Short Communication

Vitamin D in autistic children and healthy controls

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Summary

Insufficient levels of vitamin D have been demonstrated by many authors as a risk factor for autistic patients, however, the causality has not been reliably elucidated. In the present study, levels of calcidiol were determined in group of autistic children and compared with age matched healthy children as controls. Suboptimal levels of calcidiol in more than 60 % of both autistic patients as well as of control group were found. No significant differences in vitamin D between autistic children and healthy controls were observed.

For many years it was thought that the only role of calcitriol is the regulation of calcium kinetics, but in the past 15 years there have been an increasing number of studies suggesting varied actions of vitamin D (Basit 2013). Fifteen years ago it was also suggested that vitamin D was the "forgotten neurosteroid" (McGrath *et al.* 2001). Hence, there is a handful of evidence that vitamin D plays an important role in the brain and nervous system health and disease (DeLuca *et al.* 2013; Ramakrishna 1999). Low pre- and perinatal saturation with vitamin D may be a candidate risk factor for the later development of multiple disease including multiple sclerosis (MS), depression, seasonal affective disorders, schizophrenia, Alzheimer´s disease (AD), Parkinson´s disease (Eyles *et al.* 2013; Littlejohns *et al.* 2014) and others. Among the mental disorders stands out child autism (autism spectrum disorder; ASD) characterized by deficits in social communication, and which incidence has constantly risen in recent years. Typical manifestation of the disease is the repetitive and restricted patterns of behavior (Wang *et al.* 2016). According to many authors, there is a danger of ASD in children with low vitamin D level at birth. Literature about this problem was summarized elsewhere (Macova *et al.* 2017), but the number of publications is constantly rising (Cannell 2017b; Wu *et al.* 2017). Depresive symptoms as irritability and agitation are possibly observed as an autism comorbidity (Ghaziuddin 2005; Stewart *et al.* 2006) and other depressive symptoms can complicate ASD diagnosis. There are many reports describing that low levels of vitamin D or calcidiol are associated with major depression (Jozefowicz *et al.* 2014) or symptoms of depression (Black *et al.* 2014; Kerr *et al.* 2015; Maddock *et al.* 2013; Milaneschi *et al.* 2014), but no effect on reducing the severity of these symptoms was found with vitamin D supplementation (Gowda *et al.* 2015; Kjaergaard *et al.* 2012). Current evidences have not yet definitively demonstrated if vitamin D deficiency is a cause or risk factor for developing depression or if vitamin D is an effective therapy for depression (Howland 2011).

In contrast to the reports on vitamin D association with depression, fewer contributions have been published on the association of anxiety disorders with the levels of vitamin D (Armstrong *et al.* 2007). Some of them even deny the relation of vitamin D deficiency with anxiety or stress (Black *et al.* 2014).

General term Vitamin D primarily means Vitamin D3 (cholecalciferol) produced after exposure to UVB radiation by skin from 7-dehydrocholesterol and taken in limited amounts from diet. Vitamin D2 (ergocalciferol) is of plant origin. Both vitamins are inactive and transported to the liver bounded to vitamin D binding protein, where they are metabolized to respective 25-hydroxyvitamin D metabolite called *calcidiol*.

Calcidiol is a stable form of vitamin D with the biological half-life 6-8 weeks and in this form it is also stored in the body. Therefore, its level measured in blood reflects the real saturation of organism by vitamin D. Calcidiol is biologically inactive until conversion to 1,25-dihydroxymetabolite (mainly in proximal tubule of kidneys) called *calcitriol,* which is biologically active form but remains stable only for several minutes.

In our study, calcidiol levels were investigated in 45 boys, 4-7 of age, suffering from ASD and compared with the levels of calcidiol in a group of 40 age matched healthy boys as controls. A commercially available ECLIA method (Roche, Switzerland) was used to determine vitamin D levels. All children were divided into groups according to season of blood collection to avoid the effect of calcidiol fluctuations throughout the year. However, the differences between each groups were not statistically significant (see Fig. and Table).

At the end of 2016, the study of National Institute of Public Health of Czech Republic on 419 healthy children (boys and girls) was finished. Participants were children between 5 and 9 years of age. Levels of vitamin D were measured during the whole year 2016 by the same method as mentioned above. The results were very similar as found in our study with autistic children (Hanzlíková *et al.* 2018).

In Czech and Slovak Republics the recommended blood levels of calcidiol are at least 75 nmol/l. This is in agreement with the USA Vitamin D Council (30 – 100 ng/ml corresponding to 75 -250 nmol/l) as well as the Endocrine Society in the USA which made recommendations to treat and prevent vitamin D deficiency (achieving serum 25(OH)D concentrations of more than 30 ng/mL (>75 nmol/l)), with the preferred range of 40–60 ng/mL (100–150 nmol/l) (Holick *et al.* 2011; Weydert 2014). Vitamin D toxicity has never been reported, in adults or children, with 25 (OH)D levels below 200 ng/ml (500 nmol/l) (Cannell 2017a; Pludowski *et al.* 2018).

There are a handful of studies dealing with the relationship of vitamin D and the development of the nervous system in Czech children. The recent study analyzed associations between vitamin D and the parameters of bone metabolism (including vitamin D) and muscle development in adolescent girls (Cirmanova *et al.* 2017).

In our opinion, it is crucial to use a more sensitive method for measuring actual vitamin D levels, as commonly used methods do not distinguish between calcidiol and mixture of metabolic products, what results in high percentage of cross-reactions. The correct answer could be given by the chromatographic method in future. Our team is currently developing a novel chromatographic method that separates and accurately determinates vitamin D metabolites.

Surprisingly, we did not observe statistically significant differences in vitamin D between autistic children and healthy controls. Our results suggest poor supply of young population with vitamin D. We found suboptimal levels of vitamin D in more than 60 % of both autistic patients as well as of control groups. Our observation raised a question about the adequate vitamin D level needed for the physiologic development in juvenile population. Studies confirm that long-term vitamin D insufficiency leads to manifestation of neurodevelopmental disorders such as depression, anxiety, RS, AD and negative influence on immuno-metabolism (Vanherwegen *et al.* 2017).

In summer, healthy children do not need supplementation by vitamin D if they are exposed long enough to the sun. In winter, it is necessary to check the level of vitamin D (calcidiol) at least 75 nmol/l is desired. Once the level is lower, it is necessary to initiate or increase usage of nutritional supplements with readily absorbable vitamin D and to increase the consumption of fish to activate the neuroimmune system and balance the body homeostasis.

In pregnant and breastfeeding women, especially during October-April, replenishment is necessary! The international consensus recommends a flat-rate supplemental dose of 500-600 IU per day, with infants still supplying 1000 mg of calcium daily (Munns *et al.* 2016).

It is not possible to overdose vitamin D (= cholecalciferol) at the dose determined by the physician and if it is not for patients with sarcoidosis, granulomatous diseases or hypercalcemia!

Conflict of Interest

There is no conflict of interest.

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REFERENCES:

ARMSTRONG DJ, MEENAGH GK, BICKLE I, LEE AS, CURRAN ES, FINCH MB: Vitamin D deficiency is associated with anxiety and depression in fibromyalgia. *Clin Rheumatol* **26**: 551-554, 2007.

BASIT S: Vitamin D in health and disease: a literature review. *Br J Biomed Sci* **70**: 161-172, 2013.

BLACK LJ, JACOBY P, ALLEN KL, TRAPP GS, HART PH, BYRNE SM, MORI TA, BEILIN LJ, ODDY WH: Low vitamin D levels are associated with symptoms of depression in young adult males. *Aust N Z J Psychiatry* **48**: 464-471, 2014.

CANNELL JJ: How safe is vitamin D? https://www.vitamindcouncil.org/how-safe-is-vitamin-d/, 2017a.

CANNELL JJ: Vitamin D and autism, what's new? *Rev Endocr Metab Disord* **18**: 183-193, 2017b.

CIRMANOVA V, ZOFKOVA I, KASALICKY P, LANSKA V, BAYER M, STARKA L, KANCEVA R: Hormonal and bone parameters in pubertal girls. *Physiol Res* **66**: S419-S424, 2017.

DELUCA GC, KIMBALL SM, KOLASINSKI J, RAMAGOPALAN SV, EBERS GC: Review: the role of vitamin D in nervous system health and disease. *Neuropathol Appl Neurobiol* **39**: 458-484, 2013.

EYLES DW, BURNE TH, MCGRATH JJ: Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. *Front Neuroendocrinol* **34**: 47-64, 2013.

GHAZIUDDIN M: Mental Health Aspects of Autism and Asperger Syndrome. Jessica Kingsley Publishers, London, 2005.

GOWDA U, MUTOWO MP, SMITH BJ, WLUKA AE, RENZAHO AM: Vitamin D supplementation to reduce depression in adults: meta-analysis of randomized controlled trials. *Nutrition* **31**: 421-429, 2015.

HANZLÍKOVÁ L, SOCHOROVÁ L, PUKLOVÁ V, ČERNÁ M, FIALOVÁ AC, VOSÁTKOVÁ M, BIČÍKOVÁ M, KUBÍNOVÁ R: Jsou české děti dostatečně zásobeny vitaminem D? *Vox Pediatriae* **18**: 34-35, 2018.

HOLICK MF, BINKLEY NC, BISCHOFF-FERRARI HA, GORDON CM, HANLEY DA, HEANEY RP, MURAD MH, WEAVER CM: Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* **96**: 1911-1930, 2011.

HOWLAND RH: Vitamin D and depression. *J Psychosoc Nurs Ment Health Serv* **49**: 15-18, 2011.

JOZEFOWICZ O, RABE-JABLONSKA J, WOZNIACKA A, STRZELECKI D: Analysis of vitamin D status in major depression. *J Psychiatr Pract* **20**: 329-337, 2014.

KERR DC, ZAVA DT, PIPER WT, SATURN SR, FREI B, GOMBART AF: Associations between vitamin D levels and depressive symptoms in healthy young adult women. *Psychiatry Res* **227**: 46-51, 2015.

KJAERGAARD M, EGGEN AE, MATHIESEN EB, JORDE R: Association between headache and serum 25-hydroxyvitamin D: the Tromso Study: Tromso 6. *Headache* **52**: 1499-1505, 2012.

LITTLEJOHNS TJ, HENLEY WE, LANG IA, ANNWEILER C, BEAUCHET O, CHAVES PH, FRIED L, KESTENBAUM BR, KULLER LH, LANGA KM, LOPEZ OL, KOS K, SONI M, LLEWELLYN DJ: Vitamin D and the risk of dementia and Alzheimer disease. *Neurology* **83**: 920-928, 2014.

MACOVA L, BICIKOVA M, OSTATNIKOVA D, HILL M, STARKA L: Vitamin D, neurosteroids and autism. *Physiol Res* **66**: S333-S340, 2017.

MADDOCK J, BERRY DJ, GEOFFROY MC, POWER C, HYPPONEN E: Vitamin D and common mental disorders in mid-life: cross-sectional and prospective findings. *Clin Nutr* **32**: 758-764, 2013.

MCGRATH J, FERON F, EYLES D, MACKAY-SIM A: Vitamin D: the neglected neurosteroid? *Trends Neurosci* **24**: 570-572, 2001.

MILANESCHI Y, HOOGENDIJK W, LIPS P, HEIJBOER AC, SCHOEVERS R, VAN HEMERT AM, BEEKMAN AT, SMIT JH, PENNINX BW: The association between low vitamin D and depressive disorders. *Mol Psychiatry* **19**: 444-451, 2014.

MUNNS CF, SHAW N, KIELY M, SPECKER BL, THACHER TD, OZONO K, MICHIGAMI T, TIOSANO D, MUGHAL MZ, MAKITIE O, RAMOS-ABAD L, WARD L, DIMEGLIO LA, ATAPATTU N, CASSINELLI H, BRAEGGER C, PETTIFOR JM, SETH A, IDRIS HW, BHATIA V, FU J, GOLDBERG G, SAVENDAHL L, KHADGAWAT R, PLUDOWSKI P, MADDOCK J, HYPPONEN E, ODUWOLE A, FREW E, AGUIAR M, TULCHINSKY T, BUTLER G, HOGLER W: Global Consensus Recommendations on Prevention and Management of Nutritional Rickets. *J Clin Endocrinol Metab* **101**: 394-415, 2016.

PLUDOWSKI P, HOLICK MF, GRANT WB, KONSTANTYNOWICZ J, MASCARENHAS MR, HAQ A, POVOROZNYUK V, BALATSKA N, BARBOSA AP, KARONOVA T, RUDENKA E, MISIOROWSKI W, ZAKHAROVA

I, RUDENKA A, LUKASZKIEWICZ J, MARCINOWSKA-SUCHOWIERSKA E, LASZCZ N, ABRAMOWICZ P, BHATTOA HP, WIMALAWANSA SJ: Vitamin D supplementation guidelines. *J Steroid Biochem Mol Biol* **175**: 125-135, 2018.

RAMAKRISHNA T: Vitamins and brain development. *Physiol Res* **48**: 175-187, 1999.

STEWART ME, BARNARD L, PEARSON J, HASAN R, O'BRIEN G: Presentation of depression in autism and Asperger syndrome: a review. *Autism* **10**: 103-116, 2006.

VANHERWEGEN AS, GYSEMANS C, MATHIEU C: Vitamin D endocrinology on the cross-road between immunity and metabolism. *Mol Cell Endocrinol* **453**: 52-67, 2017.

WANG T, SHAN L, DU L, FENG J, XU Z, STAAL WG, JIA F: Serum concentration of 25-hydroxyvitamin D in autism spectrum disorder: a systematic review and meta-analysis. *Eur Child Adolesc Psychiatry* **25**: 341-350, 2016.

WEYDERT JA: Vitamin D in Children's Health. *Children (Basel)* **1**: 208-226, 2014.

WU DM, WEN X, HAN XR, WANG S, WANG YJ, SHEN M, FAN SH, ZHUANG J, LI MQ, HU B, SUN CH, BAO YX, YAN J, LU J, ZHENG YL: Relationship Between Neonatal Vitamin D at Birth and Risk of Autism Spectrum Disorders: the NBSIB Study. *J Bone Miner Res*, 2017.

Table 1. Vitamin D in autistic (ASD) and control group (in nmol/l).

	control group	ASD
Number of subjects	40	45
Average vitD	70.348	65.066
Median vitD	64.46	65.22
Standard deviation	20.733	25.950
Min vitD	26.71	22.03
Max vitD	128.2	136.1
Lower quartile	57.495	44.47
Upper quartile	82.8	83.5

Fig. 1. Distribution of ASD patients/controls in groups according to vitamin D level.