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Review Article

Factors Causing Discrepancies in the Results of Vitamin D Assessment in Community Studies: Proper Judgement, Proper Act
A note for the policymakers

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ABSTRACT
Vitamin D was identified over one hundred years ago, yet vitamin D deficiency (VDD) is still the most prevalent nutritional problem all around the world. The National Food and Nutrition Surveillance together with other reports have documented alarming rates of VDD and its possible adverse consequences in both children and adults in Iran. However, the prevalence rates of VDD reported by various research groups show a wide range. In this short review, we discuss some of the main causes of these discrepancies and propose some strategies to minimize them. Finally, some key recommendations for future studies and combating VDD are presented for policymakers.

Keywords: Vitamin D, Vitamin D deficiency, 25-hydroxycalciferol, 25(OH)D, Food fortification

Highlights:
- Vitamin D deficiency (VDD) is a global health problem and Iran is no exception. Nevertheless, the prevalence rates of VDD reported by various research groups show a wide range.
- To have a better picture of vitamin D status in the community, standardization of definitions of vitamin D status and laboratory methods is necessary.
- One approach to minimize inter-method variations of 25(OH)D assay is harmonization.
- Using any method to assay circulating 25(OH)D, VDD prevalence rate of 50 percent and above is undoubtedly a major health problem and necessitates prompt intervention.
- Vitamin D fortification of flour is an effective strategy to improve vitamin D status of the community but must be examined in a pilot study.

Introduction
The history of medicine has witnessed several cases of human glories in the battle with infectious diseases. Eradication of smallpox from the world (1), dracontiasis from Iran (2) and very recently control of corona virus (COVID-19) pandemic (3) are just a few examples. In the latter case, the duration between identification of coronavirus and development of effective vaccines was rather short (4). Ironically and sadly, this is not true for many forms of malnutrition especially several micronutrient deficiencies including vitamin D deficiency (VDD). Vitamin D was identified by McCollum over one hundred years ago, yet VDD is still the most prevalent nutritional problem all around the world (5, 6). Suboptimal vitamin D status has been associated with many conditions including musculoskeletal disorders, cardiovascular diseases, diabetes, some forms of malignancies notably colorectal cancers, depression, pregnancy outcomes and the prognosis of COVID-19 (7-14). Several reports derived from the National Food and Nutrition Surveillance (FNS) being implemented by the National Nutrition and Food Technology Research (NNFTRI) in Iran have documented alarming rates of VDD and its possible adverse consequences in both children and adults (15-21).

Vitamin D: Vitamers, Sources and Metabolism

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Vitamin D is a common name for a group of vitamers with more or less similar functions but with different potencies in the body. Traditionally, vitamin D is known mostly with its two isoforms based on their origins i.e. ergocalciferol (D_2) which is plant-based and cholecalciferol (D_3) which is found in animals. Yet, there is another vitamer, vitamin D_4, which is photosynthesized from 22,23-dihydroergosterol which is structurally very similar to vitamin D_3 except for an additional methyl group on carbon 24 of the side chain (22).

The natural source of vitamin D for humans is sun. Provitamin D_3, 7-dehydrocholesterol, in epidermis and dermis is converted to previtamin D_3 under the influence of solar ultra violet (UV) beam in the wavelength range of 280-320 nm. To become fully activated, prevtiamin D_3 needs to undergo two steps of hydroxylation in the liver and kidney to produce 25-hydroxycholecalciferol (25(OH)D_3) and the daughter steroid hormone, 1,25-dihydroxycholecalciferol (1, 25$(OH)_2D_3$ or calcitriol), respectively. Calcitriol acts in the body through the so-called calcemic and non-calcemic functions (Figure 1) (12).

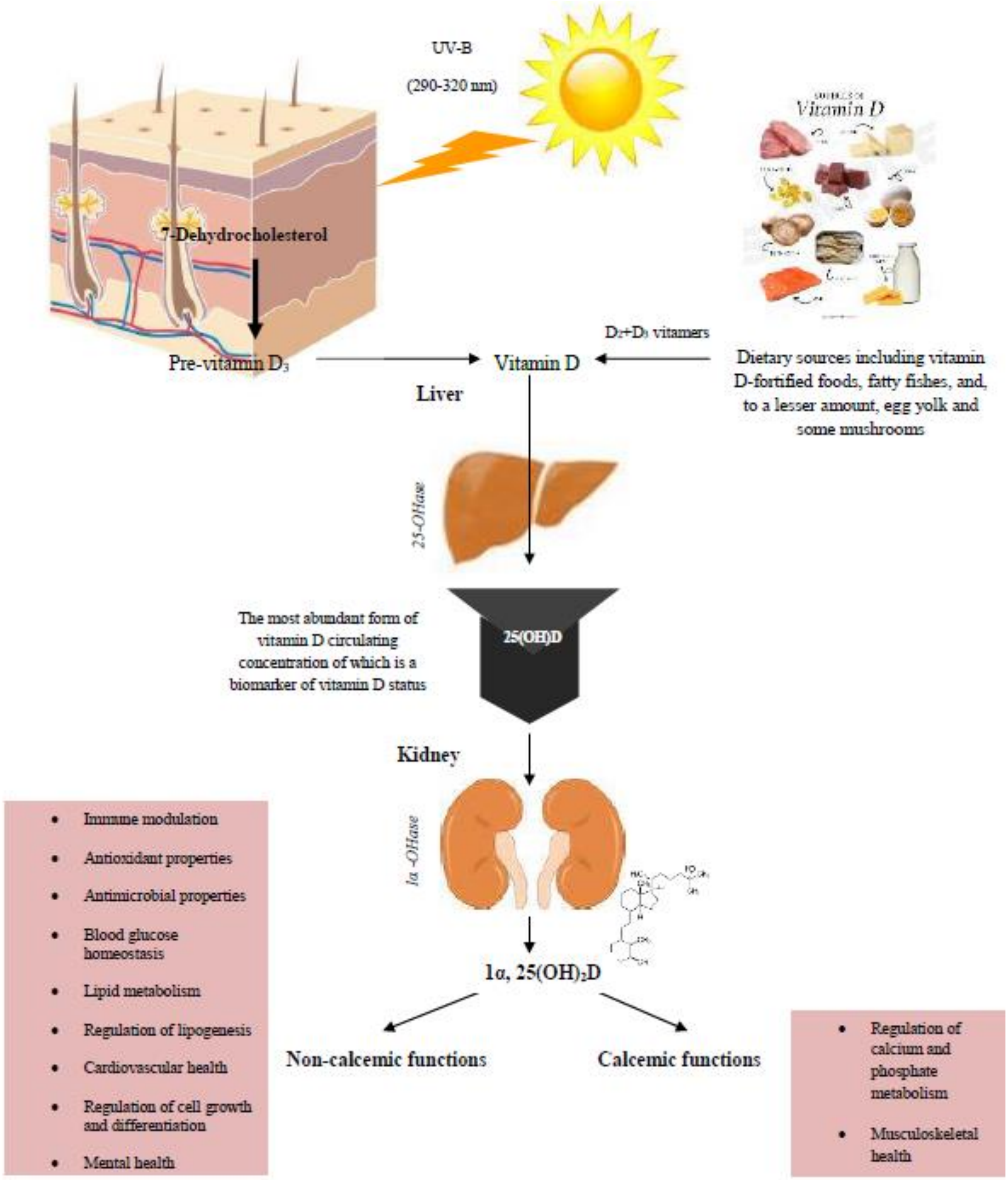

Figure 1. Vitamin D biosynthesis, metabolism and functions at a glance

Assessment Methods of Vitamin D Status: Why Results from Different Studies are Different and How to Minimize the Discrepancies

Circulating 25(OH)D_3, the most abundant form of cholecalciferol (23), has been commonly accepted as a biomarker of vitamin D status. Though technically total 25(OH)D (D_2+D_3) is assayed, the contribution of 25(OH)D_2 in some communities including Iran is actually negligible (24). There are several methods to measure serum 25(OH)D including enzyme-linked immunoassay (EIA), radioimmunoassay (RIA), high performance liquid chromatography (HPLC), electrochemiluminescence (ECL), chemiluminescent immunoassay (CLIA) and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), each with certain advantages and limitations (25). Therefore, at the first glance the issue of assessing vitamin D status in a clinical setting or at the population level seems very easy; just a small amount of (fasting or non-fasting) venous blood is needed to separate serum for 25(OH)D determination using one of the above-mentioned systems. Notwithstanding, judgement on vitamin D status based on circulating 25(OH)D concentration especially in community studies has been, and continues to be, problematic for several reasons (26-28). Firstly, there is still no consensus on definitions of vitamin D deficiency, insufficiency and sufficiency (29). This issue was the subject of a great debate in the 2nd International Conference on Controversies in Vitamin D (30). Secondly, there is a considerable disagreement among the results obtained from different methods and from different laboratories (31-34). The other issue is the season of the study. Seasonal variation in circulating 25(OH)D has been well documented (16, 35). Altogether, these have caused a wide range of prevalence rates of VDD in Iran reported by various research groups (36-39). Even two meta-analytical studies conducted separately in 2018 reported different prevalence rates (40, 41).

To have a better judgement on the situation of vitamin D status in the community, employment of reliable laboratory kits is a must. Nonetheless, this is highly challenging in Iran due to the severe sanction condition in the country which adversely affects laboratory supply (42). Consequently, medical and research laboratories actually have no selection right; they must purchase whatever available and affordable in the market instead of checking the performance characteristics of the kits and selecting the most suitable ones. One strategy to minimize discrepancies in 25(OH)D assay results among different methods is harmonization. Using this approach, results obtained from different methods are harmonized with a reference method like HPLC or, where available, LC-MS/MS (31). Nevertheless, the harmonizing equations must be calculated for the available kits.

Vitamin D Deficiency in Iran: How to Judge, How to Act?

Despite discrepancies in prevalence rates of VDD in Iran reported by different studies (15-17, 37, 39-41), and apart from the season of the assessments and the methods used for 25(OH)D assay, all studies reported high prevalence rates and considered VDD as a major public health problem. We do believe that VDD prevalence rates of 50 percent and above, regardless of the assay method, necessitates prompt attention and action. Vitamin D supplementation and food fortification are the main strategies to combat VDD. Notwithstanding, food fortification is more cost-effective and sustainable and also have a wider population coverage (43-45).

Key messages to the policy-makers:

- To have a better picture of vitamin D status in the community to be used as an evidence to design and implement proper interventions, standardization of definitions of vitamin D status , i.e. who is deficient, insufficient and sufficient, and of laboratory methods is undoubtedly necessary (29, 33). Recently, vitamin D status based on circulating 25(OH)D concentrations was defined as : deficiency [<20 ng/mL (<50 nmol/L)], suboptimal status [20–30 ng/mL (50–75 nmol/L)], and sufficiency [30–50 ng/mL (75–125 nmol/L)] (46). We do recommend using these reference intervals in both clinical and research laboratories.
- Along the same line, one approach to minimize inter-method variations of 25(OH)D assay is harmonization. Using this approach, results obtained from different methods are harmonized with a reference method like HPLC or, where available, LC-MS/MS (31). In addition, efforts must be made for inter-laboratory standardization.
- Considering the season of the community-based vitamin D studies is crucial for the relevant interventions.
- Using any method to assay circulating 25(OH)D, VDD prevalence rate of 50 percent and above is undoubtedly a major health problem and deserves further attention and presumably urgent and appropriate intervention.
- Though vitamin D supplementation could be a quick remedy, the sustainability, costs and effectiveness of this strategy, as compared with food fortification, is debatable (47, 48). Vitamin D fortification of foods, on the other hand, has been shown to be an effective strategy to improve vitamin D status of general population (49). Several vehicles have been examined in Iran (9, 50-52).

However, considering bread as a staple food and bakery wheat flour already being fortified with iron and folic acid in Iran, flour fortification with vitamin D seems more effective and feasible (44, 51). Nevertheless, it must be examined in a pilot study as soon as possible.

- More delay in proper intervention could increase social, medical and economical burden of VDD and possibly its related conditions (12).

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Abbreviations

CLIA: Chemiluminescent immunoassay
COVID-19: Coronavirus disease of 2019
ECL: Electrochemiluminescence
EIA: Enzyme immunoassay
FNS: Food and Nutrition Surveillance
HPLC: High performance liquid chromatography
25(OH)D; 25-hydroxyvitamin D
1, 25(OH)$_2$D$_3$: 1,25-dihydroxycholecalciferol
LC-MS/MS: Liquid chromatography coupled with tandem mass spectrometry
NNFTRI: National Nutrition and Food Technology Research
RIA: Radioimmunoassay
UV: Ultra violet
VDD: Vitamin D deficiency

References

1. Lane JM. Mass vaccination and surveillance/containment in the eradication of smallpox. Current topics in microbiology and immunology. 2006;304:17-29.
2. Askarian M, Mansour Ghanaie R, Karimi A, Habibzadeh F. Infectious diseases in Iran: a bird's eye view. Clinical Microbiology and Infection. 2012;18(11):1081-8.
3. Wang H, Lan Y. The global dynamic transmissibility of COVID-19 and its influencing factors: an analysis of control measures from 176 countries. BMC Public Health. 2023;23(1):404.
4. Prasad V, Haslam A. COVID-19 vaccines: history of the pandemic’s great scientific success and flawed policy implementation. Monash Bioethics Review. 2024.
5. Holick MF. The One-Hundred-Year Anniversary of the Discovery of the Sunshine Vitamin D(3): Historical, Personal Experience and Evidence-Based Perspectives. Nutrients. 2023;15(3).
6. Cui A, Zhang T, Xiao P, Fan Z, Wang H, Zhuang Y. Global and regional prevalence of vitamin D deficiency in population-based studies from 2000 to 2022: A pooled analysis of 7.9 million participants. Frontiers in Nutrition. 2023;10.
7. Charoenngam N, Holick MF. Immunologic effects of vitamin D on human health and disease. Nutrients. 2020;12(7):2097.
8. Yari Z, Nikooyeh B, Neyestani TR. Circulating 25-hydroxyvitamin D is associated with metabolic phenotypes of obesity: National Food and Nutrition Surveillance. Nutrition Research. 2023;110:14-22.
9. Nikooyeh B, Neyestani TR, Farvid M, Alavi-Majd H, Houshiarrad A, Kalayi A, et al. Daily consumption of vitamin D–or vitamin D+ calcium–fortified yogurt drink improved glycemic control in patients with type 2 diabetes: a randomized clinical trial. The American journal of clinical nutrition. 2011;93(4):764-71.
10. Kaviani M, Nikooyeh B, Zand H, Yaghmaei P, Neyestani TR. Effects of vitamin D supplementation on depression and some involved neurotransmitters. Journal of affective disorders. 2020;269:28-35.
11. Motamed S, Nikooyeh B, Kashanian M, Chamani M, Hollis BW, Neyestani TR. Evaluation of the efficacy of two doses of vitamin D supplementation on glycemic, lipidemic and oxidative stress biomarkers during pregnancy: a randomized clinical trial. BMC Pregnancy and Childbirth. 2020;20:1-11.
12. Yari Z, Nikooyeh B, Ebrahimof S, Neyestani TR. Global Burden of Disease, the Heavy Cost of Sun Deprivation: Implications for Mass Food Fortification with Vitamin D. Applications of Functional Foods and Nutraceuticals for Chronic Diseases: CRC Press; 2023. p. 67-116.
13. Ranaei V, Pilevar Z, Neyestani TR. Can raising vitamin D status slow down Covid-19 waves? Nutrition and Food Sciences Research. 2021;8(1):1-3.
14. Pal R, Banerjee M, Bhadada S, Shetty A, Singh B, Vyas A. Vitamin D supplementation and clinical outcomes in COVID-19: a systematic review and meta-analysis. Journal of endocrinological investigation. 2022;45(1):53-68.
15. Nikooyeh B, Abdollahi Z, Hajifaraji M, Alavi-Majd H, Salehi F, Yarparvar AH, et al. Vitamin D status, latitude and their associations with some health parameters in children: national food and nutrition surveillance. Journal of tropical pediatrics. 2017;63(1):57-64.
16. Nikooyeh B, Abdollahi Z, Shariatzadeh N, Kalayi A, Zahedirad M, Neyestani T. Effect of latitude on seasonal variations of vitamin D and some cardio metabolic risk factors: national food and nutrition surveillance. Eastern Mediterranean Health Journal. 2021;27(3):269-78.
17. Nikooyeh B, Abdollahi Z, Hajifaraji M, Alavi-Majd H, Salehi F, Yarparvar AH, et al. Vitamin D status and cardiometabolic risk factors across latitudinal gradient in Iranian adults: National food and nutrition surveillance. Nutrition and health. 2017;23(2):87-94.
18. Nikooyeh B, Neyestani TR. Poor vitamin D status increases the risk of anemia in school children: National Food and Nutrition Surveillance. Nutrition. 2018;47:69-74.
19. Nikooyeh B, Neyestani TR. Contribution of vitamin D status as a determinant of cardiometabolic risk factors: a structural equation model, National Food and Nutrition Surveillance. BMC public health. 2021;21:1-7.
20. Nikooyeh B, Hollis BW, Neyestani TR. Modulating effect of vitamin D status on serum anti-adenovirus 36 antibody amount in children with obesity: National Food and Nutrition Surveillance. BMC pediatrics. 2020;20:1-8.

21. Nikooyeh B, Abdollahi Z, Hajifaraji M, Alavi-Majd H, Salehi F, Yarparvar AH, et al. Healthy changes in some cardiometabolic risk factors accompany the higher summertime serum 25-hydroxyvitamin D concentrations in Iranian children: National Food and Nutrition Surveillance. Public health nutrition. 2018;21(11):2013-21.

22. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 5460703, Vitamin D4; [cited 2024 May 6]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Vitamin-D4.

23. Nikooyeh B, Anari R, Neyestani TR. Vitamin D, oxidative stress, and diabetes: crossroads for new therapeutic approaches. Diabetes: Elsevier; 2020. p. 385-95.

24. 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. International journal for vitamin and nutrition research. 2007;77(5):341-6.

25. Sempos CT, Binkley N. 25-Hydroxyvitamin D assay standardisation and vitamin D guidelines paralysis. Public Health Nutr. 2020;23(7):1153-64.

26. Hollis BW. Editorial: The determination of circulating 25-hydroxyvitamin D: no easy task. The Journal of clinical endocrinology and metabolism. 2004;89(7):3149-51.

27. Vieth R. The future of "vitamin D", i.e. 25-hydroxyvitamin D, testing. Clinical biochemistry. 2013;46(3):189.

28. Binkley N, Carter GD. Toward Clarity in Clinical Vitamin D Status Assessment: 25(OH)D Assay Standardization. Endocrinology and metabolism clinics of North America. 2017;46(4):885-99.

29. Nikooyeh B, Neyestani TR. What is the definition of" vitamin D deficiency" and who is considered" vitamin D deficient"? Urgent need for a national consensus. Nutrition and Food Sciences Research. 2017;4(2):1-5.

30. Giustina A, Adler RA, Binkley N, Bollerslev J, Bouillon R, Dawson-Hughes B, et al. Consensus statement from 2(nd) International Conference on Controversies in Vitamin D. Reviews in endocrine & metabolic disorders. 2020;21(1):89-116.

31. 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. Journal of clinical laboratory analysis. 2017;31(6).

32. Tripathi A, Ansari M, Dandekar P, Jain R. Analytical methods for 25-hydroxyvitamin D: advantages and limitations of the existing assays. The Journal of Nutritional Biochemistry. 2022;109:109123.

33. Sempos CT, Heijboer AC, Bikle DD, Bollerslev J, Bouillon R, Brannon PM, et al. Vitamin D assays and the definition of hypovitaminosis D: results from the First International Conference on Controversies in Vitamin D. British journal of clinical pharmacology. 2018;84(10):2194-207.

34. 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. Clinical laboratory. 2014;60(9):1541-50.

35. Hays H, Flores LE, Kothari V, Bilek L, Geske J, Skinner A. Vitamin D Status and Seasonal Variation: A Retrospective Single Institution Database Study of Patients Pursuing Metabolic/Bariatric Surgery. Clinical Nutrition Open Science. 2022;41:1-9.

36. Hovsepian S, Amini M, Aminorroaya A, Amini P, Iraj B. Prevalence of vitamin D deficiency among adult population of Isfahan City, Iran. Journal of health, population, and nutrition. 2011;29(2):149.

37. 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 Nutrition. 2011;15(2):324-30.

38. Faghih S, Abdolahzadeh M, Mohammadi M, Hasanzadeh J. Prevalence of vitamin d deficiency and its related factors among university students in shiraz, iran. International journal of preventive medicine. 2014;5(6):796.

39. Heshmat R, Mohammad K, Majdzadeh S, Forouzanfar M, Bahrami A, Omrani GR, et al. Vitamin D deficiency in Iran: A multi-center study among different urban areas. Iranian Journal of Public Health. 2008:72-8.

40. Vatandost S, Jahani M, Afshari A, Amiri MR, Heidarimoghadam R, Mohammadi Y. Prevalence of vitamin D deficiency in Iran: A systematic review and meta-analysis. 2018;24(4):269-78.

41. Tabrizi R, Moosazadeh M, Akbari M, Dabbaghmanesh MH, Mohamadkhani M, Asemi Z, et al. High Prevalence of Vitamin D Deficiency among Iranian Population: A Systematic Review and Meta-Analysis. Iranian journal of medical sciences. 2018;43(2):125-39.

42. Dehghani M, Mesgarpour B, Akhondzadeh S. How the US Sanctions Are Affecting the Health Research System in Iran? 2021;24(2):101-6.

43. Buttriss JL, Lanham-New SA. Is a vitamin D fortification strategy needed? Nutrition bulletin. 2020;45(2):115-22.

44. El Hoss K, Salla M, Khaled S, Krayem M, Hassan H, El Khatib S. Update on vitamin D deficiency and its impact on human health major challenges & technical approaches of food fortification. Journal of Agriculture and Food Research. 2023;12:100616.

45. Cashman KD. Vitamin D: dietary requirements and food fortification as a means of helping achieve adequate vitamin D status. The Journal of steroid biochemistry and molecular biology. 2015;148:19-26.

46. Płudowski P, Kos-Kudła B, Walczak M, Fal A. Guidelines for Preventing and Treating Vitamin D Deficiency: A 2023 Update in Poland. 2023;15(3).

47. Brett NR, Gharibeh N, Weiler HA. Effect of vitamin D supplementation, food fortification, or bolus injection on vitamin D status in children aged 2–18 years: a meta-analysis. Advances in nutrition. 2018;9(4):454-64.

48. Adebayo FA, Itkonen ST. Safety of Vitamin D Food Fortification and Supplementation: Evidence from Randomized Controlled Trials and Observational Studies. 2021;10(12).

49. Nikooyeh B, Neyestani T. Efficacy of food fortification with vitamin D in Iranian adults: a systematic review and meta-analysis. Nutrition and Food Sciences Research. 2018;5(4):1-6.

50. Neyestani T, Hajifaraji M, Omidvar N, Nikooyeh B, Eshraghian M, Shariatzadeh N, et al. Calcium-vitamin D-fortified milk is as effective on circulating bone biomarkers as

fortified juice and supplement but has less acceptance: a randomised controlled school-based trial. Journal of human nutrition and dietetics. 2014;27(6):606-16.

51. 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. The Journal of Clinical Endocrinology & Metabolism. 2016;101(6):2511-9.

52. Nikooyeh B, Zargaraan A, Kalayi A, Shariatzadeh N, Zahedirad M, Jamali A, et al. Vitamin D-fortified cooking oil is an effective way to improve vitamin D status: an institutional efficacy trial. European Journal of Nutrition. 2020;59:2547-55.