



Editorial

What is the definition of "vitamin D deficiency" and who is considered "vitamin D deficient"? Urgent need for a national consensus

Bahareh Nikooyeh, Tirang R. Neyestani*

Laboratory of Nutrition Research, National Nutrition and Food Technology Research Institute and Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Received: February 2017

Accepted: March 2017

Since early works on isolation and characterization of vitamin D by Elmer Verner McCollum and Edward Mellanby in 1900s, this molecule still seems mysterious (1-2). After several decades since discovery of the miraculous effect of cod liver oil in treatment of nutritional rickets, escalating number of reports provide a huge body of evidence for various effects of vitamin D on human body (3). The spectrum of vitamin D health effects is quite wide, from bone and muscles (so-called calcemic effects) to a variety of non-calcemic effects including cardiovascular, insulin function, adipogenesis, obesity, immunity and mental status. Consequently, having an optimal vitamin D status has attracted a huge attention (4-8).

Despite the fact that the body is equipped with the vitamin D photosynthetic machinery, undesirable vitamin D status is a global health problem due to many geographical, environmental as well as socio-cultural reasons (9-10). However, unlike most other nutrients, there is no consensus on definition of "desirable vitamin D status". It is generally accepted that circulating 25-hydroxycalciferol (25(OH)D) concentration can well reflect the body's vitamin D status (11). However, there is no agreement on desirable serum 25(OH)D level (12). While some scientific bodies recommend concentrations above 50 nmol/L (13), some vitamin D experts criticize this recommendation and suggest as high concentrations as 75 and 100 nmol/L and even above (10). As a result, different research groups usually based on their experience, recommendation of the manufacturers of the commercial assay kits and

occasionally their tastes use various cutoff points to define vitamin D status of their study population. Needless to say that the occurrence rates reported by these studies may hardly be comparable. The problem of definition of vitamin D status becomes even more complicated when considering the effect of assay system on 25(OH)D test results (14).

Table 1 shows some of the cross-sectional researches performed over a decade ago. The cutoffs used, range from 23 nmol/L (15) to as high as 87.5 nmol/L (16). Interestingly the prevalence of undesirable vitamin D status in 20-64 yr males based on 23 nmol/L was reported 81.3% (15) while in another study on mothers of newborns, the prevalence rate was as low as 5.7%, based on 25(OH)D < 50 nmol/L (17). Part of these discrepancies might be due to the methods used in various studies. Disagreement of 25(OH)D assay results obtained from different assay systems has been already reported (14, 18-22). This could potentially disturb decision making at the clinical settings (23).

At the national level, two nationwide surveys on vitamin D are noticeable. In 2001, the first National Integrated Micronutrient Survey (NIMS I), reported the prevalence rates of undesirable vitamin D status among 15-23 month children and pregnant women as 3.7% and 0.9%, respectively (24). However, after a decade the prevalence rates in these age groups tremendously increased to 23.7% and 86%, respectively, as reported by NIMS II (25). These unexpectedly worrying numbers vigorously stimulated stake-holders at Deputy of Health of the

Iran Ministry of Health (MOH) to hold several sessions in order to take an urgent action. These sessions led to recommendations and

implementations of supplementation for different population subgroups across the country.

Table 1. Occurrence rates of undesirable vitamin D status using different assay methods in different Iranian subpopulations

Author, year	Population	Method	Cutoff	Prevalence of undesirable vitamin D status
Moussavi M, [et al], 2005 (43)	153 boys and 165 girls, aged 14-18 yr	RIA	< 50 nmol/L	all subjects: 46.2% (72.1% in females and 18.3% in males)
Hashemipour S, [et al], 2004 (15)	1210 subjects 20-64 yr	RIA	Normal range: 23 to 113 nmol/L	81.3%
Salek M, [et al], 2008 (17)	88 newborns and their mothers	RIA	Mothers: < 50 nmol/L Newborns: < 31.2 nmol/L	Mothers: 5.7% Newborn: 4.5%
Kazemi A, [et al], 2009 (44)	67 full-term pregnant mothers	-	< 25 nmol/L	86% of the women and 75% of the newborns during winter and 46% of the mothers and 35% of the newborns during summer
Rahnavard Z, [et al], 2010 (16)	2396 healthy men	EIA	< 87.5 nmo/L	68.8%
Hovsepian S, [et al], 2011 (45)	1,111 healthy people- 243 men and 868 women, 20-80 yr	RIA	< 75 nmol/L	70.4%
Kashi Z, [et al], 2011 (46)	351 subjects (66.4% women, 33.6% men) aged 11 to 69	EIA	< 75 nmol/L	87.5% in winter and 78.6% in summer
Kaykhaei MA, [et al], 2011 (47)	993 apparently healthy subjects	ECL	< 75 nmol/L	94.7%
Khalesi N, [et al], 2012 (48)	100 neonates and their mothers	Not reported	Not reported	85% of neonates and 74% of mothers
Neyestani TR, [et al], 2012 (27)	1111 children aged 9-12 yr	EIA	< 50 nmol/L	91.7 %
Talaei A, [et al], 2012 (49)	420 students 10-16 yr	RIA	< 50 nmol/L	84%
Alipour S, [et al], 2014 (50)	538 women aged 20-80 years	ECL	<35 nmol/L	69%
Faghih S, [et al], 2014 (51)	254 university students (19-32 yr)	RIA	< 75 nmol/l	95.2% of males 97.5% of females
Saki F, [et al], 2015 (29)	children (n=477) aged 9-18 years	HPLC	< 75 nmol/L	96%
Abbasian M, [et al], 2016 (52)	284 pregnant women and their newborn	EIA	< 75 nmol/L	Mothers: 61.3% Neonates: 51.4%
Larijani B, [et al], 2016 (30)	444 middle and high school students	EIA	< 75 nmol/L	77.6%

Abbreviations: ECL: electrochemiluminescence; EIA: enzyme immunoassay; HPLC: high-performance liquid chromatography; RIA: radioimmunoassay.

Notwithstanding, some points have seemingly been overlooked in interpretation of NIMS II findings. Firstly, the 25(OH) D assay method used in NIMS I was radioimmunoassay (RIA) whereas in NIMS II was electrochemiluminescence binding immunoassay (ECLIA) using Elecsys system. Disagreement among different 25(OH) D systems has been demonstrated in several studies which was more prominent for RIA than the other systems (19-20, 22). More importantly, the cutoffs used to define vitamin D deficiency in NIMS I was 25(OH)D < 12 and <25 nmol/L while in NIMS II was <25 and <50 nmol/L for vitamin D deficiency and insufficiency, respectively (24-25). Obviously, this rise in cutoff points could cause a great increment in the prevalence rates.

The National Food and Nutrition Surveillance (FNS) revealed 93% of the Iranian children have suboptimal circulating 25(OH) D (< 50 nmol/L) during cold seasons (26). This occurrence rate is quite comparable with our earlier reports from Tehran children (27). Similar occurrence rates were observed in adults across latitudinal gradient (28). In these studies similar cutoff points and assay methods were employed. Other studies that used similar cutoffs but different assay systems reported different occurrence rates in almost similar age groups (29-30).

Though lots of reports suggest a pandemic of vitamin D deficiency (31-36), some researchers believe that this is a misconception (37). They believe that the Institute of Medicine (IOM) recommendation for desirable circulating 25(OH) D concentration is based on recommended daily intake (RDA) for vitamin D. However, RDA considers the population at the highest end of distribution whereas estimated average requirement (EAR) gives a more realistic picture of the population's requirement. It is noteworthy that neither RDA nor EAR considers solar exposure. According to these researchers, based on EAR half of the population would need to have circulating 25(OH)D \leq 40 nmol/L (16 ng/mL) (37). A major critique of this notion is that only calcemic effects of vitamin D were considered. Besides, it has been estimated that daily intake of 440 IU vitamin D (=11 μ g) would result in 19.4 (CI: 13.9-24.9) nmol/L increment in serum 25(OH)D (38). Therefore, daily intake of vitamin D as much as RDA (600 IU) and EAR (400 IU) would result in an average increase of

26.4 and 17.6 nmol/L in circulating calcidiol. That means neither RDA nor EAR would be efficient in raising serum calcidiol concentrations to as high as 50 and 40 nmol/L respectively. at least in those people who do not have enough solar exposure for any reason.

The situation therefore seems absolutely confounding. The key question is raised as "what is the definition of vitamin D deficiency?" In other words, which criteria are mostly suitable for the Iranian population? Answer to this question is undisputedly important. One of the viable and sustainable strategies to tackle micronutrient deficiencies (including vitamin D) is fortification of staple foods (39). Without a concrete criterion, it would be hard, if not impossible, for stake-holders to decide how to implement and how to evaluate the effectiveness of the fortification program.

The other important issue is the variability of calcidiol assay results from different assay systems. To overcome this problem, employment of assay-specific cutoff points (40) as well as harmonization and standardization of the assay results have been proposed (41-42). Based on our experience, we believe that circulating 25(OH)D concentrations of 50 nmol/L (20 ng/mL) and above based on HPLC assay system could be considered adequate. This cutoff point could vary according to the assay system (42). Nevertheless, a national consensus is urgently needed on definition of adequate vitamin D status. Until that day, that we do hope it comes very soon, the establishment of a sustainable effective strategy against vitamin D deficiency is absolutely unrealistic.

Financial disclosure

The authors declared no financial interest.

References

1. Mellanby E. An experimental investigation on rickets. *Nutrition Reviews*. 1976;34(11):338-40.
2. McCollum E, Simmonds N, Becker JE, Shipley P. Studies on experimental rickets XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. *Journal of Biological Chemistry*. 1922;53(2):293-312.
3. O'Riordan JL, Bijvoet OL. Rickets before the discovery of vitamin D. *Bonekey Reports*. 2014 Jan 8;3:478.
4. Grober U, Spitz J, Reichrath J, Kisters K, Holick MF. Vitamin D: Update 2013: From rickets prophylaxis to

- general preventive healthcare. *Dermatoendocrinol.* 2013 Jun 01;5(3):331-47.
5. Hossein-nezhad A, Holick MF. Vitamin D for health: a global perspective. *Mayo Clin Proc.* 2013 Jul;88(7):720-55.
 6. Nimitphong H, Holick MF. Vitamin D status and sun exposure in southeast Asia. *Dermatoendocrinol.* 2013 Jan 01;5(1):34-7.
 7. Wacker M, Holick MF. Sunlight and Vitamin D: A global perspective for health. *Dermatoendocrinol.* 2013 Jan 01;5(1):51-108.
 8. Wacker M, Holick MF. Vitamin D - effects on skeletal and extraskkeletal health and the need for supplementation. *Nutrients.* 2013 Jan 10;5(1):111-48.
 9. Holick MF. McCollum Award Lecture, 1994: vitamin D--new horizons for the 21st century. *The American Journal of Clinical Nutrition.* 1994;60(4):619-30.
 10. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *The Journal of Clinical Endocrinology and Metabolism.* 2011;96(7):1911-30.
 11. Hollis BW. Assessment of vitamin D status and definition of a normal circulating range of 25-hydroxyvitamin D. *Current Opinion in Endocrinology, Diabetes and Obesity.* 2008;15(6):489-94.
 12. Holick MF. Vitamin D deficiency. *New England Journal of Medicine.* 2007;357(3):266-81.
 13. IOM Updates Guidance on Vitamin D, Calcium. <http://www.aafp.org/news/health-of-the-public/20101201iomrpt-vitdcal.html>. 2010.
 14. Enko D, Fridrich L, Rezanka E, Stolba R, Ernst J, Wendler I, et al. 25-hydroxy-Vitamin D status: limitations in comparison and clinical interpretation of serum-levels across different assay methods. *Clin Lab.* 2014;60(9):1541-50.
 15. Hashemipour S, Larijani B, Adibi H, Javadi E, Sedaghat M, Pajouhi M, et al. Vitamin D deficiency and causative factors in the population of Tehran. *BMC Public Health.* 2004 Aug 25;4:38.
 16. Rahnavard Z, Eyboosh S, Homami MR, Meybodi HA, Azemati B, Heshmat R, et al. Vitamin d deficiency in healthy male population: results of the Iranian multi-center osteoporosis study. *Iran J Public Health.* 2010;39(3):45-52.
 17. Salek M, Hashemipour M, Aminorroaya A, Gheiratmand A, Kelishadi R, Ardestani PM, et al. Vitamin D deficiency among pregnant women and their newborns in Isfahan, Iran. *Exp Clin Endocrinol Diabetes.* 2008 Jun;116(6):352-6.
 18. van den Ouweland JM, Beijers AM, Demacker PN, van Daal H. Measurement of 25-OH-vitamin D in human serum using liquid chromatography tandem-mass spectrometry with comparison to radioimmunoassay and automated immunoassay. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2010 May 01;878(15-16):1163-8.
 19. Jafri L, Khan AH, Siddiqui AA, Mushtaq S, Iqbal R, Ghani F, et al. Comparison of high performance liquid chromatography, radio immunoassay and electrochemiluminescence immunoassay for quantification of serum 25 hydroxy vitamin D. *Clin Biochem.* 2011 Jul;44(10-11):864-8.
 20. Neyestani TR, Gharavi A, Kalayi A. Determination of serum 25-hydroxy cholecalciferol using high-performance liquid chromatography: a reliable tool for assessment of vitamin D status. *Int J Vitam Nutr Res.* 2007 Sep;77(5):341-6.
 21. Gallo S, Comeau K, Agellon S, Vanstone C, Sharma A, Jones G, et al. Methodological issues in assessing plasma 25-hydroxyvitamin D concentration in newborn infants. *Bone.* 2014 Apr;61:186-90.
 22. Zahedi Rad M, Neyestani TR, Nikooyeh B, Shariatzadeh N, Kalayi A, Khalaji N, et al. Competitive Protein-binding assay-based Enzyme-immunoassay Method, Compared to High-pressure Liquid Chromatography, Has a Very Lower Diagnostic Value to Detect Vitamin D Deficiency in 9-12 Years Children. *Int J Prev Med.* 2015;6:67.
 23. Lai JK, Lucas RM, Banks E, Ponsonby AL. Variability in vitamin D assays impairs clinical assessment of vitamin D status. *Intern Med J.* 2012 Jan;42(1):43-50.
 24. National Integrated Micronutrient Survey 2001 (NIMS I) Tehran: Ministry of Health and Medical Education. Nutrition Office of Iran Ministry of Health, National Nutrition and Food Technology Research Institute (National Report).2005.
 25. Siassi F, Mohammad K, Djazayeri A, Djalali M, Abdollahi Z, Dorosty A.R, et al. National Integrated Micronutrient Survey 2012 (NIMS II) Tehran: Ministry of Health and Medical Education2015.
 26. Nikooyeh B, Abdollahi Z, Hajifaraji M, Alavi-Majd H, Salehi F, Yarpardar AH, et al. Vitamin D Status, Latitude and their Associations with Some Health Parameters in Children: National Food and Nutrition Surveillance. *J Trop Pediatr.* 2017 Feb;63(1):57-64.
 27. Neyestani TR, Hajifaraji M, Omidvar N, Eshraghian MR, Shariatzadeh N, Kalayi A, et al. High prevalence of vitamin D deficiency in school-age children in Tehran, 2008: a red alert. *Public Health Nutr.* 2012 Feb;15(2):324-30.
 28. Nikooyeh B, Abdollahi Z, Hajifaraji M, Alavi-majd H, Salehi F, Yarpardar AH, et al. Vitamin D status and cardiometabolic risk factors across latitudinal gradient in Iranian adults: National food and nutritional surveillance. *Nutr Health.* 2017:[in press].
 29. Saki F, Dabbaghmanesh MH, Omrani GR, Bakhshayeshkaram M. Vitamin D deficiency and its associated risk factors in children and adolescents in southern Iran. *Public Health Nutr.* 2015 Jun 08:1-6.

30. Larijani B, Hossein-Nezhad A, Feizabad E, Maghbooli Z, Adibi H, Ramezani M, et al. Vitamin D deficiency, bone turnover markers and causative factors among adolescents: a cross-sectional study. *J Diabetes Metab Disord.* 2016;15:46.
31. Holick MF. The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action. *Mol Aspects Med.* 2008 Dec;29(6):361-8.
32. Dobnig H. A review of the health consequences of the vitamin D deficiency pandemic. *J Neurol Sci.* 2011 Dec 15;311(1-2):15-8.
33. Ferder M, Inserra F, Manucha W, Ferder L. The world pandemic of vitamin D deficiency could possibly be explained by cellular inflammatory response activity induced by the renin-angiotensin system. *Am J Physiol Cell Physiol.* 2013 Jun 01;304(11):C1027-39.
34. Robey RB, Crane-Godreau MA. "Does sunscreen promote hypertension?" and other questions. Novel interactions between vitamin D and the renin-angiotensin axis. Focus on "The world pandemic of vitamin D deficiency could possibly be explained by cellular inflammatory response activity induced by the renin-angiotensin system". *Am J Physiol Cell Physiol.* 2013 Jun 01;304(11):C1040-1.
35. Shah D, Gupta P. Vitamin D Deficiency: Is The Pandemic for Real? *Indian J Community Med.* 2015 Oct-Dec;40(4):215-7.
36. Cashman KD, Dowling KG, Skrabakova Z, Gonzalez-Gross M, Valtuena J, De Henauw S, et al. Vitamin D deficiency in Europe: pandemic? *Am J Clin Nutr.* 2016 Apr;103(4):1033-44.
37. Manson JE, Brannon PM, Rosen CJ, Taylor CL. Vitamin D Deficiency - Is There Really a Pandemic? *N Engl J Med.* 2016 Nov 10;375(19):1817-20.
38. Black LJ, Seamans KM, Cashman KD, Kiely M. An updated systematic review and meta-analysis of the efficacy of vitamin D food fortification. *J Nutr.* 2012 Jun;142(6):1102-8.
39. Nikooyeh B, Neyestani TR, Zahedirad M, Mohammadi M, Hosseini SH, Abdollahi Z, et al. Vitamin D-Fortified Bread Is as Effective as Supplement in Improving Vitamin D Status: A Randomized Clinical Trial. *J Clin Endocrinol Metab.* 2016 Jun;101(6):2511-9.
40. Souberbielle JC, Fayol V, Sault C, Lawson-Body E, Kahan A, Cormier C. Assay-specific decision limits for two new automated parathyroid hormone and 25-hydroxyvitamin D assays. *Clin Chem.* 2005 Feb;51(2):395-400.
41. Hypponen E, Turner S, Cumberland P, Power C, Gibb I. Serum 25-hydroxyvitamin D measurement in a large population survey with statistical harmonization of assay variation to an international standard. *J Clin Endocrinol Metab.* 2007 Dec;92(12):4615-22.
42. Nikooyeh B, Samiee SM, Farzami MR, Alavimajd H, Zahedirad M, Kalayi A, et al. Harmonization of serum 25-hydroxycalciferol assay results from high-performance liquid chromatography, enzyme immunoassay, radioimmunoassay, and immunochemiluminescence systems: A multicenter study. *J Clin Lab Anal.* 2017 Feb 07.
43. Moussavi M, Heidarpour R, Aminorroaya A, Pournaghshband Z, Amini M. Prevalence of vitamin D deficiency in Isfahani high school students in 2004. *Horm Res.* 2005;64(3):144-8.
44. Kazemi A, Sharifi F, Jafari N, Mousavinasab N. High prevalence of vitamin D deficiency among pregnant women and their newborns in an Iranian population. *J Womens Health (Larchmt).* 2009 Jun;18(6):835-9.
45. Hovsepian S, Amini M, Aminorroaya A, Amini P, Iraj B. Prevalence of vitamin D deficiency among adult population of Isfahan City, Iran. *J Health Popul Nutr.* 2011 Apr;29(2):149-55.
46. Kashi Z, Saeedian F, Akha O, Gorgi M, Emadi S, Zakeri H. Vitamin D deficiency prevalence in summer compared to winter in a city with high humidity and a sultry climate. *Endokrynol Pol.* 2011;62(3):249-51.
47. Kaykhaei MA, Hashemi M, Narouie B, Shikhzadeh A, Rashidi H, Moulaei N, et al. High prevalence of vitamin D deficiency in Zahedan, southeast Iran. *Ann Nutr Metab.* 2011;58(1):37-41.
48. Khalesi N, Bahaeddini SM, Shariat M. Prevalence of maternal vitamin D deficiency in neonates with delayed hypocalcaemia. *Acta Med Iran.* 2012;50(11):740-5.
49. Talaei A, Yadegari N, Rafee M, Rezvanfar MR, Moini A. Prevalence and cut-off point of vitamin D deficiency among secondary students of Arak, Iran in 2010. *Indian J Endocrinol Metab.* 2012 Sep;16(5):786-90.
50. Alipour S, Saberi A, Seifollahi A, Shirzad N, Hosseini L. Risk factors and prevalence of vitamin d deficiency among Iranian women attending two university hospitals. *Iran Red Crescent Med J.* 2014 Oct;16(10):e15461.
51. Faghieh S, Abdolahzadeh M, Mohammadi M, Hasanazadeh J. Prevalence of vitamin d deficiency and its related factors among university students in shiraz, iran. *Int J Prev Med.* 2014 Jun;5(6):796-9.
52. Abbasian M, Chaman R, Amiri M, Ajami ME, Jafari-Koshki T, Rohani H, et al. Vitamin D Deficiency in Pregnant Women and Their Neonates. *Glob J Health Sci.* 2016 Sep 01;8(9):54008.