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Vitamin D deficiency

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Spread of vitamin D deficiency in children of various ages has not been studied in the Russian Federation. Numerous studies of foreign authors indicate high importance of this issue in many regions of the world, including southern territories with high level of insolation. The review analyzes issues of optimal calcidol level and vitamin D deficiency effect on the immune system. The authors support the hypothesis that vitamin D deficiency results in derangement of congenital and adaptive immunity and may be considered one of the etiological factors of frequent respiratory morbidity in children.
Keywords: *vitamin D, vitamin D deficiency, hypervitaminosis D, children, congenital immunity, adaptive immunity.*

Until recently, Russian literature had been viewing the role of vitamin D in pediatric practice only from a perspective of its influence on calcium and phosphorus metabolism in the child's body.
The scientific discussion caused by article "Controversial theoretical and practical issues of rickets in children in the present stage" by E.V. Neudakhin and V.A. Ageykin in journal "Pediatrics" focused primarily on differences in definitions, discussion of various rickets classifications and controversial recommendations regarding preventive and therapeutic doses of vitamin D for term and premature infants [1].
Professor S.V. Maltsev summarized that discussion in 2008 [2]. The author fairly drew the reader's attention to the fact that vitamin D-deficient rickets cannot be established without determining the level of the child's body provision with vitamin D (calcidiol blood concentration – *author's note*). He noted that "infantile (neonatal) R (rickets – *authors' note*) most often develops in the event of deficiency of Ca and phosphates in food caused by nutritional defects, intestinal malabsorption, excessive urinary discharge or bone utilization thereof due to immaturity of transport systems observed at an early age. Deficiency of Ca and phosphates is the main cause of neonatal R. Vitamin D-deficient R is observed far more rarely, as it may occur only in the children with insufficient sun exposure (asocial families, interned children etc.) [2]."
This conclusion contradicts the modern data on human body provision with vitamin D in various age periods and does not correlate with the known molecular mechanisms of calcium and phosphorus homeostasis in children and adults. According to the author, "total prevention and treatment of R (in the Soviet times – *author's note*), particularly in the so called initial period, with the preventive and therapeutic doses exceeding the physiological demand tens of times led to the development of a new nosological entity – hypervitaminosis D, which is a severer disease than R itself." We believe that this erroneous statement contributed to the vitamin D-associated phobia worsening among the Russian pediatricians, whereas the articles published abroad have been extensively discussing the issue of vitamin D supply emphasizing a significant spread of

vitamin D deficiency and deficit not only among small children, but also among the older age groups as well, including adults [3].

According to L.A. Basile, daily intake of 4,000-6,400 IU by the breastfeeding mothers did not cause hypervitaminosis D in the mothers, but contributed to the antirachitic effect of their breast milk [4].

The modern conception of vitamin D metabolism in the body and study of molecular mechanisms of synthesis and degradation of its bioactive forms contributed to the understanding that hypervitaminosis D may occur only if these mechanisms are disturbed or ultra-high vitamin D doses enter the blood flow [5].

It has been established that calcitriol synthesis is strictly regulated by the levels of plasma parathyroid hormone, serum calcium and phosphorus [6].

Excessive calcitriol generation induces expression of enzyme 25-dehydroxyvitamin D 24-hydroxylase (CYP24), which catabolizes calcidiol and calcitriol into a biologically inactive, water soluble calcitroic acid [7].

According to B. Hollis, vitamin D intoxication develops if the level of blood calcidiol [25(OH)-D] > 250 nmol/l (100 ng/ml) [8]. There have been studies demonstrating that the level of 25(OH)-D may exceed 250 nmol/l without signs of intoxication in sunbathers; clinical symptoms of hypervitaminosis D are not observed at this level of calcidiol and oral intake of vitamin D drugs as well [9]. There are hard copy proofs that vitamin D intake in the single dose of 200,000 IU does not cause vitamin D intoxication in neonates, infants and older children [5]. According to M.F. Holick [10], hypervitaminosis D develops if the level of blood calcidiol exceeds 325 nmol/l (150 ng/ml). The mentioned published data convince that the Russian specialists are clearly overestimating the problem of hypervitaminosis D.

Vitamin D is unique, as it may be synthesized in skin when exposed to ultraviolet radiation or taken with food. Synthesized in skin due to ultraviolet radiation or absorbed intestinally, vitamin D transforms into 25-hydroxyvitamin D (calcidiol) in liver microsomes when affected by enzyme CYP2R1 (vitamin D-25 hydroxylase). Affected by enzyme 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1), calcidiol is further metabolized into the active hormone-like form – 1.25-dihydroxyvitamin D (calcitriol) – in renal tubules and ensures intestinal calcium absorption and calcium reabsorption in renal tubules [7, 8]. In the apical segment of duodenal epithelial cells, calcitriol stimulates transepithelial transfer of calcium ions though calcium channel TRPV5 (vanilloid receptors, ionic channels subgroup). In an epithelial cell, a calcium ion binds with a calcium-binding protein (calbindin), the synthesis of which is stimulated by interaction of calcitriol with vitamin D receptor (VDR). After that, calcium ions with calcium-binding protein move to the basolateral cell membrane and enter the extracellular space with the help of the plasma membrane's Ca^{2+}-ATPase (PMCA1b) [9]. Homologous calcium channel TRPV6 can be found in renal tubules [11].

It has been established that the level of blood calcidiol is a reliable indicator of the body provision with the vitamin [12], although there is no agreement of opinions regarding the normal level of blood calcidiol. Adequate and sufficient level of plasma vitamin D is considered to be within the range of 30-80 nmol/l [13].

Having thoroughly analyzed literature reviews in 2011, experts of the Institute of Medicine (IOM) of North America concluded that the level of serum calcidiol of 16 ng/ml (40 nmol/l) is sufficient for ca. half of the world's population, 20 ng/m (50 nmol/l) – for 97.5%. Medical practitioners are recommended to be guided by the latter level. The level of serum calcidiol below 30 nmol/l increases the risk of rickets in small children, disturbs calcium absorption and reduces bony skeleton mineralization in children and adolescents. The experts concluded that multicenter randomized studies are required to determine the optimal blood serum concentration of calcidiol in order to avoid deficiency or excess thereof [12].

According to the Canadian researchers, the concentration of 25(OH)-D was below 50 nmol/l in wintertime (from November to March) in 1/3 of the population, who did not take additional vitamin preparations. Intake of vitamin D drugs improved the vitamin status of the population

and nullified the seasonal plasma calcidiol level reduction [14]. It ought to be mentioned that dairy products, juices and instant cereals are enriched with vitamin D in the countries of North America.

The English researchers hold a more conservative opinion regarding the sufficient level of vitamin D; they consider the serum calcidiol concentration of 10 ng/ml (25 nmol/l) to be the threshold value [15].

According to most experts, the calcidiol level below 20 ng/ml (50 nmol/l) should be regarded as a vitamin D-deficient state [10, 16]. Correlation analysis of serum calcidiol and parathormone concentrations supports this hypothesis. As soon as the blood calcidiol concentration reaches 30-40 ng/ml (75-100 nmol/l), the parathormone level returns to the normal values [17, 18].

Vitamin D deficiency occurs not only in the countries situated in the northern latitudes. According to the Australian researchers, moderate (12.5-25 nmol/l) and severe (calcidiol level below 12.5 nmol/l) deficiencies were observed in more than 70% of the children with rickets and 90% of the children with hypocalcaemia. Signs of rickets were observed in 86% of the children with the calcidiol level < 20 nmol/l (8 ng/ml); hypocalcaemia was observed in 94% thereof [19].

The calcidiol level in most people living in the equatorial latitudes exceeds 30 ng/ml. Still, vitamin D deficiency is often observed even in the very sun-exposed latitudes (due to the habit of protecting body surface from solar radiation). Thus, the calcidiol level is below 30 ng/ml in 30-50% of the children and adults in Saudi Arabia, Australia, India, the United Arab Emirates and other countries [20, 21].

The level of body supply with vitamin D depends on the geographic position of the resident territory, season and length of sun exposure. The studies conducted in the USA demonstrated that only an insignificant amount of vitamin D is synthesized in the skin of the people living in the regions to the north of the 35^{th} parallel north from November to March; in Edmonton (52^{th} parallel north) – from October to March inclusive. Efficient photosynthesis of provitamin D3 from 7-dihydrocholesterol is observed in wintertime to the south (34^{th}-18^{th} parallel north) [22]. Thus, the whole territory of Europe (all the more so of the Russian Federation) is situated in the zone of high risk of vitamin D deficiency. Scarce studies of the Russian authors confirm this thesis. According to I.N. Zakharova et al., solar radiation in the area of the 55^{th} parallel north (Moscow, Nizhniy Novgorod, Kazan and several other cities of Russia) is capable of ensuring synthesis of the adequate amount of vitamin D in skin for 4 months per year only (from mid-April to mid-August) [23].

The study of the calcidiol level in 140 healthy children and adolescents of 4-18 years of age permanently residing in the middle latitudes of Russia revealed deficiency in 1/3 of the children (39%); severe vitamin D deficiency – in 3% (in summertime [!]) [24].

According to A.I. Kozlov et al., low calcidiol concentration (below 50 nmol/l) was observed in 47% of the examined adolescents in the Perm Territory; deficient concentration of vitamin D was observed in 86% of the girls and boys in the Komi Republic in autumn and in 98% - in the early spring [25].

Thus, analysis of the level of children's and adolescent's provision with vitamin D in various climate/geographic regions of Russia indicates its differences and appears extremely important. Relevance of such studies is also justified by the established pleiotropic and noncalcemic action of hormone-like metabolites of vitamin D and vitamin D receptor. It appears that vitamin D expression is observed not only in bone tissue, but also in epithelial cells of skin, respiratory tract, intestines, in parathyroid glands and immune cells; this led to the study of molecular mechanisms of extrarenal synthesis of active vitamin D metabolite and reevaluation of its biological role in the human body [26].

According to the researchers, VDR regulates expression of more than 500 out of ≈ 20,488 genes of the human genome. Without any doubt, such a large number of the VDR-regulated genes reflects influence of concentrations of this receptor and calcitriol on many organs and systems, including molecular mechanisms of VDR and calcitriol participation in the natural immune

response, their influence on the function of T and B lymphocytes, which determine development of adaptive immunity [27-29].

It has been proven that activation of macrophage TLRs (toll-like receptors) with lipopolysaccharides or stimulation thereof with interferon (IFN) γ leads to induction of enzyme 1.25-alpha-hydroxylase (CYP27B1) and macrophage synthesis in macrophages. Interaction of macrophage TLRs with bacteria enhances VDR and 1α-hydroxylase expression, which induces synthesis of natural defensins, especially of cathelicidin, resulting in intracellular microbicidal activity enhancement. This explains one of the effects of vitamin D on congenital immunity. It has been established that insufficient body provision with vitamin D correlates with frequent infectious diseases, particularly with the high level of respiratory diseases. It has been assumed that influenza epidemics might be caused by vitamin D deficiency [30, 31].

Antimicrobial proteins (cathelicidin and defensins) do not only produce a microbicidal effect, but may also serve as signaling molecules activating the immune system. Several studies demonstrated that α-defensins and cathelicidin serve as chemoattractants for leukocyte cells, including dendritic cells, T lymphocytes, monocytes and neutrophils. These antimicrobial proteins stimulate expression of numerous cytokines and chemokines, which serve as an additional mechanism of vitamin D effect on acquired (adaptive) immunity [32]. Hormone-like vitamin D metabolite (calcitriol) directly modulates receptors of T lymphocytes. Low phospholipase Cγ1 (PLC) expression in naïve lymphocytes correlates with low response of the T-cell receptor. It has been established that phospholipase Cγ1 induction depends on vitamin D and receptor thereof. Naïve T lymphocytes do not express VDR; however, initiation of the T cell receptor signaling pathway via an alternative route (mitogen-activated protein kinase p38) leads to induction VDR and phospholipase Cγ1 required for the subsequent successful launch of the classic T cell signal and activation of T lymphocytes [33].

In whole, impact of calcitriol on the immune system includes modulation of the T cell receptor, reduction in Th1/Th17 CD4(+) T lymphocytes, increase in regulatory T lymphocytes, suppression of the T lymphocyte-mediated immunoglobulin (Ig) G synthesis and worsening of differentiation of dendritic cells. Subpopulation Th17 of T lymphocytes provides antimicrobial protection of epithelial/mucous barriers. Interleukin (IL) 22 secreted by Th17 lymphocytes stimulates synthesis of antimicrobial proteins by epithelial cells. It ought to be mentioned that calcitriol ensures tolerance by suppressing activity of the adaptive immune response and simultaneously enhancing natural immune response [34].

It has been established that stimulation of naïve [CD4(+) CD25(-)] T lymphocytes in the presence of calcitriol inhibits synthesis of anti-inflammatory cytokines, including IFN γ, IL 17 and IL 21 without any considerable impact on proliferation of T lymphocytes. Leaving aside the inhibiting effect on anti-inflammatory cytokines, calcitriol stimulates CTLA-4 (cytotoxic T-lymphocyte antigen 4) and FoxP3 expression (essential transcription factor for generation and normal function of T regulatory lymphocytes, Threg). The latter requires IL 2. Thus, calcitriol and IL2 produce a synergic effect on the activation of T lymphocytes acting as potential anti-inflammatory molecules and physiological stimulants of adaptive regulatory T lymphocytes [35, 36].

Analysis of the literature on children's and adults' provision with vitamin D leaves no doubt regarding relevance of this issue for Russian pediatricians. Determination of calcidiol level not only in small children, but in the older age groups as well appears to be extremely important. We may assume that the recommended "physiological demand of vitamin D – 400 IU" does not satisfy the real demand not only of preschoolers and schoolchildren, but also of small children. Multicenter studies of serum calcitriol concentration in children residing in various climate/geographic regions of Russia allow calculating the optimal physiological demand of vitamin D. It is especially important from the point of view of noncalcemic action of active vitamin D metabolites and effect thereof on the immune system.

Given the role of vitamin D in regulation of congenital and adaptive immunity function, we may draw correlation between reduction in the rate of respiratory diseases in small children and

increase in vitamin D delivery thereto, e.g. by means of enriching dairy products with vitamin D (regular practice in the countries of North America and a rage of European countries). It is reasonable to reevaluate prescriptive recommendations on preventive doses of vitamin D for children of various age groups depending on the region of residence. It seems unlikely that small children residing in the southern regions and in Siberia need the same dose of vitamin D (400 IU).

REFERENCES

1. Neudakhin E.V., Ageikin V.A. *Pediatriya - Pediatrics*. 2003; 3: 95–98.
2. Mal'tsev S.V. *Pediatriya - Pediatrics*. 2008; 87(2): 120–123.
3. Thomas K.K., Lloyd-Jones D.M., Thadhani R. I., Shaw A.C., Deraska D. J., Kitch B.T., Vamvakas E.C., Dick I.M., Prince R.L., Finkelstein J. S. Hypovitaminosis D in medical in patients. *N Engl J Med.* 1998; 338 (12): 777–83.
4. Basile L. A., Taylor S. N., Wagner C. L., Horst R. L., Hollis B. W. The effect of high-dose vitamin D supplementation on serum vitamin D levels and and milk calcium concentration in lactating women and their infants. *Breastfeed Med*. 2006; 1(1): 27–35.
5. Jacobus C. H., Holick M. F., Shao Q., Chen T. C., Holm I. A., Kolodny J. M., Fuleihan G.El-H., Seely E. W. Hypervitaminosis D Associated with Drinking Milk. *N Engl J Med*. 1992; 326: 1173–1177.
6. Holick M. F. Resurrection of vitamin D deficiency and rickets. *J Clin Invest*. 2006; 116: 2062–2072.
7. DeLuca H. F. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr.* 2004; 80: 1689–1696.
8. Hollis B. Overview of the proceedings from Experimental Biology 2004 symposium: vitamin D insufficiency: a significant risk factor in chronic diseases and potential disease-specific biomarkers of vitamin D sufficiency. *J Nutr.* 2005; 135 (2): 301–303.
9. Zhuang L., Peng J. B., Tou L., Takanaga H., Adam R. M., Hediger M. A., Freeman M. R. Calcium-selective ion channel, CaT1, is apically localized in gastrointestinal tract epithelia and is aberrantly expressed in human malignancies. *Lab Invest.* 2002; 82: 1755–1764.
10. Holick M. F., Chen T. C. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr.* 2008; 87 (4): 1080–1086.
11. Hoenderop J. G., Bindels R. J. Epithelial Ca2+ and Mg2+ channels in health and disease. *J Am Soc Nephrol*. 2005; 16: 15–26.
12. Ross A. C., Manson J. E., Abrams S. A., Aloia J. F., Brannon P. M., Clinton S. K., Durazo-Arvizu R. A., Gallagher J. C., Gallo R. L., Glenville J., Kovacs C. S., Mayne S. T., Rosen C. J., Shapses S. A. The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know. *J Clin Endocrinol Metab.* 2011; 96(1): 53–58.
13. Lips P. Which circulating level of 25-hydroxyvitamin D is appropriate? *J Steroid Biochem Mol Biol*. 2004; 89–90: 611–614.
14. Whiting S. J., Langlois K. A., Vatanparast H., Greene-Finestone L. S. The vitamin D status of Canadians relative to the 2011 Dietary Reference Intakes: an examination in children and adults with and without supplement use. *Am J Clin Nutr.* 2011; 94(1): 128–135.
15. Cashman K. D., Hill T. R., Cotter A. A., Boreham C. A., Dubitzky W., Murray L., Strain J. J., Flynn A., Robson P. J., Wallace J. M., Kiely M. Low vitamin D status adversely affects bone health parameters in adolescents. *Am J Clin Nutr.* 2008; 87 (4): 1039–44.
16. Bischoff-Ferrari H. A., Giovannucci E., Willett W. C., Dietrich T., Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr.* 2006; 84: 18–28.
17. Thomas M. K., Lloyd-Jones D. M., Thadhani R. I., Shaw A. C., Deraska D. J., Kitch B. T., Vamvakas E. C., Dick I. M., Prince R. L., Finkelstein J. S. Hypovitaminosis D in medical inpatients. *N Engl J Med*. 1998; 338: 777–783.

18. Chapuy M. C., Preziosi P., Maamer M., Arnaud S., Galan P., Hercberg S., Meunier P. J. Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int.* 1997; 7: 439–443.
19. Robinson P. D., Hogler W., Craig M. E. The re-emerging burden of rickets: a decade of experience from Sydney. *Arch Dis Child*. 2006; 91: 564–568.
20. Marwaha R. K., Tandon N., Reddy D. R., Aggarwal R., Singh R., Sawhney R. C., Saluja B., Ganie M. A., Singh S. Vitamin D and bone mineral density status of healthy schoolchildren in northern India. *Am J Clin Nutr.* 2005; 82: 477–482.
21. McGrath J. J., Kimlin M. G., Saha S., Eyles D. W., Parisi A. V. Vitamin D insufficiency in south-east Queensland. *Med J Aust.* 2001; 174: 150–151.
22. Webb A. R., Kline L., Holick M. F. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab*. 1988; 67: 373–37.
23. Zakharova I.N., Korovina N.A., Dmitrieva Yu.A. *Praktika pediatra – Pediatrician's practice*. 2012; 3: 34–40.
24. Vitebskaya A.V., Smirnova G.E., Il'in A.V. *Osteoporoz i osteopatii – Osteoporosis and osteopathy*. 2010; 2: 4–9.
25. Kozlov A.I., Ateeva Yu.A., Vershubskaya G.G., Ryzhaenkov V.G. *Pediatriya – Pediatrics*. 2012; 1: 144–148.
26. Bouillon R., Garmyn M., Verstuyf A., Segaert S., Casteels K., Mathieu C. Paracrine role for calcitriol in the immune system and skin creates new therapeutic possibilities for vitamin D analogs. *Eur J Endocrinol*. 1995; 133(1): 7–16.
27. Bhalla A. K., Amento E. P., Clemens T. L., Holick M. F., Krane S. M. Specific high-affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. *J Clin Endocrinol Metab*. 1983; 57(6): 1308–1310.
28. Carlberg C. Current understanding of the function of the nuclear vitamin D receptor in response to its natural and synthetic ligands. *Recent Results Cancer Res*. 2003; 164: 29–42.
29. Staples J. A., Ponsonby A. L., Lim L. L., McMichael A. J. Ecologic analysis of some immune-related disorders, including type 1 diabetes, in Australia: latitude, regional ultraviolet radiation, and disease prevalence. *Environ Health Perspect*. 2003; 111(4): 518–23.
30. Laaksi I., Ruohola J. P., Tuohimaa P., Auvinen A., Haataja R., Pihlajamaki H., Ylikomi T. An association of serum vitamin D concentrations < 40 nmol/l with acute respiratory tract infection in young Finnish men. *Am J Clin Nutr*. 2007; 86(3): 714–717.
31. Karatekin G., Kaya A., Salihoglu O., Balci H., Nuhoglu A. Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers. *Eur J Clin Nutr.* 2009; 63(4): 473–477.
32. Oppenheim J. J., Tewary P., De La Rosa G., Yang D. Alarmins initiate host defense. *Adv Exp Med Biol*. 2007; 601: 185–194.
33. Von Essen M. R., Kongsbak M., Schjerling P., Olgaard K., Odum N., Geisler C. Vitamin D controls T cell antigen receptor signaling and activation of human T cells. *Nat Immunol.* 2010; 11: 344–349.
34. Kamen D. L., Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. *J Mol Med.* 2010; 88: 441–450.
35. Kang S. W., Sang H. K., Naeun L., Won-Woo L., Kyung-A. H., Min S. S., Seung-Hyun L., Wan-Uk K., Insoo K. 1,25(OH)2 vitamin D3 promotes FOXP3 expression via binding to vitamin D response elements in its conserved non-coding sequence region. *J Immunol.* 2012; 188(11): 5276–5282.
36. Jeffery L. E., Burke F., Mura M., Zheng Y., Qureshi O. S., Hewison M., Walker L. S., Lammas D. A., Raza K., Sansom D. M. 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. *J Immunol.* 2009; 3: 5458–5467.