003**
FolicAcid	+56.63	17.28	30	**0.003**	−65.86	16.76	29	**0.001**	−11.28	16.34	29	0.496
VitaminB12	+90.65	10.44	112	**0.000**	−23.41	12.31	107	0.060	+67.86	11.93	107	**0.000**
Biotin	+224.86	47.61	30	**0.000**	−126.07	48.35	29	0.014	+108.41	49.91	29	**0.038**
VitaminE	+3.43	0.64	30	**0.000**	−1.24	0.84	29	0.151	+2.29	0.81	29	**0.009**
Coenzym Q10	+0.41	0.05	112	**0.000**	+0.02	0.06	107	0.718	+0.44	0.05	107	**0.000**
Copper(serum)	−0.02	0.02	30	0.399	−0.01	0.02	29	0.567	−0.03	0.02	29	0.148
Mangan(serum)	+0.19	0.31	30	0.554	−0.14	0.25	29	0.587	+0.07	0.29	29	0.814
Zinc(serum)	+0.18	0.11	30	0.113	+0.48	0.07	29	**0.000**	+0.63	0.12	29	**0.000**
Cholesterol	+0.29	2.54	112	0.908	−0.19	2.26	107	0.934	+0.36	2.59	107	0.888
Chol/HDLratio	+0.09	0.07	112	0.234	−0.19	0.08	107	**0.028**	−0.10	0.10	107	0.282
Homocystein(HCY)	−2.17	0.29	112	**0.000**	+0.50	0.22	107	**0.025**	−1.48	0.23	107	**0.000**
a-Oxidants,FORD	−0.02	0.08	53	0.786	+0.08	0.07	53	0.234	+0.04	0.07	52	0.560
a-Ox.Cap.,FORT	−13.15	12.53	53	0.299	+77.21	14.32	53	**0.000**	+66.58	12.34	52	**0.000**
Lipidperoxidat.	−113.80	27.20	109	**0.000**	+52.18	20.41	107	**0.012**	−61.00	32.12	105	0.060
Nirotryrosine	+7.61	4.34	105	0.083	−10.24	3.27	107	**0.002**	−2.95	3.27	101	0.369
SOD	−0.08	0.40	112	0.834	+0.19	0.25	106	0.437	+0.18	0.47	106	0.697

In contrast, in the verum group many paramaters indicated statistically significant changes. During the first three month (periode-1) ten parameter changes were significant. Between 3 and 6 months (periode-2) nine parameter changed significantly. During the complete term – after 6 months therapy – eleven parameters changed to the extent of statistical significance (Table 10).

Parallel to the increase of Vitamins, the serum HCY level decreased significantly already after 3 months, and remained well below the baseline values for the rest of the observational period (Table 10).

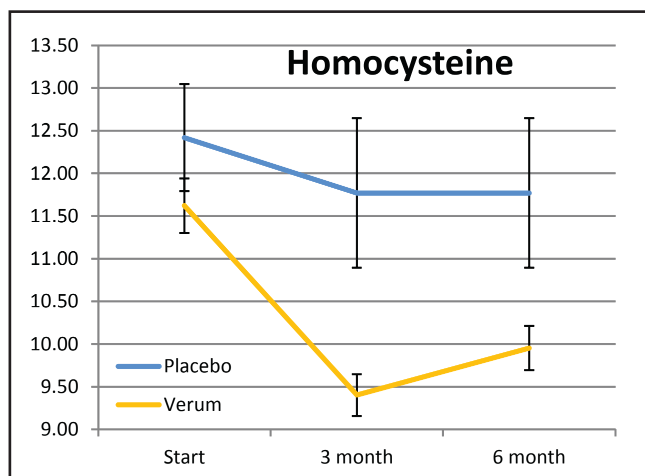

Fig. 2. LaVita and placebo consumption, Mean and Standard error of means of serum homocystein levels. After 3 month LaVita consumption the serumlevel was significantly lowered and remained low as the supplementation was continued to month 6.

The verum intake increased most vitamin serum levels already at M3 to remain up until the end of the trial. In Contrast the zinc serum levels increased slower, only during the the second period and over the whole six month the increase was significant (Table 10).

DISCUSSION

The blood analysis before, during and after the regular ingestion of verum revealed a potential neuroprotective impact of the test substance. Most likely the combination of significant increase of B-Vitamin serum levels, and coenzym Q10 and antioxidative capacity contributed to the effective lowering of HCY and SOD (Tables 6 and 7).

Most likely the increased antioxidant vitamin E and the increased coenzym Q10 triggered the reduced lipid peroxidation, the rise of the antioxidative capacity, and nitrotyrosine changes indicative of reduction of ROS and NOS (Table 10).

Reports on B vitamin supplementation trials demonstrated a slowing of brain atrophy and improvement in some domains of cognitive function (Herrmann & Obeid 2011). Meta-analysis of secondary prevention trials showed that B vitamins supplementation caused a decrease in plasma HCY and a trend for lowering the risk of cardovascular events like stroke (Herrmann & Obeid 2011). Folate is an important extrinsic signal for the development of the central nervous system, and neural stem cell proliferation and differentiation (Zhang *et al.* 2009). We conclude that mainly the significant increase of B-Vitamines and to a lesser extend the increase of vitamin E, corroborate the neuroprotective potential of the ViteC.

The serum levels of Vitamin E – a strong antioxidant agent – were raised significantly in the verum group (Table 8), therefore the protection of lipidphase membranes in the mitochondria can contribute to the postulated neuroprotective potential (Shen & Ji 2012; Marin *et al.* 2014; Muthukumaran *et al.* 2014; Lee *et al.* 2014; Haorah *et al.* 2013).

Coenzym Q10 was among the ingredients that – after 3 months – increased significantly. The serum level remained high until the end of the oberservational period (Table 7 and Table 10). Because coenzym Q10 blocked the upregulation of specific protein expression associated with oxidative stress in neurons and significantly prevented apoptotic cell death by decreasing Bax protein expression or by increasing pBad protein expression (Lee *et al.* 2014), solely the finding of increased coenzym Q10 serum level constitutes a neuroprotective potential.

Serum levels of Copper and Mangan remained steady throughout the complete 6 month term. Zinc serum levels increased slowly, the increase was statistically significant in the second halve of the trial. At the end of the observations (from baseline to end, after six months) the zinc serum had increased significantly. Among the metabolic changes it was the anti-oxidative capacity (FORT) to reach significance only after more than three months (Table 10).

HCY – a sustainable biomarker of prospective neurodegeneration – is a neurotoxic substance with deteriorating effects in mitochondrial respiration. In laboratory animals elevated plasma levels of HCY increase the BBB permebility (Kamath *et al.* 2006), by increasing proinflammatory responses (Hohsfield & Humpel 2010). Because regular ViteC intake decreases serum homocystein levels, and increases the neuroprotective coenzym Q10 as well the antioxidative capacity all these mechanisms may synergistically contribute to the postulated neuroprotection.

The disrupted physiological balance between the generation and elimination of ROS/RNS has been directly linked to dyfunctions of the BBB or neurodegenerative disorders (Lehner *et al.* 2011). Clinical trials have shown oxidative stress associated inflammatory response in patients with neurodegenerative disease (Gilgun-Sherki *et al.* 2003; Wang *et al.* 2014). Therefore serum parameters reflecting oxidative stress, such as lipidperoxidation and antioxidative capacity are reasonable endpoints in our study. Antioxidants are well documented for neuroprotection either by scavenging free radicals or inducing antioxidant enzymes (Mancuso *et al.* 2012). The significant increase of Vitamin E in our verum cohort (Table 8) underlines the preventive potential of our test substance. Our results confirm previous findings, i.e. an increase of antioxidative capacity by regular consumption of an anti-oxidant rich juice (Diaz-Rubio *et al.* 2014).

Several lines of evidence suggest that mitochondrial dysfunction, and overproduction of reactive oxygen species (ROS) and an imbalance between pro-oxidant and antioxidant systems resulting in oxidative damage to proteins, lipids and DNA play important roles in

 Copyright © 2015 Neuroendocrinology Letters ISSN 0172–780X • www.nel.edu

neurodegeneration triggering neuronal cell death (Gil-Mohapel *et al.* 2014). The composition of various antioxidants and their natural origin seem to be important to promote or ameliorate these subcellular effects (Lopez-Erauskin *et al.* 2011).

The B vitamins (Folic acid, Pyridoxin and Cobalamin) may trigger the short term reduction of mitochondriatoxic HCY levels in the central nervous system. SOD was among the slow responding parameters. The changes were borderline significant after three month (Table 6), however after 6 months the difference of SOD serum levels between placebo and verum was highly significant. As this enzyme activity is closeley related to intracellular peroxid creation, a combined effect of neuroprotective B vitamins along with lipid phase protecting antioxidants may support mitochondrial integrity in the central nervous system contributing to neurodeprotection.

CONCLUSION

We studied the neuroprotective potential of the multivitamin and trace element composition (LaVita®) in a prospective, randomized, double blind, placebo controlled trial, in healthy volunteers with no obvious neurological symptoms.

The quantitative analysis of blood parameters from both groups (intervention and control) pointed out the nutritional value of the tested composition. Neuroprotective vitamins (Folic acid, Pyridoxin) and antioxidative ingredients (Tocopherol) were significantly increased by the end of the trial term. The observed bioavailability of the composition and the metabolic changes during the intervention substantiates the potential of the testsubstance LaVita® to prevent neurodegeneration. We conclude from our results that the subsitution of vitamins and traceelements with natural source in a proper manner may be effective for neuroprotection in healthy populations.

ACKNOWLEDGMENTS

The study design and manuscript preparation was done with the aid of SCIgenia Science support GmbH Vienna (www.scigenia.com). The trial was organizied by the International Research Group for Applied Preventive Medicine Vienna (A) (I-GAP). The study was financed by LaVita Ltd (Bavaria, Germany).

REFERENCES

1 Aliev G, Priyadarshini M, Reddy VP, Grieg NH, Kaminsky Y, Cacabelos R, Ashraf GM, Jabir NR, *et al.* (2014). Oxidative stress mediated mitochondrial and vascular lesions as markers in the pathogenesis of Alzheimer disease. Current medicinal chemistry **21**: 2208–2217.

2 Anchisi L, Dessi S, Pani A, Mandas A (2012). Cholesterol homeostasis: a key to prevent or slow down neurodegeneration. Frontiers in physiology **3**: 486.

3 Baluchnejadmojarad T, Roghani M (2013). Coenzyme q10 ameliorates neurodegeneration, mossy fiber sprouting, and oxidative stress in intrahippocampal kainate model of temporal lobe epilepsy in rat. Journal of molecular neuroscience : MN **49**: 194–201.

4 Bhadri N, Sanji T, Madakasira Guggilla H, Razdan R (2013). Amelioration of behavioural, biochemical, and neurophysiological deficits by combination of monosodium glutamate with resveratrol/alpha-lipoic acid/coenzyme Q10 in rat model of cisplatin-induced peripheral neuropathy. TheScientificWorldJournal **2013**: 565813.

5 Bjelakovic G, Nikolova D, Gluud C (2014). Antioxidant supplements and mortality. Current opinion in clinical nutrition and metabolic care **17**: 40–44.

6 Caldwell CC, Yao J, Brinton RD (2014). Targeting the Prodromal Stage of Alzheimer's Disease: Bioenergetic and Mitochondrial Opportunities. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics.

7 Choi SI, Ju WK, Choi EK, Kim J, Lea HZ, Carp RI, Wisniewski HM, Kim YS (1998). Mitochondrial dysfunction induced by oxidative stress in the brains of hamsters infected with the 263 K scrapie agent. Acta neuropathologica **96**: 279–286.

8 Diaz-Rubio ME, Perez-Jimenez J, Martinez-Bartolome MA, Alvarez I, Saura-Calixto F (2014). Regular Consumption of an Antioxidant-rich Juice Improves Oxidative Status and Causes Metabolome Changes in Healthy Adults. Plant foods for human nutrition.

9 Enciu AM, Gherghiceanu M, Popescu BO (2013). Triggers and effectors of oxidative stress at blood-brain barrier level: relevance for brain ageing and neurodegeneration. Oxidative medicine and cellular longevity **2013**: 297512.

10 Gasparova Z, Janega P, Stara V, Ujhazy E (2012). Early and late stage of neurodegeneration induced by trimethyltin in hippocampus and cortex of male Wistar rats. Neuro endocrinology letters **33**: 689–696.

11 Gasparova Z, Stara V, Janega P, Navarova J, Sedlackova N, Mach M, Ujhazy E (2014). Pyridoindole antioxidant-induced preservation of rat hippocampal pyramidal cell number linked with reduction of oxidative stress yet without influence on cognitive deterioration in Alzheimer–like neurodegeneration. Neuro endocrinology letters **35**: 454–462.

12 Gil-Mohapel J, Brocardo PS, Christie BR (2014). The role of oxidative stress in Huntington's disease: are antioxidants good therapeutic candidates? Current drug targets **15**: 454–468.

13 Gilgun-Sherki Y, Melamed E, Offen D (2003). Antioxidant treatment in Alzheimer's disease: current state. Journal of molecular neuroscience : MN **21**: 1–11.

14 Gonzalez-Escamilla G, Atienza M, Garcia-Solis D, Cantero JL (2014). Cerebral and blood correlates of reduced functional connectivity in mild cognitive impairment. Brain structure & function.

15 Guallar E, Stranges S, Mulrow C, Appel LJ, Miller ER, 3rd (2013). Enough is enough: Stop wasting money on vitamin and mineral supplements. Annals of internal medicine **159**: 850–851.

16 Haorah J, Rump TJ, Xiong H (2013). Reduction of brain mitochondrial beta-oxidation impairs complex I and V in chronic alcohol intake: the underlying mechanism for neurodegeneration. PloS one **8**: e70833.

17 Herrmann W, Obeid R (2011). HCY : a biomarker in neurodegenerative diseases. Clinical chemistry and laboratory medicine : CCLM / FESCC **49**: 435–441.

18 Hohsfield LA, Humpel C (2010). HCY enhances transmigration of rat monocytes through a brain capillary endothelial cell monolayer via ICAM-1. Current neurovascular research **7**: 192–200.

19 Kamath AF, Chauhan AK, Kisucka J, Dole VS, Loscalzo J, Handy DE, Wagner DD (2006). Elevated levels of HCY compromise blood-brain barrier integrity in mice. Blood **107**: 591–593.

20 Kruman, Ii, Culmsee C, Chan SL, Kruman Y, Guo Z, Penix L, Mattson MP (2000). HCY elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. The Journal of neuroscience : the official journal of the Society for Neuroscience **20**: 6920–6926.

21 Kruman, Ii, Kumaravel TS, Lohani A, Pedersen WA, Cutler RG, Kruman Y, Haughey N, Lee J, *et al.* (2002). Folic acid deficiency and HCY impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience **22**: 1752–1762.
22 Lee D, Shim MS, Kim KY, Noh YH, Kim H, Kim SY, Weinreb RN, Ju WK (2014). Coenzyme Q10 inhibits glutamate excitotoxicity and oxidative stress-mediated mitochondrial alteration in a mouse model of glaucoma. Investigative ophthalmology & visual science **55**: 993–1005.
23 Lehner C, Gehwolf R, Tempfer H, Krizbai I, Hennig B, Bauer HC, Bauer H (2011). Oxidative stress and blood-brain barrier dysfunction under particular consideration of matrix metalloproteinases. Antioxidants & redox signaling **15**: 1305–1323.
24 Lopez-Erauskin J, Fourcade S, Galino J, Ruiz M, Schluter A, Naudi A, Jove M, Portero-Otin M, *et al.* (2011). Antioxidants halt axonal degeneration in a mouse model of X-adrenoleukodystrophy. Annals of neurology **70**: 84–92.
25 Maler JM, Seifert W, Huther G, Wiltfang J, Ruther E, Kornhuber J, Bleich S (2003). HCY induces cell death of rat astrocytes in vitro. Neuroscience letters **347**: 85–88.
26 Mancuso C, Barone E, Guido P, Miceli F, Di Domenico F, Perluigi M, Santangelo R, Preziosi P (2012). Inhibition of lipid peroxidation and protein oxidation by endogenous and exogenous antioxidants in rat brain microsomes in vitro. Neuroscience letters **518**: 101–105.
27 Marin T, Contreras P, Castro JF, Chamorro D, Balboa E, Bosch-Morato M, Munoz FJ, Alvarez AR, *et al.* (2014). Vitamin E dietary supplementation improves neurological symptoms and decreases c-Abl/p73 activation in Niemann-Pick C mice. Nutrients **6**: 3000–3017.
28 Mattson MP, Kruman, Ii, Duan W (2002). Folic acid and HCY in age-related disease. Ageing research reviews **1**: 95–111.
29 Muthukumaran K, Leahy S, Harrison K, Sikorska M, Sandhu JK, Cohen J, Keshan C, Lopatin D, *et al.* (2014). Orally delivered water soluble Coenzyme Q10 (Ubisol-Q10) blocks on-going neurodegeneration in rats exposed to paraquat: potential for therapeutic application in Parkinson's disease. BMC neuroscience **15**: 21.
30 Shen L, Ji HF (2012). Vitamin E: supplement versus diet in neurodegenerative diseases. Trends in molecular medicine **18**: 443–445.
31 Wang P, Xie K, Wang C, Bi J (2014). Oxidative stress induced by lipid peroxidation is related with inflammation of demyelination and neurodegeneration in multiple sclerosis. European neurology **72**: 249–254.
32 Zhang XM, Huang GW, Tian ZH, Ren DL, J XW (2009). Folate deficiency induces neural stem cell apoptosis by increasing HCY in vitro. Journal of clinical biochemistry and nutrition **45**: 14–19.