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THE CONTENTS OF VITAMIN D3 AND 25-HYDROXYVITAMIN D3 IN DIFFERENT VARIETIES OF DAIRY PRODUCTS IN RUSSIAN MARKET

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Abstract. Vitamin D has received global attention because of its many health benefits. Although there is general agreement about the importance of vitamin D for bone health, there remains skepticism about the nonskeletal health benefits of vitamin D. There is general agreement that vitamin D deficiency is a worldwide health problem. This is due in part to a lack of appreciation that sunlight is an important source of vitamin D. Recent findings in western countries have made new recommendations for vitamin D intake to prevent vitamin D deficiency. Vitamin D deficiency is defined as a 25-hydroxyvitamin D3 level below 20 ng/ml and vitamin D insufficiency is defined as 21–30 ng/ml. This research focuses on the reasons for vitamin D deficiency and demonstrates the results of the contents of vitamin D3 and 25-hydroxyvitamin D3 in dairy products including natural and pasteurized goat milk; natural, baked, and pasteurized cow milk; fortified cow milk (pasteurized and ultra-pasteurized); butter; homemade butter; and butter made from baked milk (ghee). The greatest concentrations of vitamin D3 and 25(OH)D3 in milk were discovered in goats' milk, according to our findings. Generally speaking, the larger the quantity of butter in a recipe, the higher the concentration of Vitamin D3 and 25(OH)D3.
Keywords: 25-hydroxyvitamin D, dietary intake, vitamin D3

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СОДЕРЖАНИЕ ВИТАМИНА D3 И 25-ГИДРОКСИВИТАМИНА D3 В РАЗЛИЧНЫХ ВИДАХ МОЛОЧНОЙ ПРОДУКЦИИ НА РОССИЙСКОМ РЫНКЕ

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Аннотация. Витамин D привлек внимание всего мира из-за его многочисленных преимуществ для здоровья. Хотя существует общее мнение о важности витамина D для здоровья костей, остается скептицизм в отношении полезных свойств витамина D для здоровья, не относящегося к скелету. Существует общее мнение, что дефицит витамина D является проблемой здравоохранения во всем мире. Отчасти это связано с недостаточным пониманием того, что солнечный свет является важным источником витамина D. Исследования зарубежных ученых последних лет позволили разработать рекомендации по потреблению витамина D для предотвращения его дефицита. Дефицит витамина D определяется как уровень 25-гидроксивитамина D3 ниже 20 нг/мл, а недостаточность витамина D определяется как 21–30 нг/мл. Это исследование посвящено причинам дефицита витамина D и демонстрирует результаты содержания витамина D3 и 25-гидроксивитамина D3 в молочных продуктах, включая натуральное и пастеризованное козье молоко; натуральное, топленое и пастеризованное коровье молоко; витаминизированное коровье молоко (пастеризованное и ультрапастеризованное); сливочное масло; домашнее сливочное масло; масло из топленого молока (гхи). По нашим данным, наибольшие концентрации витамина D3 и 25(OH)D3 в молоке обнаружены в козьем молоке. Вообще говоря, чем больше количество масла в рецепте, тем выше концентрация витамина D3 и 25(OH)D3.

Ключевые слова: 25-гидроксивитамин D, потребление с пищей, витамин D3

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Introduction

Vitamin D deficiency is now documented as pandemic with a myriad of health consequences [1]. Low vitamin D status has been linked with cardiovascular disease, certain cancers, cognitive decline, depression, diabetes, pregnancy complications, autoimmune diseases, and even associated with frailty and all-cause mortality [2, 3, 4].

Recently scientists documented vitamin D deficiency as pandemic with a broad spectrum of health-related problems like dementia, depression, diabetes, abortion, general weaknesses, insomnia, and cardiovascular diseases [1].

In spite of many important health benefits from vitamin D there is controversy regarding the definition of vitamin D deficiency and what the vitamin D requirement should be. This is partially due to the lack of well-designed controlled clinical trials [5].

Vitamin D presented in the 3 main forms: (D2, D3, and 25(OH)D3) is a secosterol component produced either endogenously throughout human's body skin or can be obtained mainly from animal fats which are the main sources of vitamin D as well as such food like cod liver, salmon, tuna, and mushrooms subjected to ultraviolet radiation [4, 6].

Around 50–90 % of the recommended daily intake of vitamin D comes from skin's synthesis of this vitamin. However, that depends on the duration of exposure to sunlight, area of body exposed, skin's color and pigmentation, and latitude [7, 8].

The production of vitamin D3 in the skin depends on sunshine exposure, latitude, skin-covering clothes, the use of sun block, and skin pigmentation [9].

In Russian federation and most of north European countries where people are rarely exposing their skins to direct natural UV light throughout the year are up to 80 % vitamin D insufficient. [10].

An observational study for the people living in north-west regions of Russia declared that the majority of the population recorded the serum concentration of 25(OH)D below 20 ng/ml. However, those families that have the habit of eating fish dishes like code fish and salmon once or twice per week are associated with higher serum levels of 25(OH)vitamin D [11].

Special attention for pregnant and lactating women should be considered as statistics shows that the prevalence of insufficiency of 25(OH)vitamin D can be from 9–100 % [11]. The reason behind that is the higher demand of the infant for vitamin D and the fact that 25(OH)vitamin D is dissolved in breast milk during lactation which increase the withdrawal of this metabolite [11–13].

Only few foods varieties naturally contain or are fortified with vitamin D. Thus, the major cause of vitamin D deficiency is inadequate exposure to sunlight [14].

The main groups of people suffering from insufficiency of vitamin D can be categorized into: pregnant and lactating women, children during intensive growth periods, elderly, and long time office workers [15].

Whenever vitamin D inters the blood stream, it will bind to a protein called "vitamin D-binding protein" which in turns will transport it into the liver where it will be hydroxylated into 25-hydroxy-vitamin D or 25(OH)D [16, 17].

The 25(OH)D metabolite endures another hydroxylation reaction by the 25(OH)D-1a-hydroxylase enzyme or (CYP27B1) when it inters kidneys in order to formulate the secosteroidal hormone [1,25(OH)2D] which is straightly regulated by a cascade of factors: 1-parathyroidal hormone; 2-calcium and phosphorus levels in body serum; 3- fibroblast growth factor [18,19]. This constricted relationship among Ca, P, and vitamin D active metabolites has led some researchers to nominate food that contain these 3 elements like mother's breast milk and Parmigiano Reggiano cheese a "superfood".

The active metabolite of vitamin D existing in the serum can transfer from mother's placenta into fetus's blood stream in the form of 25(OH)D [20, 21]. Considering the average half-life of this metabolite is 20 days, meaning that the newborn infant can still be vitamin D sufficient for few weeks considering his mother was vitamin D sufficient.

Experts and clinicians from the International Endocrine Society defined patients with 25(OH)D blood concentrations of 21–30 ng/ml or 50 mmol/ml as vitamin D insufficient [22].

Their guidelines suggested that daily intake of up to 2000 IU of vitamin D supplements is necessary to achieve serum 25-hydroxyvitamin D3 concentrations consistently >30 ng/mL in male/female adults to prevent vitamin D deficiency [12, 13].

Scientists from china studied the prevalence of vitamin D and calcium deficiency among pregnant women and their newborns claimed that lactating women breast milk as well as milk obtained from unfortified cows have very little vitamin D [23].

Only daily dosage of 4000 to 6000 IU of vitamin D transferred the daily requirements of vitamin D from mother's breast milk to her child [24].

It's not an easy mission to achieve the daily requirements of 15 mcg of active vitamin D for female/male adults over sixty years old through diet existing in markets of northern countries like Russia. Nevertheless, even if it was achieved by a well-planned diet, the body would barely achieve its needs due to the low bioavailability and absorption efficiency of vitamin D whether it was obtained from natural food products or supplements [17, 22].

Even if fortified diary products like milk were added to daily meals. Here we demonstrate briefly what recently investigators in human nutrition recommended options for healthy human to be considered for supplementing our bodies with the satisfied requirements of vitamin D [25, 26]:

– A daily vitamin D capsule providing a 200 IU vitamin D which can be achieved from markets, pharmacies, online store as Rx and free prescribed supplementations at low costs;

– Vitamin (D2) 500 IU in combination for with calcium (phosphate or gluconate) at doses range of 400–800 mg/day;

– An oral dose of 100.000 IU of vitamin D provided every 4 months;

– Surprisingly, An annual dose of 600.000 IU of vitamin D taken by injection is under study on 50 patients in Australia suffering from osteoporosis normalized the active metabolite of vitamin D in the serum to an average of 114 mmol/L which is considered to be bone-supportive against osteoporosis [27, 28].

However, this study has not been proved yet. Still, a meta-analysis of randomized controlled trials for hospitalized elderly patients supplemented with high doses for vitamin D resulted in reducing the risk of hip fractures [29, 30].

The aim of this research is to analyze the contents of vitamin D3 and 25-hydroxyvitamin D3 in a variety of milk, processed milk, and milk commodity products of different fat contents obtained from Russian markets.

Materials and methods

To achieve the goal of the work, the following types of dairy products were identified, the technologies of which used various technological methods: thermal and mechanical processing and enrichment with vitamins D. The sample included: goat milk (Natural and Pasteurized) – Fortified/Enriched milk (Pasteurized and Ultra-pasteurized) – baked milk (Pasteurized) – cow milk (Pasteurized) milk (Cows received 4000 IU vitamin D3/day) – whole milk (organic) – butter and butter made from baked milk (ghee).

Sample preparation

The lipid fraction of cow's milk, which contains fat-soluble vitamin D, consists mainly of triglycerides with much smaller amounts of sterols, carotenoids, phospholipids and other minor components.

All of these compounds have solubility properties similar to those of vitamin D, so they are a potential source of interference.

Part of the vitamin D found in milk and infant formula is bound to the lipoprotein complex, so fat-protein bonds must be broken to release the vitamin.

Vitamin preparations added to milk are often coated with a protective gelatinous shell that must be dissolved at the beginning of the analysis.

Thus, the determination of vitamin D in milk and infant formula requires tedious sample preparation to homogenize, isolate and concentrate the vitamin from its association with the protein and eliminate interfering substances as much as possible.

The analysis was carried out in a closed laboratory without windows, equipped with special closed lamps.

Gaseous nitrogen (N2) was used to replace air before the saponification step. Ergocalciferol (vitamin D3) 99 % and 25-hydroxyvitamin D3 (25OHD3) 99 % were of HPLC purity and served as standards.

Measures such as pre-cooling the sample were taken into account to eliminate the effect of temperature rise during this procedure. Samples prepared for testing were analyzed without delay and protected from light.

Saponification

A 50 g sample for each body part was carefully ground in a suitable mill and mixed well.

Approximately 50 mg of the homogenized sample was transferred into a 10 ml glass tube fitted with an appropriate cap. 3 ml of 96 % ethanol and 0.15 ml of 95 % KOH, 100 ml of 1 % pyrogallol, 0.25 mg of ascorbic acid, 0.1 ml of 99 % cholecalciferol and 0.1 ml of 99 % ergocalciferol were added as internal standards and shaken in within 2 minutes.

The tube was transferred to a water bath at a temperature of 80 °C and kept for 40 minutes. Close the lid, add 1 ml of bidistilled water and 3 ml of n-hexane and then shake gently.

The saponified mixture was centrifuged at 8000 rpm for 5 minutes. The top layer was collected and the extraction was again carried out for better results with 3 ml of n-hexane without water, and then centrifuged.

Cleaning Samples

Washed with hexane with 2 ml of bidistilled water, followed by centrifugation. The aqueous phase was separated and discarded. To the washed hexane phase was added 1 ml of 99.9 % 2-propanol. Samples were purified using solid phase extraction (SPE) columns. The procedure was carried out in a stream of nitrogen gas.

Purification of fatty acids of samples from proteins was performed using a C18 cartridge (45 μm, 3 ml/500 mg).

Samples were placed in a Supelcosil LC-Si 5 μm, 15.0 cm × 4.6 mm polar silica cartridge to separate vitamin D3 and its metabolites according to their polarity.

The extracts were purified from sugars by successively connecting 2 amino columns for better separation. 2.3.4. Quantification of vitamin D3 and 25-hydroxyvitamin D3: Elution was carried out as follows: 12 ml of a 0.4 % solution (dichloromethane/2-propanol) was added to 40 μm from the extracts of the TFE columns.

The samples were evaporated in a stream of nitrogen gas to achieve dryness.

The mixture was reconstituted with 350 μl of 0.4 % dichloromethane/isopropyl alcohol solution.

Exactly 100 μl was injected into the device at a flow rate of 1.0 ml/min. 0.5 % (isopropyl alcohol/n-hexane) served as the mobile phase.

Quantitative determination of vitamin D3 and 25OHD3 was carried out by injecting the extracts onto a RP C18 SPE column. Vitamin D3 and 25OHD3 were detected on a compact UV photodiode array at 264 nm.

The content of vitamin D3 was determined by the ratio of the areas of the peaks of both vitamin D3 and vitamin D2. An external standard calibration curve was used to calculate the 25OHD3 content in samples [31–33].

Results and Discussion

Vitamin D3 and its active metabolite 25(OH)D3 can be found naturally only in few foodstuffs most of them are animal products like diary products, fish, and less concentrations in meat. In our experiment, we analyzed edible dairy products existing in Russian markets. From table, we can notice that the concentrations of both vitamin D3 and 25(OH)D3 are remarkable lower in pasteurized and ultra-pasteurized milk compared to whole natural milk. Which indicates that vitamin D3 and its metabolite is degraded due to pasteurization process. Natural goat milk showing the highest contents of both vitamin D3 and 25(OH)D3 recording 0.6 ± 0.02 and 1.38 ± 0.02 (μg/L), respectively.

The contents were dropped almost 80 % in goat milk after being pasteurized.

Pasteurized baked cow's milk seems to hold almost the same contents of the analyzed vitamins like in goat's milk. However, it seems to retain between 50-75 % more vitamin D3 than the pasteurized cow's milk.

Pasteurized milk with higher percentage of fat retained higher concentrations of both vitamin D3 and 25(OH)D3.

We could also provide natural whole milk from cows from cow's farm (Zao Kalininskoye, Tver, Russia) receiving daily dosage of vitamin 4000 IU vitamin D3.

The analyzed milk samples from fortified cows recorded elevation in vitamin D3 and 25(OH)D3 contents accumulating 0.043, 0.075, 0.092 μg/L of vitamin D3 in milk containing 1.0, 2.5, 3.5 % fat respectively. The contents of 25(OH)D3 in milk of fortified cows failed to recorded remarkably higher levels compared with all other samples. The highest content of 25(OH)D3 in milk varieties can be seen in natural goats' milk with fat concentration of 3.5 and 3.8 % with content of 1.38 and 1.35 μg/L respectively.

Four butter samples were also analyzed for their content of vitamin D3 and 25(OH)D3.

The contents of vitamin D3 and 25(OH)D3 in 4 butter samples were proportional to their fat percentages except for the butter made from baked milk (ghee butter) which contains about 98 % fat and seems to contain 10.7 μg/L of vitamin D3.

The highest concentration of both vitamin D3 in all the examined samples was for the homemade butter sample with 12.8 10.7 μg/L.

Table

The content of Vitamin D3 and 25(OH)D3 in diary products

Product	Fat Percentage, %	D-3, (μg/L)	25(OH)-D3, (μg/L)
Natural goat milk	4.8	0.6 ± 0.02	1.38 ± 0.02
Pasteurized goat milk	3.5	0.12 ± 0.03	1.35 ± 0.22
Fortified/Enriched milk (Pasteurized)	3.50	0.08 ± 0.025	0.22 ± 0.05
Fortified/Enriched milk (Ultra-pasteurized)	3.50	0.08 ± 0.01	0.19 ± 0.044
Baked milk (Pasteurized)	4.30	0.12 ± 0.01	0.24 ± 0.038
Milk (Pasteurized)	1.0	0.022 ± 0.08	0.132 ± 0.05
Milk (Pasteurized)	2.50	0.021 ± 0.0	0.20 ± 0.06
Milk (Pasteurized)	3.50	0.03 ± 0.012	0.32 ± 0.032
Milk (Pasteurized)	4.80	0.06 ± 0.015	0.34 ± 0.07
Milk (Cows received 4000 IU vitamin D3/day)	1.0	0.043 ± 0.0	0.373 ± 0.042
Milk (Cows received 4000 IU vitamin D3/day)	2.50	0.075 ± 0.04	0.25 ± 0.042
Milk (Cows received 4000 IU vitamin D3/day)	3.50	0.092 ± 0.026	0.77 ± 0.025
Whole milk (organic)	4.80	0.076 ± 0.011	0.61 ± 0.035
Butter	72.5	7.25 ± 0.28	0.41 ± 0.06
Butter	82.5	10 ± 0.22	0.42 ± 0.12
Butter made from baked milk (ghee)	98	10.7 ± 0.46	0.52 ± 0.0
Butter homemade	85.9	12.8 ± 0.62	1.09 ± 0.18

Conclusion

There are general agreements that vitamin D in all its forms is of highly importance for maintaining healthy skeleton, and hormonal regulations for fetus, newborns, adults, and elderly.

Natural food containing vitamin D altogether with foodstuffs enriched with this vitamin barely can satisfy body needs on daily basis without direct exposure to UV radiations from the sun.

Pasteurized and ultra-pasteurized milk contains less concentrations of vitamin D3 and 25(OH)D3. Goats milk contains higher concentrations of vitamin D3 and 25(OH)D3 compared to cow's milk. The higher the percentage of butter the higher content of Vitamin D3 and 25(OH)D3.

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