RESULTS OF A RANDOMISED, PLACEBO-CONTROLLED STUDY ON THE IMPACT OF VARIOUS SPRAY FORMS OF VITAMIN D ON THE LEVEL OF VITAMIN D3 IN THE BLOOD

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This *in vivo* study is, to a certain extent, a continuation of the *in vitro* study published in the October 2020 issue of the magazine "Latvijas Ārsts". The objective of the study was to determine the overall accumulation efficiency of vitamin D in the body based on usage doses and duration of use, as well as to compare the relative differences in accumulation in the body of two different vitamin D3 products with different base formulations in the body, also correlating these indicators with information about food habits and lifestyle.

Materials and methods

The duration of the study was one month (30 days) as of the commencement of administration of vitamin D3 (first group 10.02.2020–12.03.2020), which occurred on the day that followed the first blood test.

Exclusion criteria: pregnant women, minor individuals, people with mental disabilities, use of additional vitamins and dietary supplements, as well as people, whose initial level of vitamin D did not comply with the requirements for the study.

Laboratory analysis of blood samples was performed by Centrālā laboratorija at Šarlotes street 1B, Riga, Latvia. The study was conducted by using the *InCell* Ltd bases with the support of *Pharma&Med* Ltd.

The subjects were divided into 3 groups:

- Group 1: subjects whose vitamin D levels were 9–32.5 ng/ml and who took cholecalciferol integrated in a microemulsion (*LYL micro*®, hereinafter also referred to as "Group A") 4000IU/2x/d after a meal in the morning and evening, shaking the product vigorously prior to use.
- Group 2: subjects whose vitamin D levels were 7.9–31.2 ng/ml and who took cholecalciferol integrated in nano particles (*LYL EFFUSIO*®, hereinafter also referred to as "Group B") 4000IU/2x/d after a meal in the morning and evening, shaking the product vigorously prior to use.
- Control group: subjects whose vitamin D levels were 14.6–34.9 ng/ml and who took a placebo 2x/d after a meal in the morning and evening, shaking the product vigorously prior to use (hereinafter "placebo").

Assayable material: venous blood with anti-coagulant (*K2EDTA*, 5.4 mg) and serum.

Equipment: *Abbo Architect* (clinical chemistry, immunochemistry, detection of 25-OH vitamin D), manufactured in Illinois, USA.

Statistical processing of data

The primary results of the study were analysed using the t-test and ANOVA analysis. Where applicable, the Pearson correlation coefficient was calculated. *GraphPad Prizm* 8 software was used for data analysis. Statistical processing of survey data was performed using computer software SPSS (*Statistical Package of the Social Science*) v.23.0. and MS *Excel* 2017:

- study data was described using descriptive statistics with central tendency and dispersion measures;
- relationships were assessed using correlation analysis – the *Spearman's Rho* test;
- mean indicators for groups were compared using the *Mann-Whitney* U test;
- groups were compared against each other through the *Pearson chi-squared* test;
- statistical significance assumed at $p<0.05$.

All participants of the study signed a special consent form. During the study (both at the beginning and at the end) subjects were surveyed as to their eating habits and lifestyle in order to correlate this information with their overall blood levels of vitamin D. Results of previously performed tests were neither requested, nor used. For the purposes of the study, blood was collected in two special test tubes, 6 ml each – one at the start of the study, and one – at the end. All individual test results obtained during the study were encoded and were only made available to the researchers performing the study, and at the end of the study, the individual test results were sent to the participants upon request. A permission for the conduct of the study was obtained from the Scientific Research Ethics Committee of the Institute of Cardiology and Regenerative Medicine of the University of Latvia.

Analysis and discussion of results

Within the framework of the study, 158 participants (male and female, aged 18 to 60 years) were invited for the first visit. Based on the results of the initial testing and the inclusion criteria, 116 participants were invited to the second test, and testing was repeated for 114 subjects. Based on the exclusion criteria, 111 subjects qualified for the study, 84 of whom took vitamin D3 with a different base content. These included, 41 subjects, who

took cholecalciferol integrated in a microemulsion (Group 1 or A), and 43 subjects, who took cholecalciferol integrated in nano particles (Group 2 or B), while 27 subjects did not take either the specified, or any other products containing vitamin D and were included in the placebo group (Group 3 or the control group).

Based on available literature, it can be concluded that, even though the number of subjects of the study is relatively small, several other studies have employed a similar or even lower number of subjects with a relatively short duration of the study to draw relevant conclusions. It must be noted that several studies have employed a significantly different methodology and quality, which, as several authors have noted, often encumbers performance of meta-analysis. For example, in a randomized study published in 2019, Negar Naderpoor et al. focused on the modern issue of faecal microbiota, namely, its link to vitamin D supplementation, and this study included just 32 subjects. [1] Manjunath Havalappa Dodamani et al. studied vitamin D supplementation for patients with asthma complicated by allergic bronchopulmonary aspergillosis, including even less – just 30 subjects in the study. [2] In a randomized controlled trial of vitamin D and omega-3 in the medical treatment of children with autism spectrum disorder Hajar Mazahery et al. had initially included 111 children, however, later on this number was reduced by 34%, and literature provides plenty of other similar examples. [3]

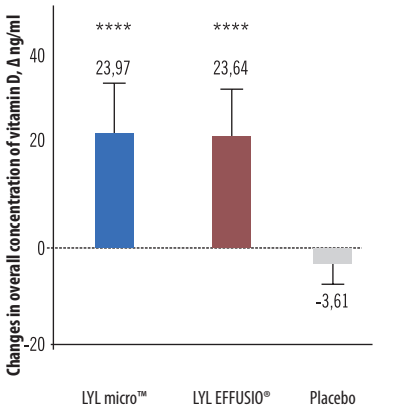
Of the total number of study subjects, 31 were male and 80 were female. The most widely represented age group was 30–39, which constituted 54.80% of the total male and 36.30% of the total female population of the study. The next age group by number of representatives was 40–49, accounting for 19.40% of the males and 26.30% of the females included in the study (*see Image 1*). The subjects of various age groups were distributed evenly among the groups by product used. The study did not identify a statistically relevant impact of demographic indicators on the changes in vitamin D levels.

Changes in vitamin D concentration

At the beginning of the study, the overall concentration of vitamin D in the serum for subjects in Group A (cholecalciferol integrated in a microemulsion) ranged from 9.0 to 32.5 ng/ml (on average 21.7± 7.19 ng/ml). The overall concentration of vitamin D in the serum for subjects in Group B (cholecalciferol integrated in nano particles) ranged from 7.9 to 31.2 ng/ml (on average 21.5± 5.7 ng/ml). The overall concentration of vitamin D in the serum for subjects in the control (placebo) group ranged from 14.6 to 34.9 ng/ml (on average 24.7± 6.36).

At the end of the study, the overall concentration of vitamin D in the serum for subjects in Group A (cholecalciferol integrated in a microemulsion) ranged from 21.3 to 66.1 ng/ml (on average 45.7± 10.02 ng/ml). An increase in the concentration of vitamin D was observed for all subjects in the group. The overall concentration of vitamin D in the serum for subjects in Group B (cholecalciferol integrated in nano particles) ranged from 24.9 to 72.5 ng/ml (on average 45.1± 9.73 ng/ml). An increase in the concentration of vitamin D was observed for all subjects in the group. The

Image 3. **Changes in overall concentration of vitamin D (difference in concentration at the beginning and end of the study) by group, ng/ml. The dashed line represents the situation at the beginning of the study (**** p<0.0001)**



overall concentration of vitamin D in the serum for subjects in the control (placebo) group ranged from 11.8 to 31.8 ng/ml (on average 21.1± 5.94 ng/ml). A decrease of the overall level of vitamin D in the serum was observed in all subjects of this group, except three.

Compared to the beginning of the study, the overall level of vitamin D increased by 5.9 to 44.4 ng/ml (on average 23.97± 9.81 ng/ml) for Group A, by 5.5 to 49.3 ng/ml (on average 23.64± 9.27 ng/ml) for Group B, while in the control group a decrease of 0.1 to 10.9 ng/ml (on average 4.0± 3.5 ng/ml) was observed for 25 subjects. A slight increase of vitamin D levels of 1.3, 0.9 and 0.1 ng/ml was observed in three subjects of the control group. The overall difference in vitamin D concentration between the placebo group and the two groups of subjects, who used trial products, was statistically significant (*see Image 2 and 3*).

Furthermore, the correlation between

Image 1. **Study subject age structure and distribution by gender**

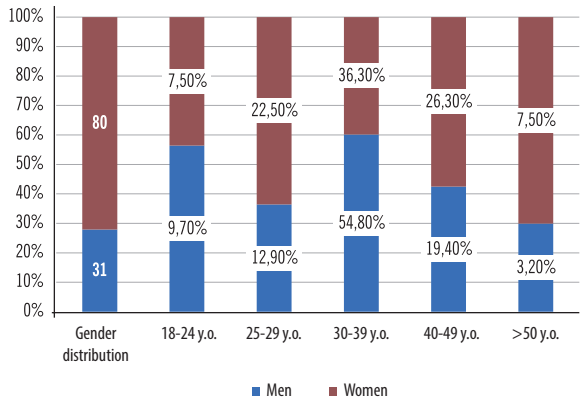
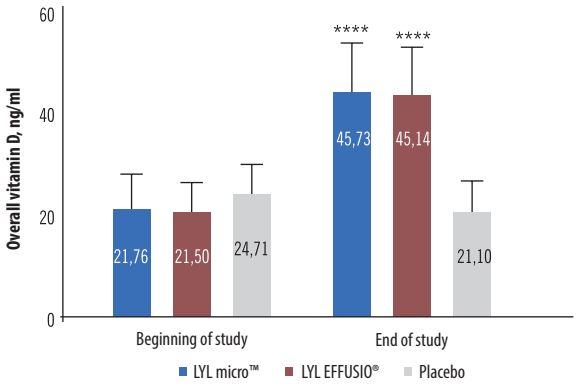


Image 2. **Overall concentration of vitamin D in the serum at the beginning and end of the study (ng/ml ****p<0.0001)**



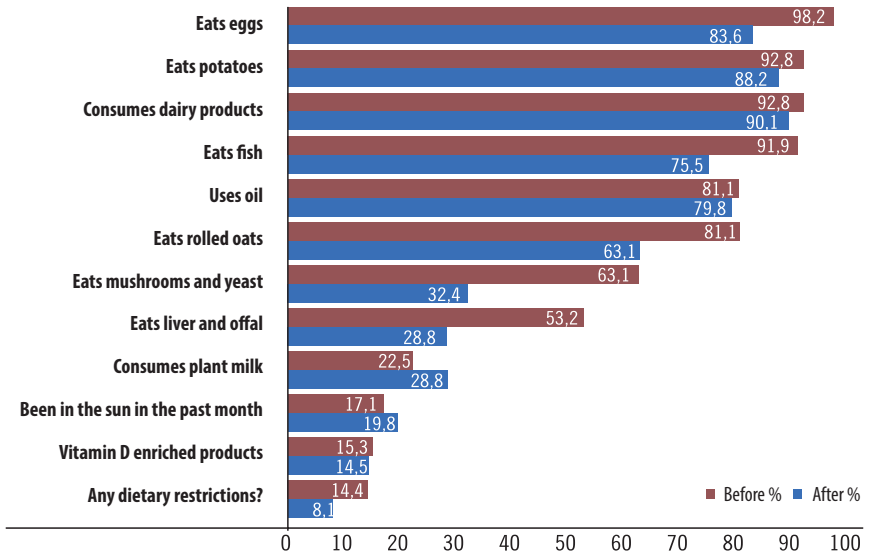
the overall level of vitamin D and its increase at the end of the study was also analysed. For both Group A and Group B a negative correlation was identified (the Pearson correlation coefficient being r = -0.773 and r = -0.718, respectively, at a statistical significance of p < 0.0001), meaning that in case of a lower initial level, a more pronounced increase of the overall level of vitamin D was observed.

As mentioned in the previous article, despite being commercially available, little is known about the efficiency of peroral vitamin D, which is mostly absorbed in the oral cavity, under the tongue and in the mucous membranes of the palate, rather than the gastrointestinal tract. The latest research has demonstrated that ingestible vitamin D can ensure an accelerated path of absorption in comparison to capsules, and can be effective for people suffering from digestive tract malabsorption. [4] Therefore, more and more researcher groups are publishing their analyses about the efficiency of this relatively new form of vitamin D. For example, an Irish research group must be noted, which performed a randomised study, albeit with less subjects, and found that vitamin D3 in oral spray form is an equally effective alternative to capsule forms for healthy adults. [5]

Comorbidities

By surveying the subjects, information was collected on subject comorbidities that could affect vitamin D indicators in the blood. Overall, almost a third or 30 subjects indicated that they did not have any comorbidities, while 19 (18.6%) had one, and 17 (16.7%) had two pre-existing conditions. The main comorbidities noted by the subjects of the study were back pain (38%), fatigue and exhaustion (50.1%), joint pain and stiffness (23.9%), hair loss (19.5%) and muscle cramps (18%). The comorbidities and their combinations in the subjects were logical and no atypical correlations were observed. Overall, the medical histories did not demonstrate any distinct correlation with vitamin D levels in the blood.

Image 4. **Changes in food habits of subjects during the study**



Food habits

Based on the analysis of the food habits of the subjects, it can be concluded that overall the subjects had a diverse diet: 85% of subjects indicated that they used at least 6 different products listed on the questionnaire.

Based on the food habits questionnaire, it can be concluded that the diversity of the diet of the subjects decreased during the course of the study (at the end of the study, at least 6 of the products listed on the questionnaire were consumed by only 58% of subjects, i.e., a decrease of 27% compared to previous result), as did the daily consumption of products containing vitamin D. Despite the fact that during the study, the subjects ate less fish and eggs than at the beginning of the study, vitamin D levels still increased, which is an additional proof of the efficacy of the formulations, as well as of the fact that the increase in vitamin D levels was not supplemented through food (*see Image 4*).

Interestingly, even though 95.5% of the subjects confirmed that they believed that vitamin D ought to be supplemented daily and that this should be done all year long (94.5%), a survey of the subjects as to regular vitamin D supplementation revealed that most of them (57.7%) only take vitamin D supplements during the darkest and coldest months of the year. 28.8% of the subjects had never before taken vitamin D supplements, while 13.5% or 15 of the subjects did so throughout the year. This fact demonstrates the need to raise public awareness about the important role of vitamin D for the body and about the possibilities to supplement it in our climatic conditions. It must also be noted that 85.6% of the subjects agreed with the statement that vitamin D in an aerosol or spray form is easier to use than vitamin D capsules, tablets or drops.

Conclusions

Cholecalciferol integrated in a micro-emulsion (*LYL micro*®) and cholecalciferol integrated in nano particles LYL *EFFUSIO*®), taken twice a day, effectively increases the overall level of vitamin D in the serum within one month of use. Moving forward, more extensive studies are needed that would allow for the accrual of a broader base of evidence regarding changes in concentration of vitamin D levels.

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