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Bioavailabilty of a liquid Vitamin Trace Element Composition in healthy volunteers – a randomized, double blind, placebo controlled clinical study

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

1 Internationale Gesellschaft für angewandte Präventionsmedizin I-GAP, Wien, Austria
2 Inst. f. Krebsforschung, KIM-1, Medizinische Universität, Austria
3 Medizin-Labor Endler, Wien, Austria

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

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Abstract

BACKGROUND: Many Vitamins and minerals for dietary supplements lack a standard scientific and regulatory definition that accurately reflects the bioavailabilities in humans. Especially the bioavailability of natural compounds in complex mixtures, where the different ingredients may interfere with each other, is unknown.

METHODS: To learn more about the bioavailability of the ingredients in the complex compound LaVita® we examined blood levels of subjects, who ingested the multivitamin and trace element composition for 6 month continuously. Blood samples for the analysis of the ingredients were taken before, during, and after administration.

RESULTS: Our data indicated a significant increase of most ingredients after 3 month, and additional three months, except for Vitamin (B9 Folic acid). The semivitamins Q10 and carnitine increased in the first 3 month (both $p<0.001$). While carnitine dropped during the second term, Q10 levels increased further slowly. After three months a significant increase was observed for iron (serum $p=0.039$; blood cells $p=0.025$), Selenium (serum $p=0.048$; cells $p=0.006$), and chromium (serum $p=0.029$). Zinc – known to interfere with the iron resorption – increased slowly in the first term of 3 months, but was raised significantly after 6 months (serum and blood cells, each $p<0.001$). The Copper/Zink ratio dropped accordingly ($p<0.001$).

CONCLUSION: We conclude that resorption interference between specific ingredients, and after resorption redistribution of specific ingredients to various tissue compartments precludes a linear increase of the respective serum parameters. We observed no deleterious resorption competitions for individual compounds. No parameter reached critical levels. We conclude that the test substance (LaVita®) is a sufficiently safe composite for long term consumption.

ORIGINAL ARTICLE

INTRODUCTION

For a healthier life and disease prevention the compensation for real or assumed dietetic shortcomings is a major spur for the consumption of nutritional supplements. The market responds to this demand with a wide array of single substance supplements or complex synthetic or natural compositions. Legislation of such substances was introduced in many countries to reinforce regulatory compliance of declared concentrations of vitamins and other micronutrients in food products and dietary supplements (Blake 2007). It is a Public Health issue, that distribution and consumption of food supplements follows product safety guidelines. Therefore, regulatory measures should be based on – or consider – bioavailability data. However, this product-information is still scarce.

The bioavailability of any food constituent depends on various physiologic pathways, including distribution to different tissue compartments (reservoirs) after intestinal absorption. Up to now, the concept of vitamin and mineral bioavailability for dietary supplements lacks standard scientific and regulatory definitions. To judge absorption, bioavailability, redistribution in tissue compartments, and metabolisation direct and dynamic measurements are required (Heaney 2001).

The bioavailability of substances from a complex mixture may be influenced by the competition for intestinal resorption as different derivatives may have dif-

Tab. 1. Raw materials for the manufacturing of LaVita®.

	Conventional Name	Botanical Name
22 Herbs	anise	Pimpinella anisum
	ramson	Allium ursinum
	basil	Ocimum basilicum
	burnet	Sanguisorba minor
	stinging nettle	Urtica dioica
	rosehip	Fruct. rosa canina infus
	hop	Gen. Humulus
	ginger	Zingiber officinale
	lime blossom	Tilia platyphyllos flor.
	dandelion	Taraxacum officinale
	holy thistle	Silybum marianum
	melissa	Gen. Melissa
	parsley	Petroselinum crispum
	rosemary	Rosmarinus officinalis
	sage	Salvia officinalis
	yarrow	Eupithecia millefoliata
	buckhorn	Plantago lanceolata
	common centaury	Centaureum erythrea
	thyme	Thymus vulgaris
	septfoil	Potentilla erecta
	woodruff	Galium odoratum
	common horsetail	Equisetum arvense
22 Fruits	acerola	Malpighia glabra
	pineapple	Ananas comosus
	apple	Malus domestica
	chokeberry	*Aronia* melanocarpa
	banana	Musa
	barberry	Gen. Berberis
	cranberry	Vaccinium macrocarpon
	strawberry	Fragaria × ananassa
	pomegranate	Punica granatum
	rosehip	Rosa canina fruct.
	blueberry	Vaccinium myrtillus
	elderberry	Sambucus nigra
	honeydew	Cucumis melo
	currants	Ribes vulgare
Fruits *cont.*	cherry	Prunus avium
	maracuya	Passiflora edulis
	oranges	Citrus ×aurantium
	papaya	Carica papaya
	seabuckthorn	Gen. Hippophaë
	sloes	Prunus spinosa
	grapes	Vitis vinifera
	lemon	Citrus limon
12 Vegetables	artichoke	Cynara scolymus
	broccoli	Brassica oleracea
	cucumber	Cucumis sativus
	carrot	Daucus carota
	pepper	Capsicum annuum
	parsnip	Pastinaca sativa
	beetroot	Beta vulgaris
	sauerkraut	Brassica oleraceae
	spinach	Spinacia oleracea
	tomato	Lycopersicon esculentum
	sunchoke	Helianthus tuberosus
	onion	Allium cepa
7 Fermented Juices	cucumber	Cucumis sativus succus ferm.
	carrot	Daucus carota succus ferm.
	pepper	Capsicum annuum succus ferm.
	parsnip	Pastinaca sativa succus ferm.
	beetroot	Beta vulgaris succus ferm.
	sauerkraut	Brassica oleraceae succus ferm.
	onion	Allium cepa succus ferm.
4 Oils	borage seed oil	Borago officinalis
	pumpkin seed oil	Curcubita semen oleum
	linseed oil	Linum usitatissimum semen oleum
	grape seed oil	Vitus Vinifera semen oleum
4 Else	aloe vera juice	Aloe barbadensis
	green tea	Camelia sinensis
	maté	Ilex paraguariensis fol.
	mare's milk	

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ferent resorption rates. In the case of Selenium also the chemical compound is important, as organic Selenium (from natural foods) has a higher bioavailability compared to inorganic Selenium (Wang *et al.* 2012).

The regular intake of various substances in a fixed ratio implies the risk for overdosing certain ingredients. Therefore different effects need to be taken into account 1) interference of vitamins with mineral resorption, 2) interference of nutrients in the metabolism in the specific organism (Vannucchi 1991). Vitamins and minerals can reduce or enhance the individual nutritional components' bioavailability (Wolber *et al.* 2013). In a complex mixture the probability of interaction increases by the number of ingredients.

Ascorbic acid (Vitamin C) can enhance the absorption of supplemented iron to a high degree (Stewart *et al.* 2012). Because ascorbic acid is the most efficient enhancer of non-haem, Teucher *et al.* (2004) investigated how organic acids enhance the iron absorption and conclude that ascorbic acid is more potent than other organic acids due to its ability to reduce ferric to ferrous iron. However, high vitamin C intake does not cause iron imbalance in healthy persons (Gerster 1999). There is consensus, that adverse effects do not occur in healthy subjects ingesting large amounts of vitamin C (Diplock 1995).

In view of possible interaction between ingredients, products should deliver sufficient evaluation before marketing, also for safety reasons (Bjelakovic *et al.* 2008). We therefore measured the serum levels of the individual compounds before, during, and after intake of the complex Vitamin-Trace Element Composition LaVita®. We analysed the serum levels at different timepoints, to determine the bioavailability, and control for possible exaggerations (critical serum levels) after regular intake by healthy persons.

MATERIAL AND METHODS:

Test-substance

The test substance, LaVita® is a vitamin and trace element composition produced from fruits and vegetable fortified with minerals and trace elements (Table 1). It contains secondary plant constituents, like enzymes, amino acids, minerals, trace elements, vitamins, and semi-vitamins such as L-carnitine and Coenzyme Q10 (Table 2).

Study size and design

The bioavailability of most ingredients was investigated in 30 or up to 116 recruited patients. The specific number of cases for each investigated substance is indicated in the result-tables.

The study protocol was designed by a research-consulting company (SCIgenia, Vienna, Austria, www.scigenia.com). Study design and realisation including monitoring by this company complied with research guidelines like GCP (Good Clinical Practice).

Participants recruitment

Medical physicians recruited healthy volunteers living a steady life (N=116), according to predefined inclusion and exclusion criteria over a time period of three years (2011 to 2013). The exclusion criteria eliminated participants with diseases, which could constitute a study bias. The required age of our participants was over 18 and under 90 years. Specific Exclusion criteria were:

- 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

Tab. 2. 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 acidy	30 mg

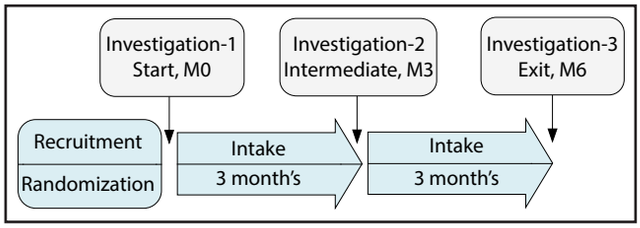

Fig. 1. Sequence of trial specific activities.

- disease with remissions and relapses (Arthritis, Multiple sclerosis, etc.) to exclude endpoint variance related to disease associated conditions
- dementia, medically treated neurodegeneration
- receiving cortison treatment or any other acute medical intervention (antibiotics) to exclude interference and study bias due to medical condition treatment
- participation at another trial (exclude interference with other trials)

Study course

Figure 1 summarizes the trial specific activities. After recruitment and baseline value determination, the participants were recalled after three months (timepoint M3), for the second visit and the second blood sample to be taken. The intake of the study substance continued for another three month term. At the exit visit (visit 3) the subjects contributed the third and last blood sample 6 months after participation start.

The blood sample analyses were performed by the accredited laboratories Endler (Vienna, Austria) and Biovis MVZ GmBH (Limburg, Germany) with standardized and certified methods (Table 3).

Statistics

The differences between the baseline laboratory parameters at start, after three months, and after 6 months (timepoints M0, M3, and M6) were analysed with the softwarepackage IBM-SPSS (Version 22). We used the students T-test for paired data, and Analysis of vari-

Tab. 3. Laboratory parameters analysed in this study.

Parameter	Abbrev	Method, analysis	Unit	Range M/F
Vitamin A	VIT A	HPLC	ng/ml	560–1280
B-Carotene		HPLC	ng/ml	150–1200
Vitamin B1	VITB1EB	HPLC	µg/l	35–99
Vitamin B2	VIT B2	HPLC	g/l	180–295
Vitamin B3	VIT B3	LCMS	g/l	8.0–52
Vitamin B5	VIT B5	LCMS	g/l	25–80
Vitamin B6	VITB6E	HPLC	ng/ml	4.1–43.7
Folsäure Speicherstatus	FOLEBMOD, AltFolsex	Competitive Chemoluminescence	ng/ml	280–800
Vitamin B12	VIT B12	Competitive Chemoluminescence	pg/ml	193–982
Vitamin E	VITE	ELISA	mg/l	5–20
Biotin	BIOTIN	ELISA	ng/l	>200
Vitamin K	VIT K	HPLC	ng/l	90–2100
Carnitine Total	CarnitinGes	LCMS	mol/l	29.0–61.0
Carnitin soluble i	CarnitinFrei	LCMS	mol/l	20.0–47.0
Coenzym Q10	Q10	HPLC	mg/l	0.88–1.43
Iron, whole blood	FeHB	ICP-MS	mg/l	440–480
Iron, cellular	FeHK	Computational	mg/l	440–480
Selenium, whole blood	SelenHB	ICP-MS	g/l	100–140
Selenium, cellular	Selen HK	Computational	g/l	100–140
Chromium, Whole blood	ChromiumHBEX	ICP - MS	g/l	<3.0
Mangan Whole blood	ManganVollblut	ICP-MS	µg/l	5–15
Copper, whole blood	CUHB	ICP-MS	mg/l	1.10–1.20
Copper, celluar	CUHK	Computational	mg/l	1.10–1.20
Zinc. Whole blood	ZinkHB	ICP - MS	mg/l	7.30–7.70
Zinc, celluar	ZinkHK	Computational	mg/l	7.30–7.70
Ratio Cu/Zn cellular	QCuZnHK	Computational	No unit	0.135–0.165

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ance taking the baseline Values (M0) as covariable (ANCOVA). Statistical significance was assumed if the alpha error was $p<0.05$.

RESULTS

The age and gender distribution of the recruited volunteers are summarised in Table 4. The overall dropout rate after 3 or 6 month was below 10% (Table 5). The participants' did not report any adverse side effects or allergic reaction. We obtained the serum levels of the single substances at baseline (visit-1, before intake, M0), at visit-2 after 3 month (M3) and at visit-3 after another three month (M6).

The vitamin baseline levels and the changes between the respective 3 month terms and the complete trial period of 6 months are given in Table 6. After three months intake, the tested vitamins revealed increased levels. Except for Vitamins A, B5 and K, the increase was highly significant ($p<0.005$). There was a further increase of the mean during the second three months term for beta-carotene and riboflavin (Vitamin B2). Although, the parameter means for Vitamin B1, Vitamin B9, Biotin and Vitamin A decreased during the second 3 month term, after 6 month intake the blood levels for all vitamins – except Vitamin A – were increased. Except for the vitamins B5, B9 and K, this increase was statistically significant (Table 6).

The results of the vitaminoids are summarized in Table 7. All parameters increased in the first 3 month term (between visit-1 to visit-2). After significant increase of carnitine in the first 3 month term, prolonged intake did not change the serum levels sustainably. Unbound Q10 increased significantly during the first 3 months ($p<0.001$, Table 7) and continued to increase in the second term, so that after 6 month the serum levels were significantly and sustainably improved ($p<0.001$).

All investigated minerals and trace elements (full blood or blood cell compartment) increased except copper (Table 8). After 3 months the increase was statistically significant for iron, selenium, and chromium (Table 8). After 6 months at least one parameter for iron, selenium, chromium, and zinc were above baseline. The Copper/Zink ratio was lowered ($p<0.001$).

Tab. 4. Age and group distribution of recruited volunteers.

	Mean	Std	N
Male	40	16.5	46
Female	44	14.1	70

Tab. 5. Participants dropout rat.

	Male	Female	total
B1, Start, M0	46	70	116
B2, Middle, M3	44	67	111
B3, End, M6	41	65	106
Drop out rate after 6 month (%)	10.9	7.14	8.6%

Tab. 6. Vitamins at baseline (M0, before intake), changes after 3 months and after 6 months.

	Visit 1, Baseline			From Visit 1 to Visit 2 (3 month, term-1)				From Visit 2 to Visit 3, (3 month, term-2)				From Visit 1 to Visit 3 (6 month, term-1 & 2)			
Parameter	Mean	Std	N	Mean Diff	Std-Diff	N	Sig.	Mean Diff	Std-Diff	N	Sig	Mean Diff	Std-Diff	N	Sig.
B-Carotene	541.741	574.970	112	+347.277	484.518	112	0.000	+68.626	458.174	107	0.124	+432.477	486.761	107	0.000
Vit A	755.268	186.528	112	+29.682	145.329	112	0.033	–62.698	142.992	107	0.000	–30.701	124.531	107	0.012
Vit B1	37.857	8.995	14	+34.576	16.338	14	0.000	–21.210	11.628	14	0.000	+12.589	14.015	13	0.007
Vit B2	85.824	14.930	29	+90.666	86.103	29	0.000	+6.594	25.874	29	0.181	+93.055	99.546	28	0.000
Vit B3	11.348	22.719	112	+12.439	40.944	112	0.002	–3.753	46.790	107	0.409	+8.717	37.222	107	0.017
Vit B5	74.633	127.634	30	+8.693	111.998	30	0.674	–4.541	49.330	29	0.624	+3.700	139.538	29	0.887
Vit B6	28.740	23.446	30	+41.837	43.715	30	0.000	–10.931	47.588	29	0.226	+32.176	53.446	29	0.003
Vit B9, Fol.acid	348.200	105.220	30	+56.633	94.636	30	0.003	–65.862	90.242	29	0.001	–11.276	88.002	29	0.496
Vit B12	445.342	194.266	112	+90.650	110.497	112	0.000	–23.408	127.330	107	0.060	+67.864	123.392	107	0.000
Vit E	12.240	2.864	30	+3.430	3.488	30	0.000	–1.241	4.525	29	0.151	+2.286	4.361	29	0.009
Biotin	206.519	163.220	31	+224.860	260.762	30	0.000	–126.068	260.392	29	0.014	+108.408	268.788	29	0.038
Vit K	318.172	268.739	29	+276.724	746.073	29	0.056	–74.034	473.633	29	0.407	+210.464	642.687	28	0.095

Legend: Mean- arithmetic mean of parameters; Std – standard deviation of parameters; N – number of parameters (subjects); Mean Diff – mean of differences between the parameters of one subject at different timepoints; Std-Diff – standard deviation of differences; Sig – Significance of the differences by means of the students t-test for parameter pairs.

Tab. 7. Vitaminoids (semi-Vitamins) at baseline (before intake); changes after 3 months and after 6 months.

	Visit 1, Baseline			From Visit 1 to Visit 2 (3 month, term-1)				From Visit 2 to Visit 3, (3 month, term-2)				From Visit 1 to Visit 3 (6 month, term-1 & 2)			
Parameter	**Mean**	**Std**	**N**	**Mean Diff**	**Std-Diff**	**N**	**Sig.**	**Mean Diff**	**Std-Diff**	**N**	**Sig.**	**Mean Diff.**	**Std-Diff**	**N**	**Sig.**
Carnitine total	59.983	14.967	30	+4.993	14.448	30	0.068	–5.155	10.298	29	0.012	–0.197	12.417	29	0.933
Carnitine, unbound	39.563	8.730	30	+6.683	8.565	30	0.000	–5.979	6.000	29	0.000	+1.134	8.461	29	0.476
Q10	1.097	0.487	112	+0.412	0.574	112	0.000	+0.021	0.587	107	0.718	+0.439	0.537	107	0.000

Note that the laboratory paramters of all ingredients increased during the term-1 to slow down or even drop during term-2.
Legend: Mean- arithmetic mean of parameters; Std – standard deviation of parameters; N – number of parameters (subjects);
Mean Diff – mean of differences between the parameters of one subject at different timepoints; Std-diff – standard deviation of differences;
Sig – Significance of the differences by means of the students t-test for parameter pairs.

Tab. 8. Minerals and Trace Elements at baseline (M0, before intake); changes after 3 months and after 6 months.

Parameter (Laboratory Acronym)	**Visit 1, Baseline**			**From Visit 1 to Visit 2 (3 month, term-1)**				**From Visit 2 to Visit 3, (3 month, term-2)**				**From Visit 1 to Visit 3 (6 month, term-1 & 2)**			
	Mean	**Std**	**N**	**Mean Diff**	**Std-Diff**	**N**	**Sig**	**Mean Diff**	**Std-Diff**	**N**	**Sig.**	**Mean Diff**	**Std-Diff**	**N**	**Sig.**
Iron serum (FeHB)	450.54	41.27	112	+5.37	27.23	112	0.039	+0.76	23.87	107	0.743	+6.69	26.23	107	0.010
Cellular Iron (FeHK)	440.48	18.08	112	+6.01	27.99	112	0.025	–3.28	19.43	107	0.084	+3.29	22.37	107	0.132
Selen serum (SelenHB)	93.97	27.09	30	+14.83	39.38	30	0.048	–9.24	19.68	29	0.017	+5.59	39.28	29	0.450
Celluar Selen (Selen HK)	67.00	9.39	15	+45.17	53.46	15	0.006	–8.65	17.36	29	0.012	+40.90	37.33	14	0.001
Chromium serum (ChromHBEX)	0.60	0.50	30	+0.33	0.79	30	0.029	+0.03	0.44	29	0.707	+0.33	0.65	29	0.010
ManganWhole blood	8.51	2.29	30	+0.19	1.71	30	0.554	–0.14	1.35	29	0.587	+0.07	1.56	29	0.814
Copper serum (CUHB)	1.21	0.29	30	–0.02	0.13	30	0.399	–0.01	0.13	29	0.567	–0.03	0.12	29	0.148
Celluar copper (CUHK)	1.21	0.29	30	–0.02	0.12	30	0.496	–0.01	0.13	29	0.598	–0.03	0.12	29	0.229
Zinc serum (ZinkHB)	6.43	0.94	30	+0.18	0.59	30	0.113	+0.48	0.40	29	0.000	+0.63	0.63	29	0.000
Celluar Zinc (ZinkHK)	6.34	0.82	30	+0.12	0.47	30	0.187	+0.41	0.33	29	0.000	+0.49	0.49	29	0.000
Ratio Cu/Zk (QCuZnHK)	0.19	0.06	30	–0.01	0.02	30	0.158	–0.01	0.02	29	0.006	–0.02	0.02	29	0.000

Legend: Mean- arithmetic mean of parameters; Std – standard deviation of parameters; N – number of parameters (subjects);
Mean Diff – mean of differences between the parameters of one subject at different timepoints; Std-diff – standard deviation of differences;
Sig – Significance of the differences by means of the students t-test for parameter pairs.

DISCUSSION

In vitro and animal models do not allow to accurately determine human bioavailability; therefore, human testing is the gold standard (Gerster 1999). In our trial we followed a common study design and used blood parameters to investigate bioavailability (Heaney 2001). In accordance with earlier findings, in our cohort the bioavailability did not linearly increase the blood parameters of the various ingredients (Srinivasan 2001; Solomons & Slavin 2001; Krebs 2001).

Because foods contain components that reduce or enhance the individual nutritional components' bioavailability (Wolber *et al.* 2013), our field trial was prone to unknown confounders from the volunteers' nutrition style. However, our clients demonstrated an

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overall sufficient compliance during our investigation period (Table 5), so that we were able to receive representative test results.

All investigated vitamin blood levels were raised already after 3 month compared to baseline values. This implies that even healthy persons can benefit from a regular supplementation with naturally derived vitamins. No tendency of overdosing was marked for any ingredient over the period of 3 and even 6 month of consumption, which underpins the products' safety. We hypothesize that redistribution into tissue compartments (reservoirs) and metabolism contributed to the homeostatic regulation of traced ingredients. Many substances circulate in the blood within a tight concentration range and are redistributed to tissue reservoirs, which lowers the accuracy how blood measures reflect the changes after regular intake (Duyff 2006; Didriksen *et al.* 2015).

After oral ingestion, blood levels of specific substances reflect bioavailability from intestinal resorption, reservoir filling, and excretion. Quite frequently, we observed higher serum and blood levels after three month, than after 6 month indicating a fair bioavailability. The pattern of rapid raise, followed by plateau building or decrease as the intake of is continued indicates that tissue reservoirs are filled up by redistribution into tissue, before the excretion by the kidney contributed to a steady state blood level.

It may be relevant that LaVita® is a fluid. The relative bioavailability from fluids is generally higher as compared to the ingestion of tablets or capsules (Patel *et al.* 1984).

WATER SOLUBLE VITAMINS

Vitamin B1, Thiamine

Mean Vitamin B1 serum levels revealed an undulating tendency with significant heights after 3 months continuous consumption (Table 7, $p<0.001$) and decay of mean levels during the second 3 month period, resulting in a still significant level above baseline after 6 month. Our data of mean Thiamine serum levels represent typical metabolic pathways of this ingredient. Both, active transport and passive diffusion limit the active uptake of thiamine by blood cells and various tissues. In some tissues, thiamine uptake and secretion appears to be mediated by a soluble thiamine transporter that is dependent on Na^+ and a transcellular proton gradient (Bettendorff *et al.* 1996).

Vitamin B2, Riboflavin

Bioavailability starting from baseline serum levels were raised with high significance after 3 month, the increase was slower in the second three month term, however after 6 month the level remained highly significant above baseline (Table 7), indicating a very good bioavailability of Vitamin B2. A first pass effect after resorption in the liver may have had a considerable impact on the raising Riboflavin levels (Dainty *et al.* 2007). Some authors describe their preference to determine the vitamin B2 bioavailability from urine samples, it was suggested to determine bioavailability by analysing urine samples, as renal extinction reflects bioavailability as it increases after tissue saturation (Gibson 2005; Rutishauser *et al.* 1979). However, the observed significant increase of Vitamin B2 of mean serum levels, are in harmony with the concept of good bioavailability of Vitamin B2 in the tested composition.

Vitamin B3, Niacin

Mean serum levels showed the same pattern as Riboflavin with a significant increase in the first 3 month period. The increase was slightly reversed during the second 3 month term, but still significant at the end and at the end of the trial (Table 6). Again, this course can be attributed to tissue saturation and intracellular metabolism of the agent. Again, others have investigated the bioavailability of niacin by measuring the urine excretion of its metabolites. Both metabolites, M´methyl-2pyridone-5-carboamide and N´methyl-4-pyridone-3-carboxamide express a high creatinine-corrected correlation between niacin intakes and the urinary extinction (MacKay *et al.* 2012). In our study – as the blood levels showed consistent results, we hypothesize that a continuous control of serum niacin levels also provides sufficient bioavailability data from natural food sources.

Vitamin B5, Panthothenate

The profile was typical for substances with a high rate of intracellular metabolism after oral ingestion and intestinal resorption. Starting from baseline the mean serum levels increased after 3 month although not with statistical significance (Table 6). Most likely tissue saturation was reached before three month; and from this moment on an insignificant decrease revealed a serum level slightly above baseline after 6 month (Table 6). The bioavailability of vitamin B5 from the "average diet" is estimated to be as low as 50% (Tarr *et al.* 1981). After oral ingestion and absorption in the small intestine Vitamin B5 leaves blood rapidly become metabolised intracellularly by a series of enzymatic reactions to Coenzyme A. High tissue rates have been found in laboratory-animals liver, muscle and blood cells (Böhmer & Roth-Maier 2007). However such invasive measurements were beyond the capacity of our human study.

Vitamin B9, Folic acid

Varying definitions and issues about the ingested folate reference substance and dose from food and clinical samples limit the validity of many bioavailability studies (Wright *et al.* 2010). Genetic polymorphisms of folate/homocysteine metabolic enzymes add to the complexity to interpret the effects of intervention on folate status and bioavailability (Antoniades *et al.* 2009). The bioavailability of food folate is commonly estimated to

be around 50% (Nordic Nutrition Recommendations – Integrating Nutrition and Physical Activity 2005).

We prefer the definition of folate bioavailability as the resorbed fraction of ingested folate that can be used for metabolic processes (Melse-Boonstra *et al.* 2004). In our study we observed a significant increase of the mean serum level after 3 month (p=0.003, Table 6). The increase reversed during the second three month term to end up with serum levels in the range of baseline (p=0.496) after 6 month. Possibly the blood fluid compartment saturation was accomplished early and prompted substance redistribution into different body tissues as well as metabolic adaptions.

Despite Folic acid has no known function that would increase the likelihood of a causal role in disease development (Obeid & Herrmann 2012), concerns emerged whether or not increased folate intake may be associated with unexpected adverse effects (Hu *et al.* 2015). High serum levels of unmetabolized folic acid may inhibit dihydrofolate reductase and other enzymes (Choi *et al.* 2014). During pregnancy however, supplemented folic acid does not accumulate in umbilical cord blood, therefore unmetabolized folic acid is not likely to accumulate in the fetus. In contrast, 5-MTHF and THF accumulate in the fetus (Obeid *et al.* 2010). Our findings indicate that regular intake of our test substance did not cause folic acid at critical levels. Furthermore, we could show that serum levels of folic acid are a sufficient parameter to directly monitor the bioavailibilty of folic acid.

Vitamin B12

Watanabe (2007) reported that vitamin B12 bioavailability significantly decreases with increasing intake of vitamin B12 per meal with an average between 42–89% even under physiologic conditions. We considered to employ urinary excretion and measure metabolized Vitamin B12, but then opted for serum level determination to observe the kinetics and saturation directly (Unglaub *et al.* 1958). In our healthy human subjects Vitamin B12 serum levels increased rapidly after 3 month (p<0.001, Table 6), dropped in the second term between 3–6 month, revealed an significant increase after 6 month compared to from baseline (p<0.001). Evidently tissue saturation occurred during the early intake phase.

Biotin

Ingested Biotin is completely absorbed, even when pharmacologic doses are administered (Zempleni & Mock 1999). Therefore the fast rise in the first period (Table 6), followed by an insignificant decay, and high levels after 6 months (p<0.038) is well within the expectation. Animal trials have shown that Biotin can be detected in kidney, liver and brain specifically (Wang & Pevsner 1999). We assume that after a certain saturation phase biotin may be further enriched in such tissue, which may contribute to the observed decay of serum levels.

Vitamin C

Because there is no issue about bioavailability and toxicity with Vitamin C (Diplock 1995), we did not need to analyse this parameter. However, as ascorbic acid has the ability to reduce ferric to ferrous iron (Teucher *et al.* 2004), it is worth to mention that high vitamin C intake does not cause iron imbalance in healthy persons (Gerster 1999).

LIPID SOLUBLE VITAMINS

Fat soluble vitamins can accumulate in the tissue, and elicit adverse effects. Therefore, we monitored these Vitamins, to judge the benefit/risk ratio under constant intake.

Vitamin A

The Vitamin A absorption rate is approximately 80% (You *et al.* 2002). The bioavailability of this Vitamin is highly variable; it depends on dietary factors, fiber in the diet, as well as on characteristics of the target population, such as vitamin A status, nutrient deficiencies, gut integrity, and genetic polymorphisms associated with β-carotene metabolism (Haskell 2012). In our study Vitamin A increased after 3 month and dropped below baseline after 6 month, underlining the variability of this substance. It is known that lipid soluble Vitamin A accumulates in body stores after absorption. Tissues extract carotenoids from circulating chylomicrons in blood, but most retinyl esters are stripped from the chylomicron remnant, hydrolysed, and taken up primarily by parenchymal liver cells. If not immediately needed, retinol is re-esterified and retained in fat-storing cells (adipocytes, liver stellate or Ito cells). The liver parenchymal cells also take in substantial amounts of carotenoids. Whereas most of the body's vitamin A reserve remains in the liver, carotenoids are also deposited in fatty tissues throughout the body (Blomhoff *et al.* 1991). Considering the variability of the Vitamin A metabolism, our results may reflect the typical accumulation in fat tissue and intracellular binding (Parker 1996; Novotny *et al.* 1995; Green & Green 1994).

Vitamin E

Vitamin E is another lipid soluble agent with different isoforms and with widely still unknown bioavailability. Lately intestinal proteins have been discovered to be involved in the absorption of vitamin E (Borel *et al.* 2013). Animal trials corroborate the role of tissue saturation; maximum accretion or depletion of alpha-tocopherol occurred in plasma and liver before 6 weeks, but accretion in muscle required 3 month and depletion 180 days; further accumulating tissues were lung, subcutaneous fat, omental fat, perineal fat, kidney, diaphragm, and spinal cord (Arnold *et al.* 1993).

In our study Vitamin E serum levels increased fast. Compatible with the concept of body compartment saturation, the significant raise after the first 3 month

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period, was followed by drop levels to leave them significantly above baseline after 6 month (p=0.009, Table 6).

Vitamin K

The pattern of the Vitamin K dynamics is comparable to the other lipid soluble vitamins. Though, compared to baseline the changes did not reach significant levels after 3 and after 6 month (Table 6). Because liver keeps a huge reservoir of this vitamin, and high concentrations were also found in adrenal glands, lungs, bone marrow, kidneys and lymph nodes high redistribution rates may substantially contribute to overall low serum levels (Kindberg & Suttie 1989).

VITAMIN TOXICITY ISSUES

Vitamin A in high doses can be teratogenic. Safe doses are 10,000 IU/d or less, doses over 10,000 IU/d have been reported to cause malformations in a single epidemiologic study. No study reports adverse effects of 10,000 IU/d preformed vitamin A supplements, which is more than the Recommended Dietary Allowance (RDA) for pregnant women (2670 IU or 800 RE/d). Neither teratogenicity nor vitamin Toxicity has been observed in multiple species exposed to high doses of beta-carotene (Miller *et al.* 1998). Based on results in monkeys, a dose of 30,000 IU/day can be considered as non-teratogenic in man (Wiegand *et al.* 1998).

Based on a meta-analysis, supplementation with vitamin E appears to have no effect on all-cause mortality at doses up to 5,500 IU/d (Abner *et al.* 2011). It has been suggested that vitamin E, as ingested in the diet or in supplements that are rich in gamma- and delta-tocopherols, is cancer preventive; whereas supplementation with high doses of alpha-tocopherol is not (Yang *et al.* 2012). No toxicity has been reported for Vitamin K (Greer 2010).

SEMI-VITAMINS

Both, total L-Carnitine and free Carnitine starting at baseline showed an increase in serum levels after 3 and 6 month. The dynamics were similar to fat soluble vitamins. With an increase of mean average blood levels in the first section and a lowering tendency during the second 3 month period the blood level indicate a tissue saturation, a few month after regular intake. Renal clearance of L-carnitine contributes to keep the blood concentrations within the physiologic (Evans & Fornasini 2003).

In a Study on the pharmacokinetics of Ubiquinone (Coenzyme Q10) two plasma peaks were observed after oral administration, the first at 2–6 hours and the second about 24 hours after administration. Enterohepatic recycling and redistribution from the liver to the blood compartment can explain this pattern over time.

In our study Q10 showed a significant increase after 3 month (p<0.001) and a further increase after 6 month. However, because after 3 month the increase was slower we attribute this result to the enhanced bioavailability of our solubilized CoQ10 formulation and the tissue saturation in various reservoirs (Bhagavan & Chopra 2006).

MINERALS AND TRACE ELEMENTES

Iron

About 65% of the iron in the body is bound in haemoglobin in red blood cells whereas only 4% is bound in muscle tissue. Around 30% of the iron in the body is further stored as ferritin or hemosiderin in the spleen, the bone marrow and the liver. None of this iron is directly accessible by testing the serum (Arosio & Levi 2002): In our study both, mean serum Iron (Ferrum) and red blood cell depots were significantly increased after 3 month. The increase slowed or reversed during the second 3 month period. While after 6 month the serum levels were raised significantly, the red blood cell levels indicated saturation (Table 8). Therefore we conclude that reservoirs which are not accessible to blood analyses may benefit from the regular intake for longer time periods.

Selenium

The bioavailability of Selenium depends on the organic or inorganic presentation, genotypes, enzyme activities, etc. which modulate the bioavailability of selenium from different food sources (Fairweather-Tait *et al.* 2010). In our study Selenium showed a significant increase after 3 month (Table 8), proving that the Selenium in our fluid test composition is bioavailable. The dynamics in the second 3 month term indicate tissue saturation over the entire test period, an observation in harmony with other bioavailability tests of selenium from different food sources (Schrauzer 2000). Because most of the absorbed selenium is retained in the body and not excreted in the urine (Bügel *et al.* 2004), the significant decline of selenium serum levels during our second 3 month term may reflect the normal pathway of saturation and redistribution to body reservoirs.

Chromium

Chromium (III) is regarded an essential trace element since long, but the biochemical function and even basic transport pathways in the body remain unclear. Animal studies have shown a rather low bioavailability, which depends on the compounds and chemical status of the chromium supplement (Laschinsky *et al.* 2012). It is therefore interesting, that our study showed a continuous increase of the Chromium serum levels over the whole study period (Table 8). Known chromium reservoirs are liver, spleen, soft tissue, and bone (Lim *et al.* 1983). Again, the increase was slower in the second term, possibly indicating that the uptake rate approached saturation and possible redistribution to various body tissues, or excretion. The overall increase

proved a sufficient bioavailability of chromium from our test substance (Table 8).

Zinc

Most of absorbed zinc is distributed into the brain, muscle, bones, kidney, and liver, with the highest concentrations in the prostate and parts of the eye to maintain steady status of this essential trace element (Krebs 2000).Our results demonstrate that zinc resorption from our test substance was slow but constant (Table 8), reflecting the limits of Zinc absorption by numerous inhibitory factors (Lönnerdal 2000).

CONCLUSIONS

The need for robust bioavailability data is particularly important in the light of supplement intake for health promotion and disease prevention. Product safety requires basic data related to the continuous intake over an extended period.

We investigated the bioavailability of ingredients from a liquid composition from natural origin by interval blood analysis at baseline, and after 3 and 6 month to determine the bioavailability of the specific liquid formulation enriched with vitamins and trace elements. Because we observed possible interactions but an overall good bioavailability of the investigated ingredients we can offer a rationale for the continuous intake of the composition.

Specifically we conclude that,

1. The analysis of the ingredients in blood samples during the intake period of 6 month was adequate to reveal the bioavailability of the main ingredients of the tested composition.
2. In view of the low dropout rate after 6 month (Table 5) the results are representative for the recruited population.
3. The observed increase of the specific substances demonstrates a sufficient bioavailability.
4. Because some of the ingredients (Vitamins, Minerals and Trace elements) showed higher blood levels after three months, compared to six month, we conclude that the drop during further three month, either reflects the redistribution of specific parameters from the blood compartment into body tissue reservoirs, or alternatively renal excretion was triggered after tissue reservoirs have filled up.
5. The observed blood levels and the dynamics of the serum levels over time indicates that no unwanted exaggerated levels occurred.
6. Despite the complexity of the liquid composition, possible substance interferences did not cause fatal reductions of the specific bioavailability. Some low dosed ingredients raised the respective blood levels significantly.
7. Our results are relevant for the normal population, and underpin the strategy of micronutrient supplementation to fill up tissue reservoirs for e.g. preventive purposes.

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