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ORIGINAL ARTICLE

Potential of the multivitamin-mineral-trace element composition LaVita® before, during and after pregnancy

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Abstract

OBJECTIVES: Pregnancy is a period in life with a high demand of micronutrients. A prophylactic supplementation of folic acid to reduce the risk of neurological malformations in the newborn is common practice. The array of essential micronutrients during pregnancy includes neurotropic vitamins (Vitamin B6, B12 and folic acid), minerals like iron, and trace elements like zinc. As the serum level of most micronutritients is actively regulated by the organism, a prophylactic broad supplementation with a mild, but effective supplementation typically does not pose any risk for exaggerated serum levels, therefore prophylactic intake may be prefered to blood screening and specific interventions.
METHODS: To investigate the ingredients' bioavailability of the complex vitamin-mineral-trace element composition LaVita® we recruited healthy volunteers for six months and observed the changes of pregnancy relevant parameters by means of laboratory measures. The study design was prospective, double blind, placebo controlled, and included a "male group control". We determined baseline parameters of folate, vitamin B6 and vitamin B12, iron, zinc, homocysteine and Hb-alpha-1c. After three and six months of daily intake of the study substance the blood tests were repeated and compared to the baseline levels.
RESULTS: The regular intake resulted in an increase of the supplemented substances' serum levels. The metabolic parameter homocysteine decreased significantly, Hb-alpha-1c was slightly lowered.
CONCLUSION: The regular intake filled up the respective storage compartments and reservoirs in the tissues, and improved the metabolic status. Female participants tended to benefit more than male. We conclude that the composition is safe, and warrants optimized micronutrient supply during pregnancy or postnatal breastfeeding.

INTRODUCTION

Conceptivity, pregnancy and pregnancy outcome are associated with a high level metabolic state. Deficiency in key nutrients has been linked to reduced conceptivity, or pregnancy related malfunction like pre-eclampsia, restricted fetal growth, neural tube defects, skeletal deformity and low birth weight. To explore the potential of a natural vitamin, mineral and trace element composition, we accomplished a prospective study and observed the bioavailability of various pregnancy relevant ingredients, like iron, zinc, vitamin B6, folate, vitamin B12, biotin, and metabolic parameters like ferritin, Hb-alpha-1c and homocysteine, i.e. parameters that have been associated with a favourable pregnancy outcome.

Iron

According to the World Health Organization (WHO) the global prevalence of anemia is 14%. In industrialized countries the prevalence of iron deficiency anemia (IDA) in pregnant women is 17.4%, going close to 60% in less developed countries. Most of the anemic pregnant women are already anemic before conception (Roodenburg 1995; Khalafallah & Dennis 2012). Therefore the WHO issued iron supplementation programs during pregnancy to prevent maternal anaemia, puerperal sepsis, low birth weight, and preterm birth http://www.who.int/elena/titles/guidance_summaries/daily_iron_pregnancy/en/. Accordingly, in countries like the USA and France pregnant woman are advised to take iron supplements of 30 to 60 mg/d routinely, in Australia and the UK the protocol is to screen and treat those with diagnosed deficiency (Friedrisch & Friedrisch 2017).

Both, iron deficiency and associated anemia in pregnant women may be associated with pre-eclampsia, low birth weight, prematurity, perinatal mortality, delayed fetal maturation, impaired neurocognitive development and impaired motor capacity (Zhou *et al.* 2006).

The demand increases from 800 µg daily intake in the first trimester to 7500 µg/d in the last trimester of pregnancy (Scholl 2011). The dietary iron intake in gestation should be at least 27 mg and during the lactation period 10 mg, compared to 1 to 8 mg in the general adult population (Roodenburg 1995; Achebe & Gafter-Gvili 2017). Our verum test substance contains 4 mg of iron for a mild supplementation on a daily basis (Table 1).

Zinc

Zinc – an essential mineral with rather low bioavailbility – is essential for various biological functions like protein synthesis, cell division, nucleic acid metabolism, etc. Zinc deficiency can occur associated with low consumption of zinc-rich animal-source foods, and/or high intake of foods rich in phytates, which inhibits zinc resorption (Parr 1996; Chaffee & King 2012). It is estimated that the zinc intake in over 80% of pregnant women is as low as only 9.6 mg zinc per day, which is below the recommended minimum for the second and third trimester of pregnancy (Caulfield *et al.* 1998).

It has been suggested that maternal zinc deficiency is associated with impaired infant development, which is a risk for poor birth outcomes (Caulfield *et al.* 1998). Low plasma zinc concentrations during pregnancy cause low placental zinc supply (Moghimi *et al.* 2017). This condition may alter various hormone levels associated with the onset of labour. Because zinc is essential for normal immune function, zinc deficiency may contribute to systemic and intra-uterine infections (Lamberti *et al.* 2016).

Zinc supplementation conveys no particular risk and may improve pregnancy outcomes for mothers and infants. Two meta-analyses of randomised controlled trials on zinc supplementation during pregnancy conducted across five continents between 1977 and 2008, concluded that zinc supplementation was associated with a significant reduction in preterm birth. This effect of zinc supplementation on premature birth may be due to a reduction in the incidence or severity of maternal infections, which are a known risk factor for premature birth. On this basis other authors recommended that in populations at risk for zinc deficiency zinc should be supplemented during pregnancy (Hess & King 2009).

Vitamin B6

Vitamin B6 – a water-soluble vitamin – plays a vital role in numerous metabolic processes, including the development of a normal nervous system. Vitamin B6 is a cofactor for more than 140 essential enzymes involved in amino acid metabolism, like aminotransferases, decarboxylases, racemases and dehydratases. This emphasizes its role in maternal health and fetal development (Salam *et al.* 2015). B6 is a coenzyme for δ-aminolevulinate synthase, which catalyzes the first step in heme biosynthesis and for cystathionine β-synthase and cystathioninase, enzymes involved in the transsulfuration pathway from homocysteine to cysteine. Much of the total vitamin B6 in the body is involved in phosphorylase activity in muscles.

Classical clinical symptoms of vitamin B6 deficiency are seborrheic dermatitis, microcytic anemia, epileptiform convulsions, depression and confusion. Many studies in B6-depleted experimental animals – especially in severe B6 depletion – descibed findings like low levels of neurotransmitters (dopamine, serotonin, and γ-aminobutyrate).

Vitamin B6 supplementation may convey some relief from pregnancy-related nausea. In animal trials there is no indication that supplementation is associated with any teratogenic risk, even a 5- to 6-fold oversupplementation of vitamin B6 had no adverse effects (Almeida *et al.* 2015).

Folic Acid (Folate)

Folate is a generic name for the group of chemically related compounds with the folic acid structure. Dietary folate is a naturally occurring nutrient typically found in leafy green vegetables, legumes, egg yolk, liver and citrus fruit. In contrast, folic acid is a synthetic dietary supplement.

Neither folate nor folic acid are metabolically active. The predominant biologically active form in the plasma is 5-methyltetrahydrofolate (methylfolate). In the digestive system, the majority of dietary folate is converted into 5-methyltetrahydrofolate before entering the bloodstream (Patanwala *et al.* 2014). Supplemented folic acid is enzymatically converted to dihydrofolate (DHF) and tetrahydrofolate (THF). Only then THF can be converted to the biologically active l-methylfolate by the enzyme methylenetetrahydrofolate reductase (MTHFR). A key function of l-methylfolate is methyl donation for purine/pyrimidine synthesis during DNA and RNA assembly, for DNA methylation and regulation of amino acid synthesis and homocysteine metabolism (Pietrzik *et al.* 2010).

Folate deficiency during pregnancy has been linked to abnormalities like anemia and peripheral neuropathy in the pregnant mother and congenital abnormalities in the fetuses (De Wals *et al.* 2007).

Vitamin B12

Vitamin B12 (B12) is found in animal-derived foods. It is important for the synthesis and methylation of DNA and plays a role in the cells' energy metabolism (Allen *et al.* 1993). Few supplementation-studies of B12 in pregnancy investigated possible effects on birth weight and length of gestation. A meta analysis supports the conclusion that B12 deficiency affects placentation and fetal growth and increases the risk of preterm birth (Rogne *et al.* 2017). Fetal and maternal plasma concentrations of vitamin B12 correlate strongly, and oral maternal B12 supplementation effectively improves the infant vitamin B12 status as well (Frery *et al.* 1992; Allen 2012). Infants of mothers, who were in the lowest decile of B12 concentrations at the 28th week of gestation performed less well on tests of attention and memory compared with infants born to mothers of the highest decile (Bhate *et al.* 2008). Furthermore, infant B12 supplementation was associated with improved gross motor function and less frequent gastrointestinal regurgitation compared with placebo (Torsvik *et al.* 2013). Vitamin B12 may also bear a later effect on the metabolism of the developing child, low maternal B-12 plasma concentrations in pregnancy correlated with increased insulin resistance, as measured by the IR-HOMA (Insuline Resistance Homeostasis Model Assessment) in children aged 6 years (Yajnik *et al.* 2008).

Biotin

The water-soluble biotin (vitamin B7) acts as a coenzyme to carboxylases and is involved in gluconeogenesis, fatty acid synthesis, and amino acid catabolism. Reduced activity of biotin-dependent enzymes (acetyl-CoA carboxylase I and II, and propionyl-CoA carboxylase) alters lipid metabolism and may impair synthesis of polyunsaturated fatty acids and prostaglandins. In addition, biotin contributes to the modulation of gene expression by binding covalently to histones. Biotin is also an essential cofactor of methylcrotonyl-CoA carboxylase, the reduced activity of methylcrotonyl-CoA carboxylase may lead to the accumulation of its substrate 3-methylcrotonyl CoA. As acyl-CoA compounds are compartmentalized within the mitochondria, accumulation of 3-methylcrotonyl CoA may lead to a disruption of the esterified CoA to free CoA ratios and ultimately lead to potentially lethal mitochondrial toxicity (Pasquali *et al.* 2006; Zammit *et al.* 2009).

Tab. 1. Ingredients
Ingredients of the verum test substance; the daily intake was 2 x 10 = 20 ml

Ingredients	per 10 ml
ß Carotene	4000 µg
Vitamin B1	3 mg
Vitamin B2	2,5 mg
Viamin B3 (Niacine)	40 mg
Viamin 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
Coenzyme Q10 (Q10)	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 acids	30 mg

Tab. 2. Laboratory parameters
Laboratory parameters determined at three time points; before (M0, baseline) after three months (M3) and after six month (M6) of regular intake.

Parameter	Laboratory code	Unit	Reference range
Iron (cellular)	FEHK	mg/l	440 – 480
Iron (blood)	FEHB	mg/l	440 – 480
Ferritin	FERR	ng/ml	13 – 300
Zinc (cellular)	ZNHK	mg/l	7,30 – 7,70
Zinc (blood)	ZNHB	mg/l	7,30 – 7,70
Folate	AltFOLX	ng/ml	280 – 800
Vit B12	VITB12MOD	pg/ml	193 – 982
Vit B6	VITB6E	ng/ml	4,1 – 43,7
Biotin	Biotin	ng/l	> 200
Adiponectine	ADIPOEX	g/ml	> 6,1
Hb-alpha-1c (relative)	HBA1	%	4,1 - 6,0
Hb-alpha-1c (absolute)	HBA1MO	mmol/mol	22 – 42
Homocysteine	HOMO	µmol/l	6 – 12

Some early studies on the biotin status during pregnancy described significantly lower biotin plasma concentrations, compared to non pregnant women (Dostalova 1984). While mild deficiency may be common in normal pregnancy, Zempleni and Mock (2000) suggested that biotin deficiency may be teratogenic in humans.

Hyperinsulinemia in pregnancy

Pregnancy increases the risk for obesity if post partum the physiological state of hyperinsulinemia is maintained. The placenta supplies the growing fetus with nutrients and water, furthermore it produces various hormones to maintain the pregnancy. Some of these hormones, like estrogen, cortisol and human placental lactogen inhibit the activity of insulin. This physiologic inhibition usually begins at about pregnancy week 20 to 24. As the placenta grows, the hormonal situation contributes to an increased insulin resistance. Women with gestational diabetes generally have normal blood glucose levels during the critical first trimester when the baby's organs form. The signs and symptoms associated with hyperinsulinemic hypoglycemia result from 2 physiologic processes: hypoglycemia activates the autonomic nervous system and releases epinephrine, CNS glucopenia leads to neurologic manifestations.

The post-birth hyperinsulinemia increases the risk of developing gestational hypertension (Hamasaki *et al.* 1996). Furthermore gestational diabetes mellitus increases the risk for later type 2 diabetes mellitus (T2DM), obesity and cardiovascular disease in both, mother and offspring (Dörner & Plagemann 1994).

Study rationale

Without the aim to develop general rules for supplementation during pregnancy – the focus of this study was to examine whether or not the regular consumption of the natural vitamin and trace elements composition LaVita® could satisfy the increased demand of micro and macronutritients during pregnancy, but do not cause overdose.

METHODS

Study design

The study protocol and the ingredients of the study substance were described before (Muss *et al.* 2015a). Briefly, we recruited healthy male and female volunteers. Persons with acute medical treatments, that could possibly interfere with our endpoints were excluded from participation. The recruitment phase spanned all 12 months of the year to avoid bias due to seasonal effects. In total 159 eligible persons consented to the

Tab. 3. Participants
Analyzed participants (female/male) and their allocation to the groups (verum/control)

Verum / Placebo	Gender	N
Control	F	26
	M	10
	Total	36
Verum	F	66
	M	41
	Total	107
Total	F	92
	M	51
	Total	143

Tab. 4. Verum only - results
The parameters shown in this table were determined after intake of the verum substance only. The analyses explored the changes of the respective parameter from baseline (Visit 1, M0) during the first or second 3 month periods by means of the Student t-test for paired data. After Bonferoni *p* adjustment for multiple testing all *p*-values < 0,01 indicate a significant difference (indicated by bold letters).

	Visit 1 (M0)			Period M0-M3				Period M3-M6				Period M0-M6			
	Mean	**N**	**SEM**	**Mean Dif**	**SEM**	**DF**	***p***	**Mean Dif**	**SEM**	**DF**	***p***	**Mean Dif**	**SEM**	**DF**	***p***
Zinc Blood	6,43	30	0,17	+ 0,18	0,11	29	0,1130	+ 0,48	0,07	28	**0,0000**	+ 0,63	0,12	28	**0,0000**
Zinc Cell	6,34	30	0,15	+ 0,12	0,09	29	0,1870	+ 0,41	0,06	28	**0,0000**	+ 0,49	0,09	28	**0,0000**
Vit B6	28,74	30	4,28	+ 41,84	7,98	29	**0,0000**	- 10,93	8,84	28	0,2260	+ 32,18	9,92	28	**0,0030**
Biotin	208,30	30	30,25	+ 224,86	47,61	29	**0,0000**	-126,07	48,35	28	0,0140	+ 108,41	49,91	28	0,0380
Folic Acid	348,20	30	19,21	+ 56,63	17,28	29	**0,0030**	-65,86	16,76	28	**0,0010**	-11,28	16,34	28	0,4960

Legend: Mean Diff – Difference of Means between the timepoints, SEM - Standard Error of Means, DF - Degree of Freedom, *p* - statistical error probability.

terms of the study and were recruited. In an open first phase 30 participants received verum only. Then the study trial design was prospective, randomized, double-blind, placebo-controlled; it complied with GCP (Good Clinical Practice) research guidelines.

The randomization was done with the software "Rancode" (IDV Gauting, Germany http://idvgauting.de/). Because this software was no longer updated, we switched to "RandList V.1.2" (datinf GmbH Tübingen http://randomisierung.de/). The ratio verum:placebo was 3:1, the block size was 12.

To guarantee double blinding the two test substances (verum, placebo) were packed in numbered carrier bags according to the group allocation in the randomisation list. The bags were sealed with string, the free ends knotted, covered with adhesive label and stamped. The list with the unblinding codes and the sealing stamp were locked up in the office of team 01. The sealed carrier bags were arranged in ascending order and brought to the team 02 (laboratory), where samples for the laboratory analyses were taken before the participants left the laboratory with a sealed carrier bag. To defend the double blinded randomisation against deciphering, the recruited patients allocated themselves to the respective study arm by picking their personal sample out of a stack with the numbered sealed carrier bag. The number of the respective carrier bag was documented in the recruitment documents at the front desk of the laboratory. The documents were stored and periodically picked up by team 01 for further processing. At the end of the recruitment phase no participant number was documented twice, which would have indicated an allocation and/or documentation mistake.

Study substance raw materials and ingredients

Among the raw materials for manufacturing the verum study substance LaVita® there are 22 herbs, 22 fruits, 12 vegetables, 7 fermented juices, 4 vegetal oils and other ingredients like aloe vera juice, green tea, and maté. A detailed list was published before (Muss *et al.* 2015a). The verum is fortified with minerals and trace elements. Additionally, the final product contains secondary plant constituents, enzymes, amino acids, minerals, trace elements, vitamins and semi-vitamins such as L-carnitine, coenzyme Q10 and omega-3 fatty acids (Table 1). The control substance was described before (Muss *et al.* 2015b), it was a concentrate of fruit and vegetable juice without any fortification.

To investigate the possible benefits of an adequate supplementation before and during pregnancy as preventive measure, we observed healthy females and males for a 6 month intake period, and determined serum levels of ingredients and parameters associated with respective metabolic changes. The blood and serum parameters were determined by certified medical laboratories (Table 2). The baseline parameters were determined before any intake (timepoint: M0), then after 3 months of regular intake (timepoint: M3), and again after further 3 months of regular intake at the exit visit after 6 months (timepoint: M6).

Statistical analyses

Because few participants dropped out and because single parameters strained during laboratory analyses, the number of parameters may vary from parameter to parameter. The actual number of analysed parameters for each analysis is given in the respective table. To compare the baseline parameters between male and female and between verum and placebo group from the first visit (M0), with those obtained after three month (2nd visit, M3) and six month (3rd visit, M6), from the verum- and placebo-group, we used the software-package IBM-SPSS (Version 24). The obtained parameters were analysed for differences by means of the students T-test, or multivariate ANOVA with the baseline levels (M0) as covariable (ANCOVA). The specific statistical

Tab. 5. Verum placebo
Comparison of verum/placebo via multivariate General Linear Model (GLM) model ANOVA at baseline (visit 1), after three months (visit 2), and six months (visit 3);
While the supplementation let the respective parameters increase in the blood, the parameters ferritin, homocysteine, Hb-alpha-1c decreased. Adiponectine revealed no particular dynamics during the entire intake phase. The differences between the verum and the placebo group were significant for Vit B12 and homocysteine. For more specificity we continued with the analyses of gender specific differences (see Table 6).

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Parameter		Mean at Visit 1			Mean at Visit 2			Mean at Visit 3		
	Unit	Placebo	Verum	Signif. *p*	Placebo	Verum	Signif. *p*	Placebo	Verum	Signif. *p*
Iron (blood)	mg/l	444,2 ± 45,1 (40)	450,5 ± 41,3 (112)	0,419	451,5 ± 46,6 (36)	455,8±37,5 (107)	0,816	444,3 ± 54,6 (36)	456,6 ± 38,3 (107)	0,204
Iron (cellular)	mg/l	447,2 ± 29,8 (40)	440,5 ± 18,1 (112)	0,097	453,7 ± 40,1 (36)	446,9 ± 24,5 (107)	0,976	444,6 ± 45,3 (36)	443,6 ± 16,6 (107)	0,289
Ferritin	ng/ml	108,5 ± 101,6 (43)	111,5 ± 94,1 (86)	0,870	90,9 ± 104,6 (36)	112 ± 92,9 (78)	0,198	91,8 ± 103,3 (36)	103,3 ±74 (78)	0,749
Vit B12	pg/ml	532,1 ± 317,7 (40)	445,3 ± 194,3 (112)	0,111	546,4 ± 298,6 (36)	535,7 ± 217,8 (107)	0,013	557,2 ± 361,6 (36)	512,3 ± 203,7(107)	0,537
Homocysteine	µmol/l	12,4 ± 4,1 (43)	11,6 ± 3,4 (116)	0,221	11,6 ± 5,6 (36)	9,4 ± 2,6 (107)	0,040	12,1 ± 6,0 (36)	10 ± 2,7 (107)	0,126
Adiponectine	g/ml	9,8 ± 4,4 (40)	9,8 ± 5,9 (112)	0,984	10,1 ± 5,7 (36)	10,1 ± 6 (107)	0,632	10,6 ± 5,5 (36)	9,8 ± 5,7 (107)	0,490
Hb-alpha-1c (relative)	%	5,4 ± 0,5 (43)	5,3 ± 0,4 (101)	0,640	5,4 ± 0,4 (36)	5,3 ±0,4 (92)	0,884	5,4 ± 0,4 (36)	5,3 ± 0,4 (92)	0,144
Hb-alpha-1c (absolute)	mmol/ mol	35,0 ±5,3 (43)	34,7 ±4,2 (101)	0,729	35,3 ± 4,8 (36)	34,7 ± 4,2 (92)	0,738	35,1 ± 4,8 (36)	34,1 ± 4,4 (92)	0,092

test for differences between placebo/verum and gender (PV*Gender) is outlined in the legends of the respective tables. The significance level was set to $p \leq 0,05$, the value for statistical tendency was set at $p \leq 0,1$.

RESULTS

Table 3 shows the group allocation of the participants who ended their participation according to protocol, and contributed data at baseline, midterm, and after six month at the the exit visit.

Table 4 provides the baseline values and the changes after intake for three and six moths of parameters observed during the first open phase. The zinc level increased continuously over the entire observational period. The intake of the verum lead to a significant increase of vitamin B6 and biotin serum level after three months with still significantly raised values after six months. Folic acid increased, reached a peak after three month and showed no increase after six month (Table 4).

Table 5 shows an overall group comparison (placebo/verum) in both genders (male, female) of healthy participants. Since expected gender specific data ranges raise the variance, and this may obscure subtle intake related differences, we proceeded with gender specific analyses.

Gender comparison

Table 6 shows the results of the gender specific analyses. The iron levels (cellular and whole blood, Figure 1) and the iron storage protein and inflammation marker ferritin showed significantly higher mean baseline values in men. The differences in the iron parameters became smaller after three and six month due to a higher increase of serum iron in females, compared to males. The ferritin levels did not change significantly throughout the observation time, neither in male nor in female participants.

Also the mean baseline blood zinc level showed a gender specific distribution, it was lower in females than in males (p=0,017, Figure 2). And again this statistically significant differences between male and female participants leveled out after three and six month (p=0,774; Table 6).

Vit B6 – at baseline – was higher in men, in females it tripled after three months. After six months it had dropped in females, while it further increased in males (Table 6, Figure 3), possibly indicating that tissue reservoirs are filled up in females, but not in males yet. The biotin values showed a similar gender specific distribution from baseline to the end of the observation time (Table 6, Figure 4). Both, the increase of corresponding levels after 3 months of consumption and a fall in females during the second term was mostly due

Tab. 6. Gender differences

Gender comparison, multivariate ANOVA analysing the differences at baseline (visit 1) between placebo/verum and gender (male/female); ANCOVA for visit 2 (M3) and 3 (M6), the baseline data served as covariable.

Before intake (M0, visit 1) some statistically significant differences exist between male and female participants, no differences exist if bonds with the group allocation is also considered (PV*gender). After three months, the differences between female and male participants were smaller, suggesting that the female participants had more benefit from the intake, e.g. the increase of iron.

Parameter	Baseline data (M0), Mean of Visit 1			PV*Gender	After 3 Months (M3,) Mean of Visit 2			PV*Gender	Exit data (M6), Mean of Visit 3			PV*Gender
	Male	Female	Signif. *p*	Signif. *p*	Male	Female	Signif. *p*	Signif. *p*	Male	Female	Signif. *p*	Signif. *p*
Iron (Blood)	485,2 ± 30 (53)	429,4 ± 34,3 (99)	0,000	0,898	489 ± 30,2 (51)	436 ± 31,0 (92)	0,002	0,919	487 ± 30,7 (51)	435 ± 37,6 (92)	0,108	0,913
Iron (Cellular)	448 ± 15,8 (53)	439,2 ± 24 (99)	0,026	0,922	456,3 ± 22,4 (51)	444,4 ± 31,7 (92)	0,637	0,107	448,8 ± 19,1 (51)	441,2 ± 30 (92)	0,947	0,363
Ferritin	164,1 ± 114,5 (46)	80,8 ± 69 (83)	0,000	0,761	148,7 ± 106,8 (41)	81 ± 81,7 (73)	0,604	0,325	144 ± 100,5 (41)	74,7 ± 61 (73)	0,407	0,110
Zinc (Blood)	7,0 ± 0,7 (10)	6,1 ± 0,9 (20)	0,017	--	7,2 ± 1,0 (10)	6,3 ± 0,9 (19)	0,425	--	7,6 ± 1,0 (10)	6,8 ± 0,9 (19)	0,774	--
Zinc (Cellular)	6,5 ± 0,6 (10)	6,2 ± 0,9 (20)	0,348	--	6,7 ± 0,8 (10)	6,2 ± 0,8 (19)	0,218	--	7,1 ± 0,9 (10)	6,7 ± 0,7 (19)	0,330	--
Vit B6	38 ± 17,8 (10)	24,1 ± 24,9 (20)	0,127	--	67,6 ± 34,3 (10)	74,8 ± 39,4 (19)	0,614	--	88,7 ± 49,8 (10)	47 ± 40,9 (19)	0,019	--
Biotin	220 ± 177,8 (11)	194,8 ± 156,2 (21)	0,741	--	356 ± 192 (10)	489 ± 200,2 (19)	0,098	--	391,9 ± 198,4 (10)	277,6 ± 195,7 (19)	0,148	--
Folate	305 ± 45,3 (10)	369,8 ± 120,2 (20)	0,113	--	350,8 ± 47,9 (10)	430,4 ± 121,4 (19)	0,238	--	301,3 ± 90,2 (10)	356 ± 77 (19)	0,373	--
Vit B12	462,1 ± 202,6 (53)	471,5 ± 251,7 (99)	0,881	0,604	552,2 ± 218,7 (51)	530,8 ± 251,3 (92)	0,355	0,844	539,1 ± 269,4 (51)	515 ± 243,2 (92)	0,015	0,002
Homocysteine	12,7 ± 3,9 (56)	11,4 ± 3,4 (103)	0,051	0,985	10,1 ± 3,1 (51)	10,0 ± 4 (92)	0,314	0,652	11,1 ± 3 (51)	10,2 ± 4,3 (92)	0,766	0,127
Adiponectine	6,6 ± 3,3 (53)	11,5 ± 5,7 (99)	0,000	0,220	6,4 ± 3,1 (51)	12,2 ± 6,1 (92)	0,154	0,771	6,7 ± 3,8 (51)	11,9 ± 5,7 (92)	0,174	0,495
Hb-alpha-1c (relative)	5,3 ± 0,3 (50)	5,3 ± 0,5 (94)	0,799	0,874	5,3 ± 0,3 (45)	5,4 ± 0,4 (83)	0,925	0,760	5,3 ± 0,3 (45)	5,3 ± 0,5 (83)	0,224	0,942
Hb-alpha-1c (absolute)	34,7 ± 3,7 (50)	34,9 ± 4,9 (94)	0,796	0,789	34,5 ± 3,8 (45)	35,1 ± 4,6 (83)	0,584	0,627	34,3 ± 3,7 (45)	34,4 ± 4,9 (83)	0,220	0,804

to dynamic adaptions in females (Table 6). For Folate (Figure 5) and B12 the observed parameter changes rates did not show preferences for any gender.

Metabolic parameters

The analyses of biomarkers indicating a preventive effect of vitamin supplementation in pregnancy focussed on selected parameters of the amino acid and carbohydrate metabolism, like homocysteine, adiponectine and Hb-alpha-1c. The baseline homocysteine levels were higher in men (Table 6, p=0,051), after three and six month of verum intake this difference was leveled out. Starting at baseline there was a significant drop of homocysteine levels after three months in both, male and female participants. Then – in the next three months – the values in males increase slowly, while the values remained low in the female group until the end of the observation time (Figure 6).

For the effect on the carbohydrate metabolism we investigated adiponectine and Hb-alpha-1c. At baseline the mean adiponectine levels differed significantly between men and women (Table 6), there were no particular changes throughout the observation time (Figure 7).

Starting from the Hb-alpha-1c baseline levels the placebo values remained largely unchanged. In the verum group, these parameters showed a tendency to decrease during the six months observation period (Figure 8).

DISCUSSION

We studied quantitative and functional parameters for risk minimization in pregnancy by applying nutrients (Table 1) from natural biological sources in the form of a liquid composition (LaVita®) over a period of six month in a healthy population.

Our data are based on the analysis of a randomly selected cohort of healthy male and female volunteers (Table 3), who received the test substance over a period of six months and who were monitored for specific serum and blood levels (Table 2). Taking into account findings on the effect of individual active substances from the literature, our data indicate possible benefits and a risk reducing potential of our test for women before, during and after pregnancy.

Minerals iron and zinc

Iron deficiency has been attributed to several birth complications, perinatal mortality, delayed fetal maturation, neurocognitive development and motor capacity impairment of the child (Zhou *et al.* 2006).

We observed a significant increase of iron levels in our verum group, although the total amount of supplementation was 8 mg per day only (Figure 1). On the one hand our test substance contained non-heme iron only, which is known for a lower bioavailability (2–20%; Hurrell & Egli 2010). On the other hand we attribute the sufficient iron bioavailability to the content of food components enhancing iron absorption and biavailability like ascorbic acid and other antioxidants (Hallberg *et al.* 1989). We assume that the high content of vitamin C and other oxidants in our verum substance boosted the iron bioavailability and thus contributed to the observed improvement of the iron levels.

A zinc deficiency during pregnancy is associated with considerable risk (Roodenburg 1995; Parr 1996; Khalafallah & Dennis 2012). In industrialized countries an inadequately low dietary intake of zinc is less likely than the inhibition of zinc absorption by phytates present in staple foods like cereals, corn and rice. Phytates like inositol hexaphosphates and pentaphosphates are strong inhibitors of zinc absorption from mixed meals (Lönnerdal 2000).

We were not able to measure the amount of phytate intake in our participants, while we observed sufficient zinc bioavailability. The supplementation with 10 mg zinc daily from the multivitamin herbal composition was sufficient to improve the levels significantly (Table 4, Figure 2).

Vitamin B6 and biotin

Besides the potential prevention of pre-eclampsia and the prevention of preterm birth pyridoxine (Lui *et al.* 1985; Cotter *et al.* 2003) vitamin B6 has also a strong impact on the degradation of homocysteine – a general risk factor in pregnancy. In our study vitamin B6 showed sufficient bioavailability which led to an increase in serum levels after three and six months after verum intake (Figure 3). In this respect B6 contributes to the prevention potential of our verum-substance (Barnard *et al.* 1987; Brussaard *et al.* 1997a; Brussaard *et al.* 1997b). Interestingly the increase in men was steady throughout the six month observation period, while in females the storages seemed filled up, which explains the observed female specific drop in the second three months period (Figure 3). The biotin serum levels revealed a very similar pattern: while in male we observed a steady increase, in female participants – after an initial increase – the values dropped during the second three months term (Figure 4). The serum values after three months and six months in our female volunteers show that supplementation is effective, and does not pose any risk for exaggerated serum levels.

Folic acid and vitamin B12

The human gut appears to have a very efficient capacity to convert reduced dietary folates to 5-MTHF but limited ability to reduce folic acid (Patanwala *et al.* 2014). Frosst *et al.* (1995) have identified a mutation in the gene for MTHFR with a frequency of approximately 38% in the population. Therefore the supplementation of natural folate – requiring less conversions to the active form than the manufactored folic acid – may be of advantage.

Dietary supplementation with folic acid around the time of conception has long been known to reduce the

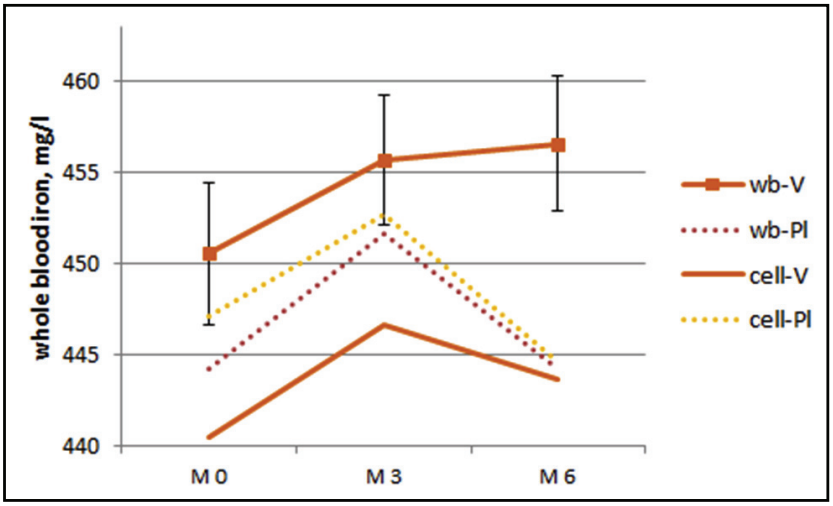

Fig. 1. Iron
Iron; whole blood (wb) and cellular, verum (V, N=112) and placebo (Pl, N=40). Both groups started with baseline values in the lower reference range (table 2). In the placebo group the values at the end of the observation time were in the range and below of the baseline values. In the verum group, after 3 months the values increased and after additional 3 months the mean scores were well above the baseline value.

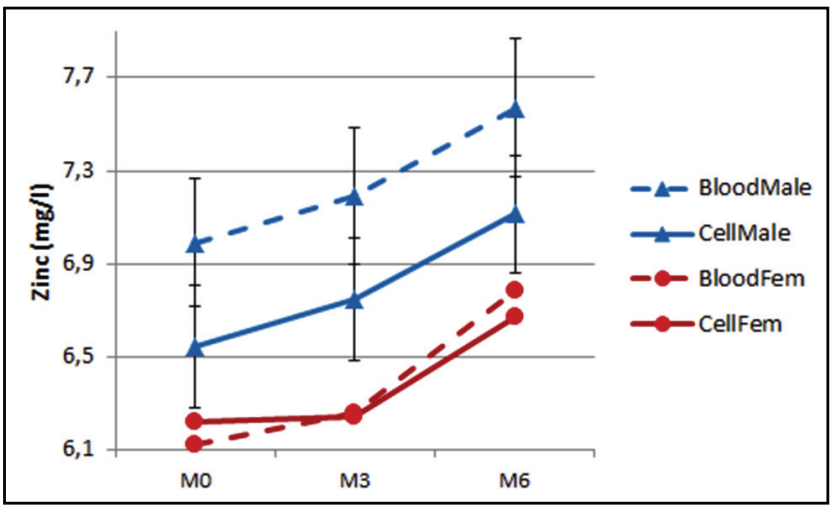

Fig. 2. Zinc
Zinc in whole blood or red blood cells at baseline (M0), after 3 months (M3) and after 6 months of intake (M6). Note that the zinc level increased continuously over the entire observational period in both, male and female participants.

offsprings' risk of neural tube defects (De Wals *et al.* 2007). Deficiency of active folate has been associated with abnormalities in both mothers (anemia, peripheral neuropathy) and fetuses (congenital abnormalities) (Greenberg *et al.* 2011).

We were able to document a significant rise of folic levels after verum intake for three month (Table 4) and a drop of serum levels most likely due to filled up storage compartments in the second 3 month observational period. In contrast to vitamin B6 and biotin the values dropped not only in females but in both gender groups to indicate saturated tissue depots (Figure 5).

The intake of 400 µg folate twice per day seemed sufficent to cause a serum level increase after three month with modulated levels again after six month close to baseline. To assure that women have adequate folate stores during pregnancy, the US National Institutes of Health (NIH) has recommended that 600 µg of folic acid be taken daily by pregnant women, and that this supplementation can be continued throughout pregnancy and reduced to 500 µg during lactation (Institute of Medicine; Standing Committee on the Scientific Evaluation of Dietary Reference, Intakes Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamine B12, Panthothenic Acid, Biotin, and choline 1998). The bioavailability of folates from fruit, vegetables and liver is approximately 80% of that of folic acid. A diet rich in food folate can improve the folate status of a population

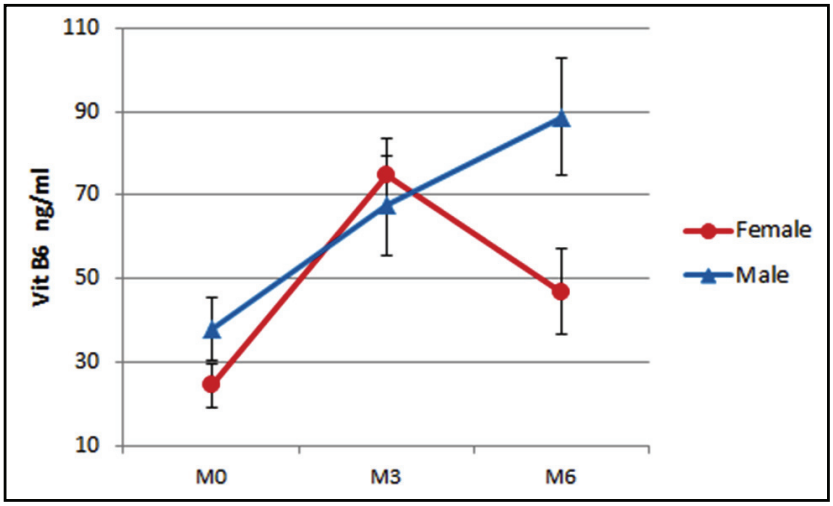

Fig. 3. Vitamin B6
Serum-Vitamin B6 at baseline (M0), after 3 months (M3), and after 6 months of verum intake (M6). Note that the intake more than doubled the serum level after 3 months, the values remained high after 6 months. The reduction of the serum level in females after 3 months may indicate that the tissue reservoirs are filled up. In any case the downregulation in female participants indicates, that the prophylactic supplementation with Vitamin B6 does not pose any risk for exaggerated serum levels.

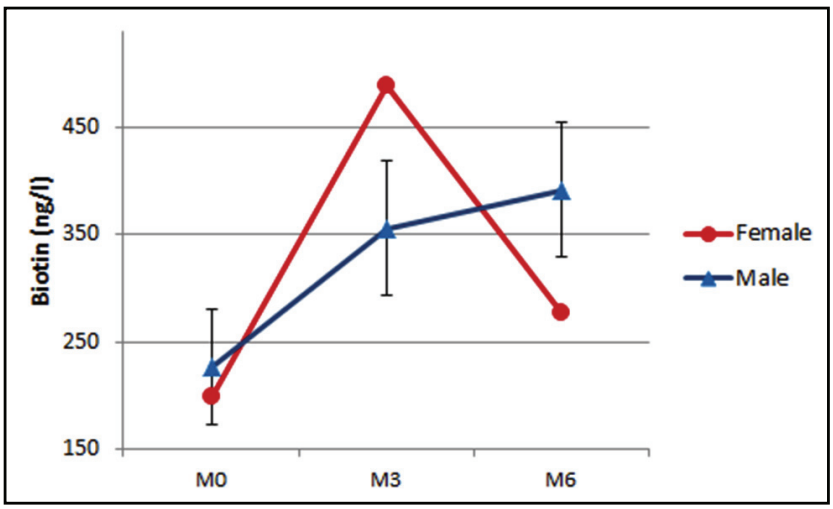

Fig. 4. Biotin
Biotin levels in male and female volunteers, while we observed a steady increase in male, the values in females dropped after three months indicating saturated tissues. Again the downregulation in the females confirms, that the supplementation with Biotin is safe.

more efficiently than is generally assumed (Winkels *et al.* 2007). As we have seen downregulation of the serum levels possibly owed to saturated storage compartments in the second three month term, our data indicate that the 800 µg in our verum may be sufficient.

Chronic liver exposure to industrially produced folic acid (not folate) in humans may induce saturation, which can explain reports of systemic circulation of unmetabolized folic acid (Patanwala *et al.* 2014). This is a cause for concern, since high levels of un-metabolized folic acid have been associated with several health problems (Sweeney *et al.* 2006). In this respect it may be of considerable advantage, that our test substance contains folate from natural sources..The observed drop (Figure 5) clearly indicates that any overdose is downregulated by the organism, making the folat-supplementation with the verum a fairly safe strategy, also because it contains folate from natural food and folic acid.

We observed increasing vitamin B12 serum levels after verum intake for three months. Probably as a result of tissue saturation or redistribution the values dropped in the second three month term, but nevertheless remained above baseline after 6 months (Table 6). In the context of state of the art folic acid

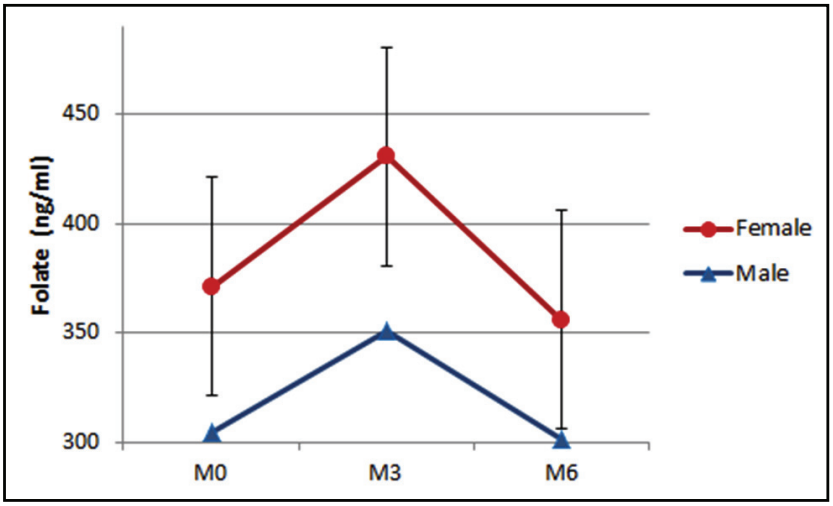

Fig. 5. Folate
Folate at baseline (M0), after 3 months (M3) and after 6 months of verum intake (M6). Note that after a significant increase of the serum level, the values dropped during the second 3 month term. After 6 month the serum level were in the range of baseline in both, male and female participants, making it fairly unlikely that an overdose or accumulation might occur.

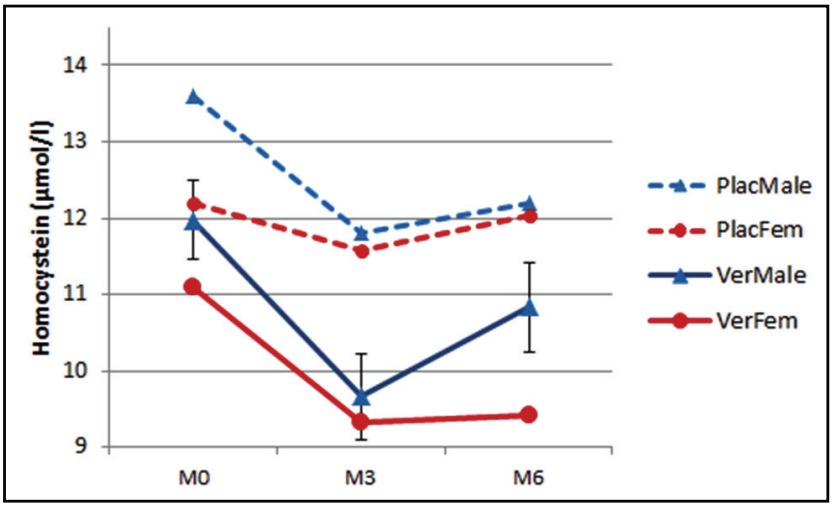

Fig. 6. Homocysteine
Homocysteine serum values; while the baseline values (M0) were similar between the verum (—) and placebo (- - -) and between males and females. The intake of the verum decreased the homocysteine values significantly. This effect very likely is associated with the increase of various Vitamin B in the serum. While in male the values increased again to some extend after 3 month, in females the beneficial decrease was maintained to the end of the observation period.

supplementation during pregnacy, and because folate therapy increases the risk for masking a vitamin B12 (cobalamin) deficiency (Sinow *et al.* 1987) our verum composition may not only considered to be a save, but also preferable to a single substance folate supplement.

Metabolic effects

To underline the potential beneficial effects of supplementation in pregnancy we measured in addition to quantitative scores of several vitamins and minerals the metabolic parameters homocysteine adiponectine and Hb-alpha 1c (Figure 6, Figure 7, Figure 8). The data indicate that the intake of the verum preparation can improve the metabolic situation of men and women, also during pregnancy.

High homocysteine is a generally recognizied risk factor of pregnancy, similar as low folate. Genetic defects of some enzymes in the homocysteine metabolism, or nutritional deficiencies of folic acid, vitamin B6 and B12 lead to an increase in homocysteine plasma levels. In pregnant women severe pre-eclampsia was associated with higher plasma homocysteine levels in early pregnancy. Low folic acid, vitamin B6 and B12 is

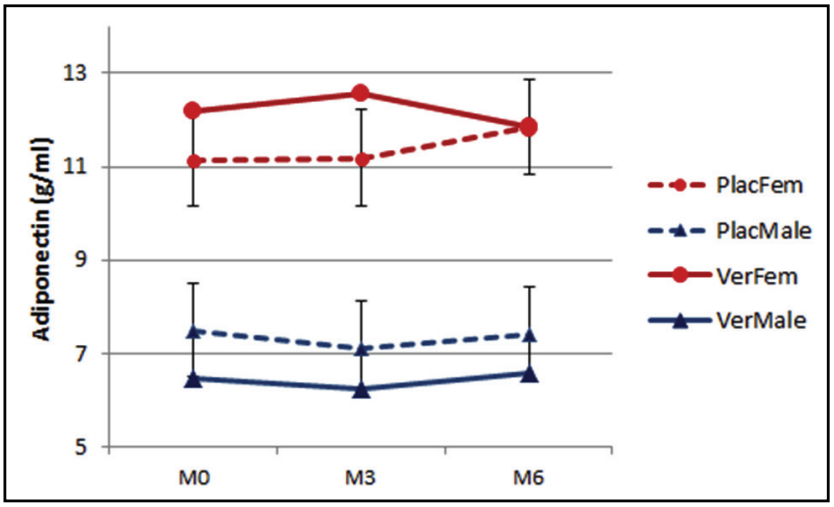

Fig. 7. Adiponectine
Adiponectine, verum and placebo, male and female, the difference between male and female was significant. During the six month intervention and observation in no gender group significant changes occurred.

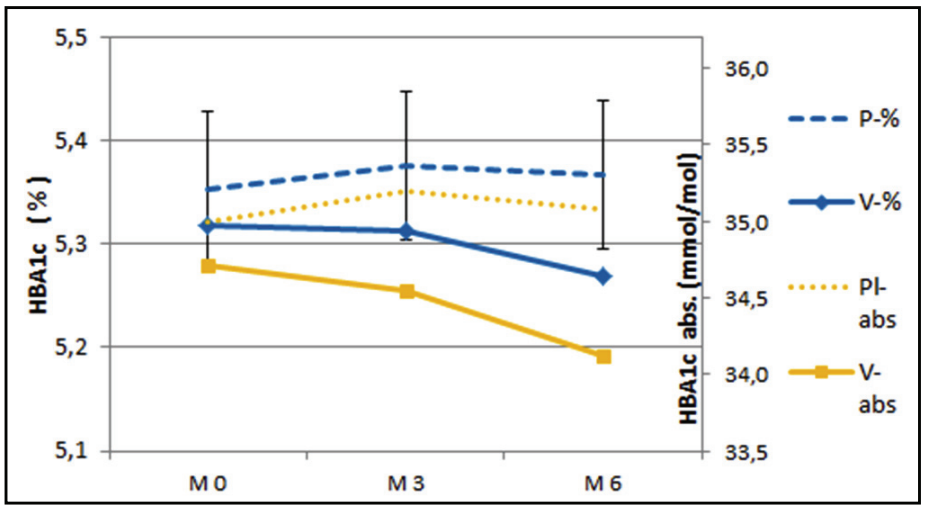

Fig. 8. Hb-alpha-1c
Hb-alpha-1c in percent and absolute measures in verum (V) and placebo (Pl); While the placebo values (- - -) remained at the same level, in the verum group (—) the average Hb-alpha-1c levels decreased gradually over the 6 months observation period.

associated with high homocysteine plasma concentration and is associated to a higher risk for cardiovascular diseases (Aleman *et al.* 2001). Our verum group revealed significantly lowered homocysteine levels than our placebo group. We attribute this effect to the demonstrated bioavailability and serum increase of vitamin B6, folic acid and vitamin B12.

Often pregnancy complications are associated with changes in metabolism. Our focus was therefore also to observe values relating to diabetic shifts. We studied the development of long-term blood sugar fluctuations (Hb-alpha-1c) and adiponectine in our cohort. Alltough not statistically significant, we found some indication for an antidiabetogenic effect, which can certainly be relevant for pregnant women.

SUMMARY

Due to concerns to perform a study with pregnant women, we conducted our study as a double blind randomized placebo controlled design with healthy volunteers. After three and six months, the ingredients proved to be bioavailable, the corresponding serum levels were raised. Furthermore, serum parameters, that can be relevant for pregnant women improved. At the same time we did not observe any cumulations towards toxic concentrations.

On the presumption that pregnancy does not adversely modify the bioavailability of specific substances our results directly relate to alimentary recommendations for the pregnancy period. The observed serum parameters, such as folic acid, vitamin B6, biotin, vitamin B12, iron and zinc, all are relevant for the prenatal and postnatal (breastfeeding) time span. In addition, we witnessed a significant reduction in mean homocysteine, and we saw some beneficial trends attributable to the carbohydrate metabolism.

Both, qualitative and quantitative results, let us suggest, that pregnant women can benefit from a multivitamin mineral and trace element composition. LaVita® is save and can be beneficial for the prenatal pregnancy and the pregnancy phase and the postnatal breastfeeding period.

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