The effect on biomarkers of tissue toxicity of a low-dose multivitamin and mineral supplement in human volunteers.

Abstract:

The benefits of multi-vitamin and mineral supplements in the (elderly) population are questioned and even adverse side effects have been reported. In the present study, the potential adverse effects of a low-dose of multivitamin and minerals is examined by a biomarker approach in human volunteers. A low dose of vitamins and minerals being 100% of the recommended daily intake (RDI) of each vitamin and 18-150% of the RDI of minerals was given for one month and 2 times this dose for another month. The renal toxicity was monitored by measurement of serum of creatinine, urea and uric acid. Changes in renal biomarkers were not observed in each of the groups. Hepatotoxicity was followed by the enzymes alanine aminotransferase and aspartate aminotransferase, albumin, total cholesterol and bilirubin. The activity of alanine aminotransferase statistically significantly increased in both women groups only. In the young group, the activities increased from 15.2 IU/L to 18.1 IU/L (P = 0.102). In the older women group, the activity increased from 19.0 to 20.9 IU/L (P = 0.017). The increase in the activity of aspartate aminotransferase occurred in the two women groups as well, but the increase was only statistically significant in the older women group with a mean increase from 20.5 to 23.4 IU/L (P = 0.013). In 16% of the women, the enzyme activities were above the upper threshold value of the clinical reference range after supplementation. This finding supports the recommendation to perform more toxicity studies on supplements before marketing.

Keywords**:** multi-vitamins; supplementation; human; biomarkers; liver toxicity.

1. Introduction

The use of vitamin and mineral supplements is increasing in the general population [1], especially in the elderly [2]. The benefits of antioxidant vitamins, however, are questioned in several studies [3-8]. In some studies, even adverse effects were reported [9-12], especially for carotenoids, vitamin E and higher doses

of vitamin A. Aging is often not considered to be an important factor in these studies. The vitamin and mineral requirements for adults change with age. Due to a decreased intake of energy, a deficiency of vitamins and minerals can occur, so that there is a greater need for adequate intake of some vitamins and minerals, which may be provided by means of supplementation.

However, few studies have examined its use in terms of nutritional risk. Not many studies in the literature studied antioxidant effects during supplementation of low doses of multivitamins and minerals on organ levels in humans. Ribeiro et al. [13] and Hoelzl et al [14] studied the antioxidant effects of multivitamins only at the DNA level. Cockle et al. [15] viewed cognitive functions and Chavange et al. [16] at infections in the elderly after multivitamin supplements. In studies of Fabian et al. [2, 17] and Huang [18] biomarker approaches were used to learn the effects of multivitamins in the elderly but in all these studies no tissue biomarkers were provided.

Therefore, in a project on aging and effects of antioxidant vitamins, we investigated potential harmful effects of supplementation of a low dose of multi-vitamins and minerals on the liver and kidney in different age groups.

2. Materials and Methods

2.1 Human volunteer study.

The technical data of the study and the characteristics of the participants including exclusion criteria have been described in detail elsewhere [19]. Some of the exclusion criteria that are important for this study were: if they had taken supplements in the last three months with one of the tested vitamins or minerals or vitamin-enriched food intake in the last three months, if they use oral contraceptive or hormonal replacement therapy use (women), if they had undiagnosed digestion problems that can negatively affect nutrient and supplements absorption, if they were excessive alcohol use (more than 1-2 standard units of alcohol per day), if they had extreme beliefs or behavior related to diet and health. In addition, a semi-quantitative questionnaire was taken on nutrition, life-style and diseases, from which no interferring habits nor large variations in nutrition were observed for all the participants.

In short, the supplementation study included 82 healthy adult participants randomly selected from Kaunas population register: 37 men and 45 women divided into two age groups; 16 men and 21 women aged 30-35 years (young groups) and 21 men and 24 women aged 60-65 years (old groups). A control group with a placebo was not used in this study because it was not possible to obtain a placebo with an identical composition of carrier materials as in the vitamin and mineral supplement. The biomarker concentrations of each of the participants at the start of the study were used as individual controls.

The study was approved by the regional ethics committee at the Lithuanian University of Health Sciences. Each participant signed the informed consent (protocol No P1-05/06) prior to the intervention. The study was completed by 12, 18, 16 and 20 of the participants for the study groups of young men (MY), old men (MO), young women (WY) and old women (WO), respectively. Figure 1 shows the study design including the numbers of participants who completed the study. The 16 participants who did not complete the whole study, gave up during the first period. Their reasons to quit cannot be attributed to the use of the supplements in this study. The different groups were identified according to gender, age and dose or duration of the multi-vitamin and mineral supplement: young men receiving 1x the RDI for the first four weeks are indicated as group MY1 and for the second period (2x the RDI) the young men are referred to as group MY2 and so on.

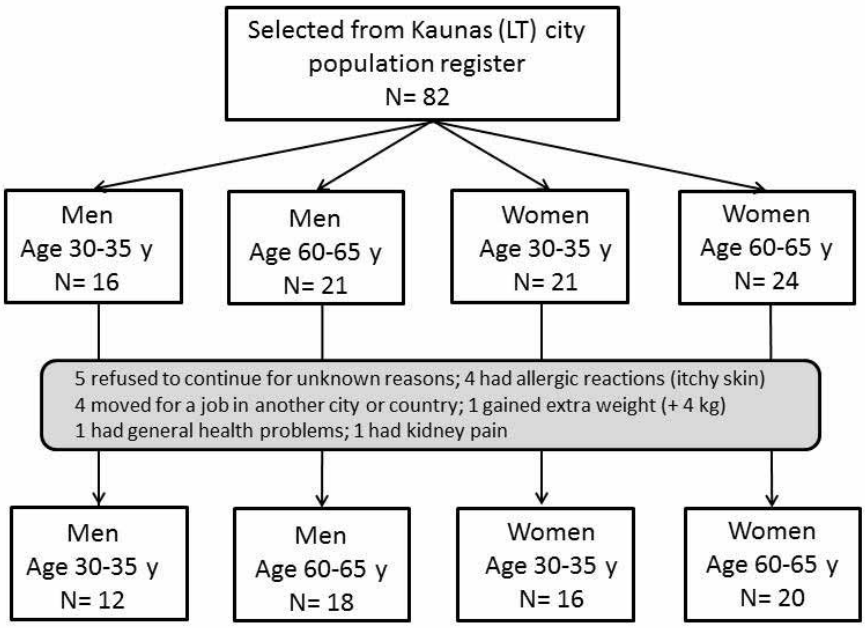

Figure 1. Design of the human volunteer study including the numbers of participants who completed the study.

The participants received a multi-vitamin and multi-mineral preparation (one pill) containing a dose of the recommended daily intake (RDI) of each component for the first four weeks and two times the same preparation (two times the RDI) for another four weeks. The multi-vitamin and mineral preparation was

obtained from a national drugstore and contained a dose corresponding to the RDI as recommended in the Netherlands. The composition of vitamins and minerals in the supplement is shown in Table 1. In addition, the following compounds were present as filler, stabilizer, glazing agent, anti-caking agent or collorant: cellulose, silicium dioxide, magnesium stearate, propylene glycol, triacetine, hydroxypropylmethylcellulose, sodium croscarmellose, iron oxide and titanium dioxide.

Blood sampling to obtain serum was performed at three points: a day before the start of the study, after 4 weeks and at 8 weeks at the end of the study. After blood withdrawal and serum preparation, all samples were frozen within 2 hrs and stored at -80 °C. After shipping on dry-ice to Bilthoven (the Netherlands), the samples were stored at -80 °C until analysis.

Table 1: Composition of the daily advised dose of the multi-vitamin and mineral supplement used in this study.

component	amount	% RDI		component	amount	% RDI
vitamin A	800 µg	100		calcium	162 mg	20
vitamin B1	1.1 mg	100		phosphor	125 mg	18
vitamin B2	1.4 mg	100		iodine	150 µg	100
vitamin B3	16 mg	100		iron	14 mg	100
vitamin B5	6 mg	100		magnesium	125 mg	33
vitamin B6	1.4 mg	100		copper	1.5 mg	150
folic acid	200 µg	100		manganese	2.5 mg	125
vitamin B12	2.5 µg	100		chromium	25 µg	63
vitamin C	80 mg	100		molybdene	25 µg	50
vitamin D3	5 µg	100		selenium	25 µg	45
vitamin E	12 mg	100		zinc	15 mg	150
biotine	50 µg	100				
vitamin K	75 µg	100				

2.2 Measurements of biomarkers.

The biomarkers in serum, creatinine (CREAT), urea (UREA), uric acid (UA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), total cholesterol (CHOL) and total bilirubin (BILI) were determined with an auto-analyzer (LX-20 Pro, Beckman-Coulter, Woerden, the Netherlands) using kits of the same company. All samples were measured in one batch on the same day. For quality control purposes three quality control sera were included before and after the measurements of the

samples. The average intra-assay coefficients of variation (N=8) of the biomarkers were: for CREAT 1.5 %, UREA 1.1 %, UA 1.7 %, ALT 1.6 %, AST 1.7 %, ALB 1.3 %, CHOL 1.8 % and BILI 4.2 %.

Vitamin E (α-tocopherol) was extracted from 250 µl plasma using hexane, after adding vitamin E nicotinate as internal standard. The hexane layer was separated and evaporated under nitrogen. The residue was dissolved in 250 µl iso-propanol and 20 µl was injected on a Hypersil BDS C-18 end-capped column (Agilent, Palo Alto, CA, USA). Isocratic elution occurred with a mobile phase which was a mixture of water and methanol (98%, v/v). Detection was performed with an UV-detector at a wavelength of 295 nm.

Concentrations of 25-hydroxy vitamin D was measured with an immune analyzer (Access-2, Beckman-Coulter, Woerden, the Netherlands) with a dedicated kit and corresponding Control materials.

2.3 Statistical analyses

For the statistical analysis, the participants were divided into 4 groups: younger men (MY), older men (MO), younger women (WY) and older women (WO). The average concentrations of the different biomarkers at the time points 30 and 60 days were expressed as percentage of the mean value at the start of the study. After a test for equal variances, a paired t-test was used to check for statistically significant differences within one group between the start and the two endpoints of the study.

3. Results

3.1 Compliance to the supplementation.

To Check for the compliance of the participants to adhere to the supplementation, plasma analyses of two fat-soluble vitamins, vitamin E and vitamin D were performed. As biomarker for the vitamin E intake, α-tocopherol was measured. To assess the vitamin D intake, 25-hydroxy vitamin D was measured, being the main metabolite in plasma. In Figure 2 and 3, the concentrations of both vitamins are shown for the 4 groups during the course of the study. It can be concluded that the levels of the vitamins increased during the study in all groups, which indicate a good compliance to the supplementation plan.

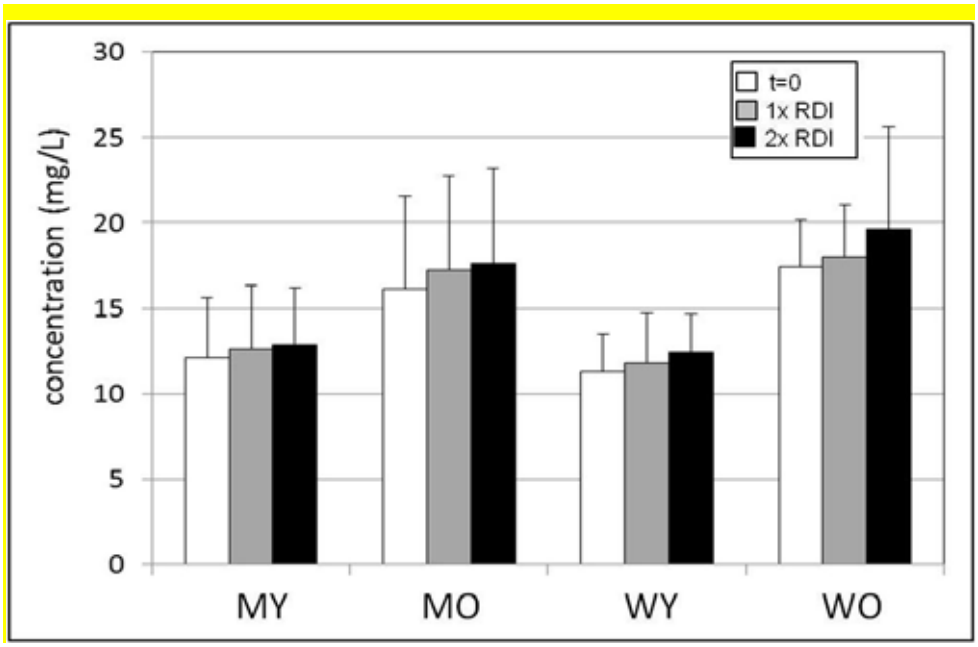

Figure 2: Vitamin E concentrations in plasma of the participants.

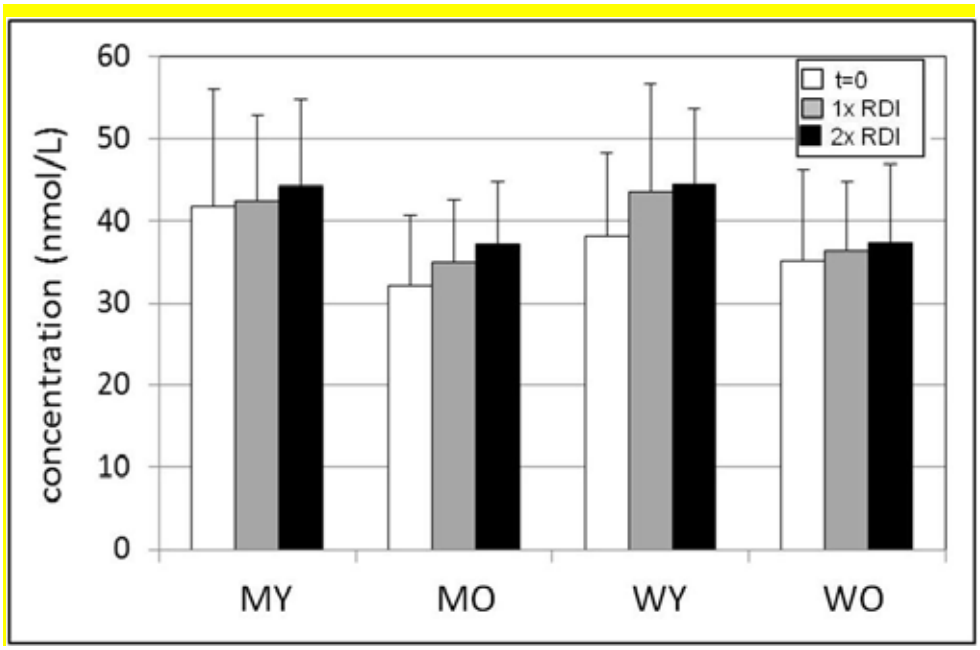

Figure 3: 25OH-vitamin D concentrations in plasma of the participants.

3.2 Baseline values of biomarkers of kidney toxicity

To assess possible effects on the kidney, three biomarkers, CREAT, UREA and UA, were measured in serum samples of the participants. The average concentrations of the biomarkers at baseline are shown in Table 2.

At baseline the following statistically significant differences were observed. The average concentration of CREAT in both male groups was 34.3 % and 17.8 % higher compared with the corresponding female groups with p-values of 0.0026 and 0.0032, respectively. No statistical difference was observed between the young and old groups of both men and women.

UREA was 9.4 % and 23.6 % higher in both elderly groups compared with the corresponding younger groups, but the difference was not statistically significant. No statistical difference was observed between the male and female groups.

The average concentration of UA was 4.6 % lower in the MO group compared with the MY and 4.7 % higher in the WO group compared with the WY group, with p-values of 0.0004 and 0.0078, respectively.

Table 2: Average concentrations of biomarkers at baseline of the participants in the various groups: MY (N=12), MO (N=18), WY (N=16) and WO (N=20). The statistically significant differences between the groups have been described in the text.

Biomarker	MY	MO	WY	WO
CREAT (µmol/L)	83.4 ± 10.8	83.3 ± 9.1	62.1 ± 11.0	70.7 ± 14.5
UREA (mmol/L)	4.2 ± 1.2	4.6 ± 1.3	3.4 ± 1.1	4.2 ± 1.6
UA (µmol/L)	352 ± 77	336 ± 81	255 ± 49	267 ± 56
ALB (g/L)	44.6 ± 2.9	42.0 ± 2.3	43.6 ± 2.1	41.4 ± 1.8
ALT (IU/L)	26.8 ± 11.4	27.6 ± 15.3	15.2 ± 4.4	16.8 ± 4.7
AST (IU/L)	24.1 ± 5.4	26.4 ± 7.5	19.0 ± 3.0	20.5 ± 4.0
CHOL (mmol/L)	5.1 ± 1.0	6.2 ± 1.3	5.2 ± 0.8	7.3 ± 1.1
BILI (µmol/L)	14.0 ± 5.2	13.4 ± 4.2	12.3 ± 4.5	9.9 ± 2.2

Abbreviations used:

MY: young men, MO: old men, WY: young women and WO: old women.

CREAT: creatinine, UREA: urea, UA: uric acid, ALB: albumin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, CHOL: total cholesterol and BILI: total bilirubin.

3.3 Baseline values of biomarkers of liver toxicity.

The potential liver toxicity was assessed by measurement of five different biomarkers: the liver enzymes ALT and AST, and the metabolic biomarkers ALB, CHOL and BILI.

At baseline, the following statistically significant differences were observed. ALB was somewhat lower (about 0.5%) in both elderly groups compared with the corresponding younger groups as is shown in Table 2. These differences were statistically significant with p-values of 0.012 and 0.0016, respectively. No statistical difference was observed between the male and female groups.

ALT showed somewhat higher activities in both elderly groups compared with the younger groups, but these differences were not statistically significant. The mean concentrations of ALT in both female groups (young and old) were about 40% lower than in the corresponding male groups, with statistically significant p-values of 0.0009 and 0.005 for the young and elderly groups, respectively.

For AST at baseline, higher activities were observed in both young and old male compared with the corresponding female groups. The difference was about 20% with p-values of 0.0035 and 0.0043, respectively for the male and female groups. No statistical difference was observed between age groups (Table 2).

The average concentrations of CHOL in the elderly groups are higher at baseline by 21.6 % for the male group and 40.4 % for the female group, with p-values of 0.02 and 0.00004, respectively. Also, a difference in the average concentration of CHOL was observed between the MO and WO groups. The WO group showed a 17% higher average concentration with a p-value of 0.012.

For BILI both elderly group show lower average values than the younger groups at baseline, but only the difference for women was statistically significant with a p-value of 0.044. There was also a higher average concentration in the MO group (35.3 %) compared with the WO group with a p-value of 0.0046, but not in the younger groups. The average concentrations of the biomarkers at baseline are shown in Table 2.

3.4 Effects of supplementation on kidney biomarkers.

After the intake of a multi-vitamin and -mineral supplement containing 1x the RDI for 4 weeks and 2x the RDI for another 4 weeks, there was no change in the concentrations of the three biomarkers of renal toxicity. In all the groups, the concentrations of the biomarkers were not changed in a statistically significant way as is shown in Figure 4.

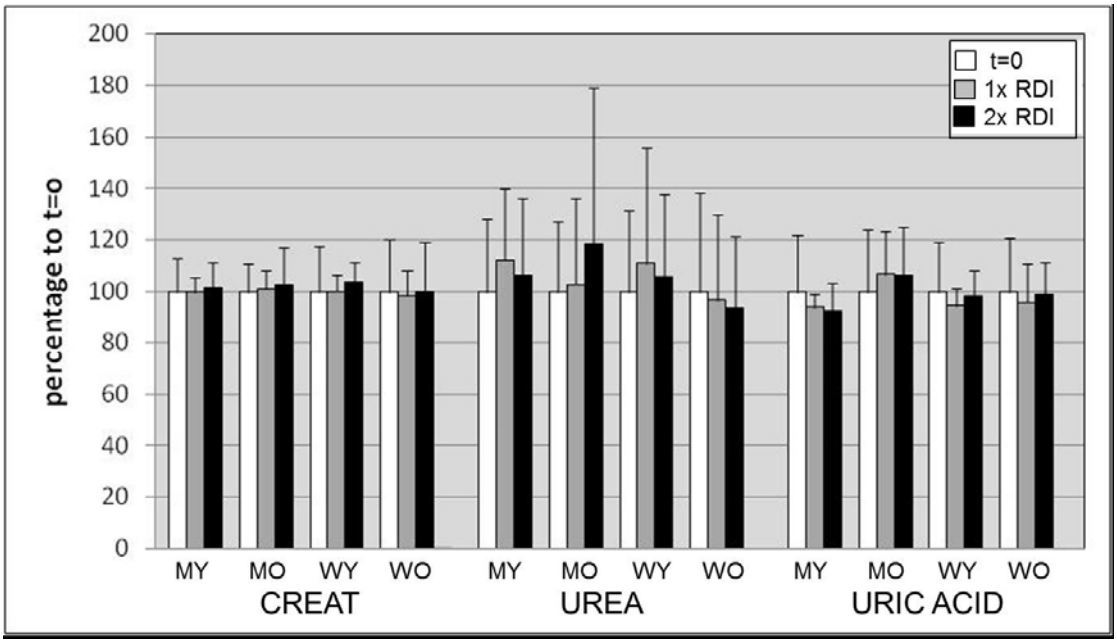

Figure 4. Concentrations of biomarkers of renal toxicity after supplementation of a multi-vitamin and -mineral supplement. The average concentrations of the different biomarkers at the time points 30 (1x RDI) and 60 days (2x RDI) were expressed as percentage of the mean value at the start of the study. After a test for equal variances, a paired t-test was used to check for statistically significant differences within one group between the start and the two endpoints of the study. No statistically significant differences were found in the renal biomarkers.

Abbreviations used:

MY: young men, MO: old men, WY: young women and WO: old women.

CREAT: creatinine, UREA: urea, UA: uric acid,

3.5 Effects of supplementation on liver biomarkers

After supplementation, the metabolic biomarkers ALB, CHOL and BILI, showed no statistically significant changes during the whole period, although in the WO group BILI increased after both periods with about 12% and almost reached statistical significance (p=0.071 and 0.057).

The activity of ALT, however, increased statistically significantly in both women groups upon supplementation. In the young group, the activity of ALT increased from 15.2 IU/L to 19.6 IU/L after 30 days and to 18.1 IU/L after 60 days, with p-values of 0.006 and 0.102, respectively. In the older women group, the activity of ALT increased from 19.0 to 20.1 IU/L after 30 days and to 20.9 IU/L after 60 days, with p-values of 0.002 and 0.017, respectively. Also in the young men group, the activities of ALT increased after 30 and 60 days, respectively, but these increases were not statistically significant (Figure 5).

An increase in the activity of AST occurred in both women groups, but the increase was only statistically significant in the older women group. In the young group the mean activity of AST increased from 16.8 IU/L to 20.1 IU/L after 30 days and to 21.5 IU/L after 60 days. In the older women group, the mean activity of AST increased from 20.5 to 22.9 IU/L after 30 days and to 23.4 IU/L after 60 days, with p-values of 0.001 and 0.013, respectively.

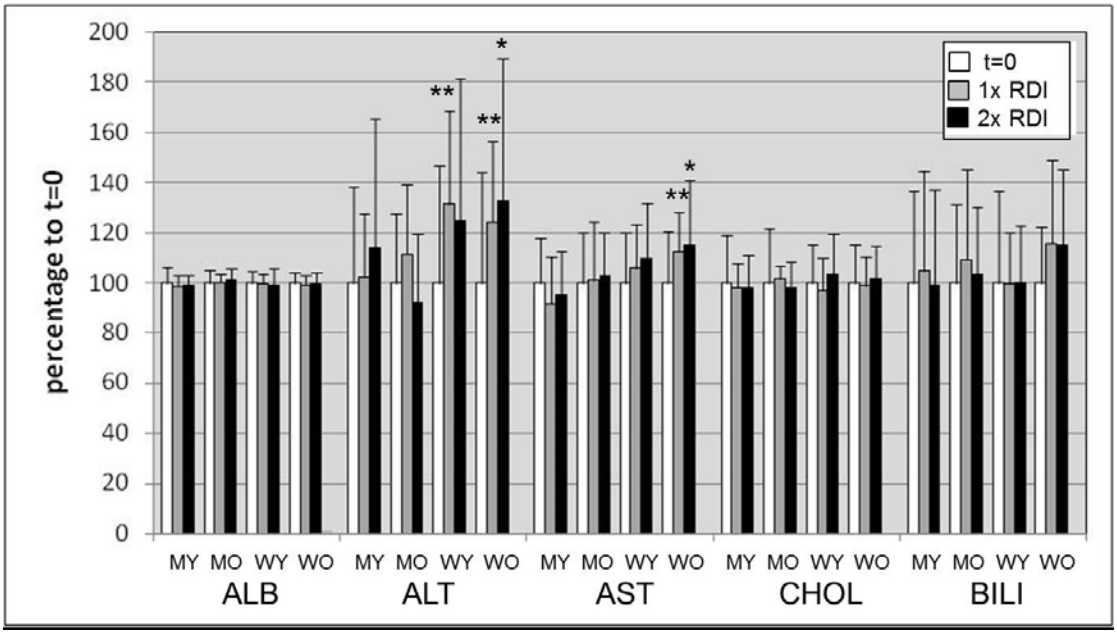

Figure 5. Concentrations of biomarkers of hepatic toxicity after supplementation of a multi-vitamin and -mineral supplement. The average concentrations of the different biomarkers at the time points 30 (1x RDI) and 60 days (2x RDI) were expressed as percentage of the mean value at the start of the study. After a test for equal variances, a paired t-test was used to check for statistically significant differences within one group between the start and the two endpoints of the study (* $p<0.05$; ** $p<0.01$).

4. Discussion

In a previous report, the positive effects on the vitamin status in serum in this study were reported, together with some age-related changes in the redox status [19]. In this report, the possible hepatic and renal toxicity of supplementation of a low dose of multivitamins and minerals was studied in healthy human volunteers.

For the liver function, five biomarkers were measured including the liver enzymes ALT and AST. An increase of ALT or AST is not expected to occur on supplementation of low doses of a multivitamin and mineral supplement. However, a statistically significant increase was observed in the two female groups for ALT and also in the older women group for AST.

Are these increased levels of the liver enzymes adverse?

In a large-scale study in literature, the median activities of ALT and AST for a healthy female population were 18 IU/L and 21 IU/L, respectively [20]. In another study, it was reported that the upper threshold concentration of the reference range for women is for ALT 35 (age range <60 y) and 28 IU/L (age range >60 y) and for AST 31 IU/L for all ages [21]. At the start of the study no female participants had activities above these values for both enzymes. After 2 months of supplementation in total 6 women (16%) had enzyme activities higher than the upper threshold level of the clinical reference range (2 women from the young group and 4 women from the old group). After 2 months of supplementation, in about 60 % of the participants of the younger female group, the activities of ALT and AST were increased and in about 80% of the participants in the older female group. Apparently, the older women are more sensitive to changes which affect liver enzymes. Although a statistical significant increase was observed in the activities of two liver enzymes, the levels of the enzyme activities were in general only moderately increased above the upper threshold value. Therefore adverse effects are not to be expected, although a long-term use of supplementation should be accompanied by periodical testing of hepatic biomarkers. The finding that only women in this study had increased liver enzymes and none of the men, remained to be explained.

The question which component of the multi-vitamin and mineral supplement can be held responsible for this effects. Since this increase in liver enzymes is observed in women only, a possible candidate can be vitamin A, because of the high sensitivity of women for this vitamin. Other candidates could be vitamin E [22,23] or iron based on their accumulation properties, although only a small increase in vitamin E levels in plasma was observed. The supplement contained however also a number of filling agents. Although it is not expected that these compounds can cause the observed effects in the liver, we cannot completely rule out this possibility, because a control group with a placebo was not used in this study. The participants were supposed to act as their own control as the biomarkers were determined at the start of the study.

The limitations of the present study are furthermore the relatively low statistical power in some of the groups. In spite of this, a significant increase in two liver enzymes was observed, indicating that already in a limited group of people for a short time an adverse effect was found. This finding has to be confirmed in other studies. Also the period of supplementation is rather short in this study (2 months). Because people tend to take this kind of vitamin supplements for longer periods on top of their diet, possible effects of chronic use should be tested also in further studies.

In conclusion, in this study we show that supplementation of a low dose of multivitamins and minerals (up to 2x the RDI) results in a statistically significant increase in the serum liver enzymes ALT and AST in female volunteers. No effect on the kidney was observed. This finding supports the conclusion of

Bjelakovic et al. [11] that vitamin and mineral supplements should be tested sufficiently before marketing.

Conflicts of Interest: The authors declare no conflict of interest.

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