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RESEARCH ARTICLE

Update on the Role of Adequate Vitamin D Provision for Avoiding Insulin Resistance and its Sequelae [Type 2 Diabetes and Cardiovascular Disease]

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ABSTRACT
Type 2 diabetes prevalence is increasing, reaching 'pandemic' proportions globally, and associated with increasing obesity rates. Concomitantly, vitamin D deficiency persists world-wide and is worsened by obesity because it reduces hepatic 25-hydroxylation of vitamin D, which reduces circulating 25(OH)D availability to target tissues; these include islet beta cells, since vitamin D is essential for adequate insulin responses to hyperglycemia. Increased insulin resistance increases type-2 diabetes risks and precedes its development by decades, reflecting changes in hepatic and muscle function that are corrected experimentally by activated hormonal vitamin D [calcitriol]; similarly, abnormal insulin resistance can be corrected in humans by correcting vitamin D deficiency using oral supplementation. Since vitamin D deficiency and inadequacy persist world-wide despite various guidelines on vitamin D intake, the possibility that correcting deficiency would contribute to reducing T2DM risks through beneficial effects on pancreatic islet beta cells and on the metabolic disorders contributing to insulin resistance through vitamin D inadequacy warrants consideration. If this is the case, then ensuring vitamin D adequacy in populations at high risk of Type-2 diabetes would be a valuable adjunct to other measures being taken to reduce that risk such as increased exercise and weight reduction. It would also reduce cardiovascular disease risks, well known to increase with insulin resistance, with or without concomitant Type-2 diabetes. Cardiovascular disease risks themselves can be reduced by adequately correcting deficiency and are lower with higher lifetime circulating 25-hydroxyvitamin D in deficient subjects from Mendelian randomization analysis. This report, therefore, reviews the evidence for increased insulin resistance as a risk factor for Type-2 diabetes, the beneficial effects of correcting vitamin deficiency on insulin resistance, Type-2 diabetes and cardiovascular risks, some basic mechanisms accounting for those benefits, the reasons for the persistence of vitamin D inadequacy globally and how best that problem could be corrected.

1. Background

1.1 vitamin D inadequacy, roles in increasing type 2 diabetes risks.

Vitamin D has been known to be essential for normal insulin secretory responses to glucose for over 40 years, both experimentally and in humans, as has the phenomenon of hormone resistance.[1,2,3,4] Circulating insulin concentrations increase as insulin sensitivity falls, followed by progressive islet beta cell failure, leading to fasting hyperglycaemia, impaired glucose tolerance and then to type 2 diabetes mellitus [T2DM]. Vitamin D repletion is now comparatively rare and T2DM prevalence has increased rapidly, even in the young, becoming a 'global pandemic' with ~463 million cases diagnosed by 2019 though undiagnosed cases are also very common.[5,6,7]

1.2 The discovery of insulin insensitivity [resistance].

Once newly available insulin entered clinical practice from the 1920s it was recognised that insulin was life-saving in those acutely ill while in many patients insulin treatment was neither essential for life nor especially effective for reducing glycaemia. These different responses to insulin treatment of diabetes were described as insulin sensitive or insulin insensitive by Harold Himsworth in the 1930s, a concept taking decades to be accepted. Later classifications used were insulin dependent [IDDM or Type-1DM] or non-insulin dependent [NIDDM or Type-2DM].[8.9.10]

1.3. Increasing insulin resistance as a precipitator of T2DM.

Increased insulin resistance is now accepted as a precipitating cause of T2DM, and can be present at least 40 years before T2DM becomes overt while hyperglycaemia can be demonstrated 10-20 years before T2DM can be diagnosed.[11.12.13] Rare genetic syndromes that include severe insulin resistance commonly include diabetes, with high CVD risks, but are excluded from this report as no reports linking them to vitamin D were found [*as of 7th March 2023*]. However, polycystic ovary syndrome [PCOS] includes insulin resistance, often leading to T2DM, is not rare and correcting deficiency of vitamin D in PCOS is reported as reducing insulin resistance and to improve fertility.[14]

1.4 Might avoiding vitamin D deficiency reduce T2DM risks?

The pertinent question, therefore, is whether ensuring vitamin D repletion across populations could reduce T2DM rates; in turn reducing the massive costs of diabetes to sufferers, and to health services, of the high rates of cardiovascular disease, renal failure, neuropathy, gangrenous amputations and visual loss caused by T2DM.[15]

2. insulin actions and insulin resistance

2.1 Assessment of insulin resistance

Insulin resistance is assessed variously, by 'gold standard' euglycaemic glucose clamp studies, by raised fasting insulin and, most commonly, by Homeostatic model analysis [HOMA] estimation of insulin resistance [HOMA-IR] from fasting and 30-minute blood glucose and insulin values during glucose tolerance tests.[16,17]

2.2. Insulin efficacy in insulin resistance

Normally, islet beta cell insulin secretion regulates hepatic gluconeogenesis and increases energy provision to tissues like liver, brain, muscle and heart by increasing glucose uptake and regulating hepatic glucose production and glycolysis which contribute to glucose homeostasis.[18] In insulin resistance both hepatic glucose and fat synthesis and output increase. However, activated vitamin D [calcitriol] added optimally to hepatocyte cultures, and correcting deficiency in intact animals under insulin resistant conditions, corrects those abnormalities. Calcitriol also reverses evidence of islet beta cell damage in experimental IR [e.g., reducing raised tissue UCP2 content and reactive oxygen species formation].[19,20] Replacing vitamin D in deficiency in IR humans, however, takes time to reduce IR. For example, supplementation of vitamin D deficient but normoglycaemic south Asian women took ~6 months and 25(OH)D concentrations that reached at least 80 nmol/l for IR correction.[21] In muscle, vitamin D reduces IR by reducing over-production of FOXO1 and through activation of vitamin D receptor driven insulin signaling pathways; for example, through enhancement of IRS-1 and VDR production in muscle tissue [22,23] IR is also well recognised as a risk factor for cardiovascular disease, probably aggravated through metabolic disorders associated with IR and T2DM development.

3. Vitamin D biology

A brief account of the mechanisms by which vitamin D protects against insulin resistance, and hence Type 2 diabetes, and of why so many people are deficient in vitamin D, follows. This is to make the reasons for aiming to reduce vitamin D deficiency rates at the population level clear, because public health measures for avoiding vitamin D deficiency will only improve if those responsible for such measures fully appreciate the need for long-term VitD repletion amongst the measures designed to

reduce T2DM and CVD risks. Vitamin D_3 [cholecalciferol; VitD], evolved ~500 million years ago in early unicellular organisms, being protective against damage from high solar irradiation by absorbing damaging UVB[24] present in most animals, VitD is provided along food chains and often synthesized in skin where it is protective against UVB damage and prevents excessive vitamin D_3 formation.[25] Fungi synthesize vitamin D_2 [ergocalciferol] similarly and D_2 is found in small amounts in animals and humans when their diet contains fungal material, for example, it is found naturally in dark chocolate.[26] Vitamin D_3 is generally considered more effective than vitamin D_2 in humans and D_2 is not considered specifically in this report, 'Vitamin D' implying vitamin D_3 throughout.

Humans obtain D_3 most efficiently from skin synthesis under UVB irradiation of wavelengths around 295-315 nm, from summer and equatorial sunshine.[25] Such UVB is only present in sunlight from spring to late summer in temperate climates. Furthermore, increasingly indoor lifestyles, sun avoidance and sunscreen use are reducing skin D_3 synthesis globally. Thus, inadequate D_3 provision is common.[27] This situation is aggravated by the low VitD content of most foods.[25] Some D_3 is found naturally in dairy products, meat, eggs and in many wild, but not farmed, oily fish but is not enough to meet human needs unless foods are fortified with D_3 and/or adequate VitD supplements are taken regularly.[25]

3.1 Vitamin D activation.

Intact D_3 is inactive but activated through two hydroxylations. Firstly, VitD is hydroxylated in the liver to form 25(OH)D, which circulates bound to D-binding proteins and albumen, though small amounts remain unbound].[28] Overall circulating 25(OH)D half-life is ~2-3 weeks. 25(OH)D is further 1-alpha-hydroxylated to form 1,25-dihydroxyvitamin D [Calcitriol] in the kidneys to provide the circulating [hormonal] calcitriol that acts to protect bone health. It is also activated, similarly, to form calcitriol in the other target tissues where it has many paracrine, apocrine and intracrine effects relevant to non-skeletal health.[29,30]

3.2 Assessment of vitamin D provision ['status'].

25(OH)D having a relatively long circulating half-life, assays measuring serum 25(OH)D are accepted as reflecting VitD 'stores'.[25] VitD measurements were firstly made by slow and costly bioassays but Berson & Yalows' 1959 competitive protein binding assays [immunoassays],[31] allowed measurement of the low concentrations of many circulating hormones and they are widely used to assess serum 25(OH)D concentrations, facilitating studies of disease risks with vitamin D provision [serum 25(OH)D, 'status'].Currently, high-pressure liquid chromatography following by tandem mass spectrographic methodology [HPLC-TMS] assays are thought the most reliable and used as a 'gold standard' for laboratory 25(OH)D assay validation, together with international quality control [QC] schemes that improve 25(OH)D assay reliability and reproducibility.[32,33]

3.3 How vitamin D acts. The multiple actions of VitD are induced through 3 main mechanisms. Firstly, genetic effects following calcitriol binding to VitD receptors [VDR] that commonly complex with retinol X receptors before binding to vitamin D response elements [VDREs] in the promotor regions of target genes in cell nuclei, of which there are many thousand.[34,35] Secondly, RXR:VDR complexes bind to various cell wall receptors and can induce rapid non-genomic effects by opening calcium channels and increasing intracellular calcium which has different effects in different tissues.[36] In islet beta cells, calcium activates intracellular endopeptidases, releasing insulin from storage granules, [phase 1 secretion in response to hyperglycaemia]. Phase 2 insulin secretion follows by 20-30 minutes], reflecting slower genetic effects. Thirdly, VitD modulates many epigenetic effects that regulate gene activity without altering their basic DNA structure [through effects on DNA methylation, chromatin histone structure and non-coding RNAs]. These epigenetic effects are important in utero and throughout development.[37,38]

4. Vitamin D deficiency and its avoidance.

Vitamin D deficiency [VitDD] is accepted as causal for rickets and osteomalacia, even though one trial has reported that giving large bolus doses in severe nutritional deficiency failed to prevent rickets.[39] The serum 25(OH)D values at which risk of rickets and osteomalacia appear have been used to diagnose deficiency in terms of bone health, e.g., <25 nmol/l by the UK Specialist Advisory Committee on Nutrition [SACN], <50 nmol/l by the American Institute of Medicine [IOM] and the European Food Safety Agency [EFSA], though the American Endocrine Society advises >75 nmol/l for optimal bone health.[40,41,42] Much effort has gone into identifying minimal adult VitD intakes necessary for avoiding 'deficiency'. Recommendations range from 400 IU/day in the UK to 600 IU/day by the IOM and 400-1000 IU/day by the EFSA.[40,41,42] IOM calculations of intake aimed to achieve 75 nmol/l in 95% of the population but were made for

achieving 75 nmol/l as a population mean and 6000 IU, not 600 IU, daily would be needed to achieve the stated target 25(OH)D value in 95% of the population.[43] This miscalculation is well recognised, but IOM advice has not been changed. Since deficiency remains common and 25(OH)D thresholds above 25 nmol/l are needed to achieve various non-skeletal health benefits.[21,44,45] most intake advice for adult populations needs revision. Furthermore, since VitDD at <25 nmol/l remains common globally despite existing guidelines, such advice must be accompanied by programmes ensuring that advised intakes are met. Intakes up to 4000 IU/day are safe, as is usually mentioned in public health guidance, e.g., from the SACN in the UK.[40] 10,000 IU/day is also reported as safe. 20,000 IU/day or more of vitamin D is produced by moderate, but safe, sun exposure. Indeed, serum 25(OH)D values of at least 120 nmol/l are recommended by many vitamin D workers since such values are seen in people with outdoor lifestyles providing large sun exposures.[45] Thus, advice suggesting total adult intakes of up to 6000 IU/day in high-risk individuals should prove generally acceptable.

4.1 Problems with very large bolus doses of vitamin D. There is a trend to increased use of large bolus doses of D_3 which raise serum 25(OH)Ds rapidly. However, very large bolus doses reduce VitD activation by reducing hepatic 25(OH)D formation and increasing fibroblast growth factor23 [FGF23] secretion which reduces calcitriol synthesis by deactivating the vitamin D-activating 1-alphahydroxylase enzyme. Those effects matter since large bolus doses have failed to prevent rickets in a trial in severe deficiency.[39] Moderate daily doses of D_3 are also more effective than large interval doses for reducing respiratory infection rates.[46] Thus, public health measures for avoiding VitDD should ensure adequate daily intakes rather than rely on interval bolus supplementation. Fortunately, when correcting deficiency is urgent, calcifediol [25(OH)D] preparations are now available which are fast-acting and do not induce the self-regulatory effects seen with large D_3 boluses.[47]

5. Mechanisms through which vitamin D induces non-skeletal health benefits.

Many mechanisms by which adequate VitD status reduces non-skeletal health risks are well understood, e.g., the reduction of IR and T2DM risks mentioned above. For risk reductions relating to obesity, CVD and hence overall mortality, these mechanisms include reducing inflammation including the remote inflammatory effects of obesity that increase atherosclerotic damage,[48] along with the well-known reductions in inflammatory processes induced by vitamin D,[49,50] which are supported by the lower CRP values found with genetically determined increases in D status in insufficiency on non-linear Mendelian randomization analysis.[51] VitD inhibits matrix-metalloproteinase [MMP]-2/9 secretion in many tissues and macrophages infiltrating arterial plaque secrete MMP2/9 which increases arterial plaque instability.[52,53] Correcting deficiency would, therefore, be expected to reduce acute thrombotic event risks resulting from plaque disruption, contributing to the reductions in overall and CVD mortality reported from RCTs of supplementation. Reductions in CVD risk are now reported with supplementation and with higher VitD status; that increased vitamin D status, as genetically determined, reduces CVD risks in deficient subjects [from non-linear Mendelian randomization analysis [MRA]], supports the validity and significance of this effect.[54,55]

Cancer risks increase with T2DM and obesity.[56] Obesity reduces vitD status,[57] and serum 25(OH)D responses to vitD intake,[57,58,59] Increases in VitDD rates contribute independently to cancer risks, as do increases in T2DM rates, since VitD inhibits many carcinogenic processes and reduces metastatic risks.[60,61] Such processes will have contributed to the reductions in overall cancer mortality seen with supplementation in the VITAL trial.[62] However, cancer incidence was only found to be reduced in non-obese subjects - probably because VitD status increases more in slim than obese subjects on comparable supplementation, due to the reduced hepatic 25-hydroxylation seen in obesity.[57,58,59]

6. Assessment of prevalence of vitamin D inadequacy.

Past VitDD prevalence must have been high when rickets was rampant, but only became assessable once 25(OH)D assays were widely available. Since then, it has continued to be common, at 48.6% of boys and 51.4% of girls respectively in a middle eastern country recently, and, when defined by a 25(OH)D < 50 nmol/l], deficiency was present in 30%-99% of adults in the US, India and Iran. Deficiency was found in 48.8% and 51.4% of boys and girls respectively in another middle eastern country.[63,64] The representative UK Biobank cohort [n ~440,000] showed deficiency in people of White European, Black African and Asian ancestry at 5.9%, 30.8 and 50.8% in spring/summer respectively, and in autumn/winter at 17.5%, 38.5% and 57.2% respectively.[65]

6.1 Risk factors for adult vitamin D deficiency. The risk factors for adult VitD deficiency [VitDD] include the generally poor dietary provision of VitD and increasing age as outdoor activity falls and skin synthesis and gut absorption become less efficient.[25,66] Modern lifestyles with increasing indoor work, exercise and leisure activity also increase deficiency risks. Obesity reduces serum 25(OH)D causally [by bi-directional MRA].[67] This is because obesity reduces hepatic 25-hydroxylation, and vitD and 25(OH)D fall with their distribution into the increased fat masses.[57,58,59] Furthermore, sun avoidance is common in both hot and temperate countries and increasing sunscreen use for skin protection blocks VitD synthesis totally when applied optimally.[68] Dark skin is a major risk factor since melanin is a natural sunblock, increasing the UVB dosages needed to match VitD production from pale skin. Those on shift work, housebound or living in residential homes, vegans and vegetarians are also well known to be at high-risk. UVB triggering VitD synthesis falls with distance from the equator and none is present between autumn and early summer in temperate climates, making latitude a risk factor.[25] Overall, chronic deficiency remains common in high-risk groups, globally, except in northern Scandinavian countries that are encouraging supplement usage or where there is an effective food fortification programme, as in Finland.[69,70]

6.2 Avoidance of vitamin D deficiency. Individuals eating ordinary food can only avoid deficiency if they regularly eat oily sea-fish and have above average summer sun exposure or take adequate VitD supplements and VitDD in adults in most countries is unlikely to be corrected by nationally advised VitD intakes,[71,40,41,42] a concept supported by the obvious persistence of VitDD despite such guidelines. Public health efforts to avoid VitDD have often failed; for example, the UK 'Sure Start' scheme, designed to provide pregnant women on benefits with access to vitD, had very low uptakes until better publicity and increased scheme availability were introduced when maternal vitamin D status increased more than the uptake of the scheme increased.[72] The UK supplemented fatty spreads from early in world war 2 [WW2] for over ~5 decades, by statute, to match the modest vitamin D content of butter but that statute was abolished ~ten years ago.[73] UK pregnant and nursing mothers and children under 5 years were supplied with '*5 welfare foods*' [extra milk, meat, eggs, orange juice and cod-liver-oil] from early in WW2 until 1964, which provided much increased VitD intakes.[74] Thus schemes designed to provide better provision of vitamin D can be effective.

6.3 Food fortification with vitamin D. Finland's diet has been improved since the early Karelia 'experiment' reduced fat intakes and subsequently reduced CVD rates.[75] VitDD being common in Finland, a voluntary dairy food fortification programme was started in 2003. Auditing population 25(OH)D concentrations showed inadequate improvement, especially in high-risk groups, after food-fortification was begun and the level of fortification was doubled in 2010 together with the provision of supplementation for high-risk groups, virtually abolishing VitDD in Finland, other than in recent immigrant groups.[70] Audit of that programme continues and public health records are being kept of the many health problems linked to VitDD.[76] Hopefully some reductions in non-skeletal disorders linked to VitDD may appear following the 10+ years of adequate VitD provision. However, whether enough of the Finnish population has achieved 25(OH)D levels high enough for this to happen remains to be seen, since, for example, 25(OH)Ds of ≥100 nmol/l were needed to reduce T2DM risks in the D2d trial.[77] Flour fortification is a cost-effective approach to food fortification suggested for the UK, since flour is a general food-staple.[78] For multi-ethnic societies this would have to cover all flours and to include gluten free preparations.

7. Potential health benefits of ensuring adequate vitamin D status for reducing rates of type-2 diabetes and cardiovascular disease.

Potential health benefits at the population level from ensuring vitamin D adequacy in adults with deficiency can be judged from the data for correcting deficiency in relevant disorders and knowing the prevalence rates of deficiency. For example, calculated risk ratios [RR; 95% CIs] for T2DM risk with supplementation achieving a 25(OH)D of ≥100 nmol/l during years 1-4 of the D2d trial significantly reduced T2DM risks with a RR = 0.48 [95% CI; 0.29-0.8] for achieved 25(OH)Ds of 100-124 nmol/l and a RR = 0.20 [95% CI;0.17-0.5] for achieved 25(OH)Ds of 125 nmol/l or above.[77] UK-biobank data revealed increased T2DM risks in south Asians and Europeans with vitamin D deficiency but no effect of T2DM on vitamin D status on bi-directional MRA which supports the causality of VitDD for T2DM.[79] The RR for all-cause mortality over 14 years in UK-Biobank subjects was increased in those with 25(OH)Ds of 25 nmol/l as compared to those with values of 50 nmol/l, RR= 1.25 [95%CI;1.16-1.55] in White

Europeans.[81,82] Where deficiency [25(OH)D <25 nmol/l] is present at 20% the overall % reductions in T2DM could, potentially, reach between 10-16%, depending on the prevalence of deficiency. For all-cause mortality, the expected reductions if population 25(OH)Ds could be brought up to at least 40 nmol/l could, potentially, reach 25% in those with proven deficiency. Estimated dose-response relationships for vitamin D with CVD events and all-cause mortality using a combination of observational and MRA data has suggested overall population-wide reductions of ~4.4% in those disorders if serum 25(OH)D concentrations in deficiency were to be raised to 40-50 nmol/l.[82] Additionally, in all those disorders, in the UK at least, such risk reductions would be expected to be greatest in dark-skinned minority groups because their deficiency rates are highest but vitamin D intakes would need to be 2-3 times as large in overweight and obese people than in those who are slim.[57,58]

8. Conclusions.

Available evidence from studies of the effects of correction of vitamin D status on insulin resistance suggest that avoiding vitamin D deficiency at the population level over time should reduce T2DM, CVD and all-cause mortality rates significantly, and provide a useful adjunct to measures already used to reduce T2DM risks such as weight control and increased physical activity. Ensuring vitamin D repletion through population level measures such as food fortification should prove highly cost-effective. The potential benefits of avoiding deficiency may become demonstrable in Finland where deficiency was virtually abolished over ten years ago which may be long enough for the benefits of long-term reductions in IR to become apparent. However, this postulate depends on Finnish audits of vitamin D status showing that enough of the 25(OH)D values achieved in their adult population are reaching the levels needed for reductions in T2DM rates to be likely.

References

1. Norman AW, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency inhibits pancreatic secretion of insulin. *Science*. 1980 Aug 15;209(4458):823-5. doi: 10.1126/science.6250216.
2. Clark SA, Stumpf WE, Sar M, DeLuca HF, Tanaka Y. Target cells for 1,25 dihydroxyvitamin D3 in the pancreas. *Cell Tissue Res*. 1980;209(3):515-20. doi: 10.1007/BF00234764.
3. Kadowaki S, Norman AW. Demonstration that the vitamin D metabolite 1,25(OH)2-vitamin D3 and not 24R,25(OH)2-vitamin D3 is essential for normal insulin secretion in the perfused rat pancreas. *Diabetes* 1985;34(4):315-20.
4. Verhoeven GF, Wilson JD. The syndromes of primary hormone resistance. *Metabolism*. 1979 Mar;28(3):253-89. doi: 10.1016/0026-0495(79)90072-6.
5. Tinajero MG, Malik VS. An Update on the Epidemiology of Type 2 Diabetes: A Global Perspective. *Endocrinol Metab Clin North Am*. 2021Sep;50(3):337-355. doi: 10.1016/j.ecl.2021.05.013.
6. Perng W, Conway R, Mayer-Davis E, Dabelea D. Youth-Onset Type 2 Diabetes: The Epidemiology of an Awakening Epidemic. *Diabetes Care*. 2023 Mar 1;46(3):490-499. doi: 10.2337/dci22-0046.
7. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. *Lancet*. 2017 Jun 3;389(10085):2239-2251.doi:10.1016/S0140-6736(17)30058-2.
8. Himsworth HP. Diabetes mellitus: its differentiation into insulin-sensitive and insulin-insensitive types. 1936. *Int J Epidemiol*. 2013 Dec;42(6):1594-8. doi: 10.1093/ije/dyt203.
9. Himsworth. The syndrome of diabetes mellitus and its causes. *Lancet*. 1949 Mar 19;1(6551):465-73. doi: 10.1016/s0140-6736(49)90797-7
10. Jorge de Faria Maraschin, 2013 *pubmed.ncbi.nlm.nih.gov*/23393666/ [The first worldwide accepted classification scheme for DM was published in 1979 by the National Diabetes Data Group (NDDG) and classified DM based on the pharmacologic therapy applied into two major groups: Insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM).]
11. Hulman A, Simmons RK, Brunner EJ, Witte DR, Færch K, Vistisen D, et al. Trajectories of glycaemia, insulin sensitivity and insulin secretion in South Asian and white individuals before diagnosis of type 2 diabetes: a longitudinal analysis from the Whitehall II cohort study. *Diabetologia* 2017;60(7):1252-1260.
12. Wu F, Juonala M, Pitkänen N, Jula A, Lehtimäki T, Sabin MA, et al. Both youth and long-term vitamin D status is associated with risk of type 2 diabetes mellitus in adulthood: a cohort study. *Ann Med* 2018;50(1):74-82
13. Sagesaka H, Sato Y, Someya Y, Tamura Y, Shimodaira M, Miyakoshi T, et al. Type 2 Diabetes: When Does It Start? J Endocr Soc 2018 Sagesaka H, Sato Y, Someya Y, Tamura Y, Shimodaira M, Miyakoshi T, et al. Type 2 Diabetes: When Does It Start? *J Endocr Soc* 2018;2(5):476-484.
14. Morgante G, Darino I, Spanò A, Luisi S, Luddi A, Piomboni P, et al. PCOS Physiopathology and Vitamin D Deficiency: Biological Insights and Perspectives for Treatment. *J Clin Med*. 2022 Aug 2;11(15):4509. doi: 10.3390/jcm11154509.
15. Boucher BJ. Re Niroomand M, Fotouhi A, Irannejad N et al. Does high-dose vitamin D supplementation impact insulin resistance and risk of development of diabetes in patients with pre-diabetes? A double-blind randomized controlled trial. *Diabetes Res Clin Pract*. 2019;148:1-9. Diabetes Res Clin Pract. 2019 Sep;155:107782. doi: 10.1016/j.diabres.2019.107782. Epub 2019 Jul 17.
16. Matthews, D.R., Hosker, J.P., Rudenski, A.S. et al. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28, 412–419 (1985). https://doi.org/10.1007/BF00280883
17. Gastaldelli A. Measuring and estimating insulin resistance in clinical and research settings. *Obesity (Silver Spring)*. 2022 Aug;30(8):1549-1563. doi: 10.1002/oby.23503. PMID: 35894085; PMCI.
18. Tokarz VL, MacDonald PE, Klip A. The cell biology of systemic insulin function. *J Cell Biol*. 2018 Jul 2;217(7):2273-2289. doi: 10.1083/jcb.201802095. Epub 2018 Apr 5. PMID: 29622564; PMCID: PMC6028526.
19. Cheng Q, Li YC, Boucher BJ, Leung PS. A novel role for vitamin D: modulation of expression and function of the local renin-angiotensin system in mouse pancreatic islets. *Diabetologia* 2011;54(8):2077-81.

20. Cheng S, So WY, Zhang D, Cheng Q, Boucher BJ, Leung PS. Calcitriol Reduces Hepatic Triglyceride Accumulation and Glucose Output Through Ca2+/CaMKKβ/AMPK Activation Under Insulin-Resistant Conditions in Type 2 Diabetes Mellitus. *Curr Mol Med*. 2016;16(8):747-758. doi: 10.2174/1566524016666160920111407.
21. Von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in SouthAsian women living in New Zealand who are insulin resistant and vitamin D deficient – a double-blind randomised controlled trial. *Br J Nutr* 2010;1034:549-55.
22. Chen S, Villalta SA, Agrawal DK. FOXO1 Mediates Vitamin D Deficiency-Induced Insulin Resistance in Skeletal Muscle. *J Bone Miner Res*. 2016 Mar;31(3):585-95. doi: 10.1002/jbmr.2729. Epub 2015 Nov 9. PMID: 26462119; PMCID: PMC4814301.
23. Alkharfy KM, Al-Daghri NM, Yakout SM, Hussain T, Mohammed AK, Krishnaswamy S. Influence of vitamin D treatment on transcriptional regulation of insulin-sensitive genes. *Metab Syndr Relat Disord*. 2013 Aug;11(4):283-8. doi: 10.1089/met.2012.0068.
24. Holick MF. Ultraviolet B Radiation: The Vitamin D Connection. *Adv Exp Med Biol*. 2017;996:137-154. doi: 10.1007/978-3-319-56017-5_12.
25. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007 Jul 19;357(3):266-81. doi: 10.1056/NEJMra070553.
26. Kühn J, Schröter A, Hartmann BM, Stangl GI. Cocoa and chocolate are sources of vitamin D_2. *Food Chem*. 2018 Dec 15;269:318-320. doi: 10.1016/j.foodchem.2018.06.098.
27. Roth DE, Abrams SA, Aloia J, Bergeron G, Bourassa MW, Brown KH, et al. Global prevalence and disease burden of vitamin D deficiency: a roadmap for action in low- and middle-income countries. *Ann N Y Acad Sci*. 2018 Oct;1430(1):44-79. doi: 10.1111/nyas.13968.
28. Makris K, Bhattoa HP, Cavalier E, Phinney K, Sempos CT, Ulmer CZ, et al. Recommendations on the measurement and the clinical use of vitamin D metabolites and vitamin D binding protein - A position paper from the IFCC Committee on bone metabolism. *Clin Chim Acta*. 2021 Jun;517:171-197. doi: 10.1016/j.cca.2021.03.002.
29. DeLuca HF. The kidney as an endocrine organ involved in the function of vitamin D. *Am J Med*. 1975 Jan;58(1):39-47. doi: 10.1016/0002-9343(75)90531-8. PMID: 163578.
30. Adams JS, Rafison B, Witzel S, Reyes RE, Shieh A, Chun R, et al. Regulation of the extrarenal CYP27B1-hydroxylase. *J Steroid Biochem Mol Biol*. 2014 Oct;144 Pt A:22-7. doi: 10.1016/j.jsbmb.2013.12.009.
31. Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. 1960. *Obes Res*. 1996 Nov;4(6):583-600. doi: 10.1002/j.1550-8528.1996.tb00274.x.
32. Coldwell RD, Trafford DJ, Varley MJ, Kirk DN, Makin HL. Measurement of 25-hydroxyvitamin D2, 25-hydroxyvitamin D3, 24,25-dihydroxyvitamin D2 and 25,26-dihydroxyvitamin D2 in a single plasma sample by mass fragmentography. *Clin Chim Acta*. 1989 Feb 28;180(2):157-68. doi: 10.1016/0009-8981(89)90347-1.
33. Carter GD, Berry J, Durazo-Arvizu R, Gunter E, Jones G, Jones J, Makin HLJ, Pattni P, Sempos CT, Twomey P, Williams EL, Wise SA. Hydroxyvitamin D assays: An historical perspective from DEQAS. *J Steroid Biochem Mol Biol*. 2018 Mar;177:30-35. doi: 10.1016/j.jsbmb.2017.07.018.
34. Carlberg C. Vitamin D Genomics: From *In Vitro* to *In Vivo*. *Front Endocrinol (Lausanne)*. 2018 May 23;9:250. doi: 10.3389/fendo.2018.00250.
35. Haussler MR, Jurutka PW, Hsieh JC, Thompson PD, Selznick SH, Haussler CA, Whitfield GK. New understanding of the molecular mechanism of receptor-mediated genomic actions of the vitamin D hormone. *Bone*. 1995 Aug;17(2 Suppl):33S-38S. doi: 10.1016/8756-3282(95)00205-r.
36. Hii CS, Ferrante A. The Non-Genomic Actions of Vitamin D. *Nutrients*. 2016 Mar 2;8(3):135. doi: 10.3390/nu8030135.
37. Wu YL, Lin ZJ, Li CC, Lin X, Shan SK, Guo B, et al. Epigenetic regulation in metabolic diseases: mechanisms and advances in clinical study. *Signal Transduct Target Ther*. 2023 Mar 2;8(1):98. doi: 10.1038/s41392-023-01333-7.
38. Godfrey KM, Costello PM, Lillycrop KA. Development, Epigenetics and Metabolic Programming. *Nestle Nutr Inst Workshop Ser*. 2016;85:71-80. doi: 10.1159/000439488.
39. Griffin G, Hewison M, Hopkin J, Kenny RA, Quinton R, Rhodes J, et al. Perspective: Vitamin D supplementation prevents rickets and acute respiratory infections when given as daily maintenance but not as intermittent bolus: implications for COVID-19. *Clin Med (Lond)*.

2021 Mar;21(2):e144-e149. doi: 10.7861/clinmed.2021-0035.

40. Scientific Advisory Committee on Nutrition (SACN). Vitamin D and health. 2016. https://www.gov.uk/government/groups/scientific-advisory-committee-on-nutrition. Viewed 7th March 2023
41. Institute of Medicine. Dietary reference intakes for calcium and vitamin D. Washington, DC: *The National Academies Press*; 2011.
42. European Food Safety Authority (EFSA). Dietary reference values for vitamin D. EFSA Panel on Dietetic Products, Nutrition and Allergies. 2016. https://efsa.onlinelibrary.wiley.com/ https://doi.org/10.2903/j.efsa.2016.4547.
43. Veugelers PJ, Ekwaru JP. A statistical error in the estimation of the recommended dietary allowance for vitamin D. *Nutrients*. 2014 Oct 20;6(10):4472-5. doi: 10.3390/nu6104472.
44. Kimball SM, Holick MF. Official recommendations for vitamin D through the life stages in developed countries. *Eur J Clin Nutr*. 2020 Nov;74(11):1514-1518. doi: 10.1038/s41430-020-00706-3.
45. Grant WB, Al Anouti F, Boucher BJ, Dursun E, Gezen-Ak D, Jude EB, et al. A Narrative Review of the Evidence for Variations in Serum 25-Hydroxyvitamin D Concentration Thresholds for Optimal Health. *Nutrients*. 2022 Feb 2;14(3):639. doi: 10.3390/nu14030639.
46. Jolliffe DA, Camargo CA Jr, Sluyter JD, Aglipay M, Aloia JF, Ganmaa D, et al. Vitamin D supplementation to prevent acute respiratory infections: a systematic review and meta-analysis of aggregate data from randomised controlled trials. *Lancet Diabetes Endocrinol*. 2021 May;9(5):276-292. doi: 10.1016/S2213-8587(21)00051-6.
47. Petkovich M, Melnick J, White J, Tabash S, Strugnell S, Bishop CW. Modified-release oral calcifediol corrects vitamin D insufficiency with minimal CYP24A1 upregulation. *J Steroid Biochem Mol Biol*. 2015 Apr;148:283-9. doi: 10.1016/j.jsbmb.2014.11.022. Epub 2014 Nov 22. PMID: 25446887.
48. Mehmood ZH, Papandreou D. An Updated Mini Review of Vitamin D and Obesity: Adipogenesis and Inflammation State. Open Access *Maced J Med Sci*. 2016 Sep 15;4(3):526-532. doi: 10.3889/oamjms.2016.103.
49. Chun RF, Liu PT, Modlin RL, Adams JS, Hewison M. Impact of vitamin D on immune function: lessons learned from genome-wide analysis. *Front Physiol*. 2014 Apr 21;5:151. doi: 10.3389/fphys.2014.00151.
50. Mirza I, Mohamed A, Deen H, Balaji S, Elsabbahi D, Munasser A, et al. Obesity-Associated Vitamin D Deficiency Correlates with Adipose Tissue DNA Hypomethylation, Inflammation, and Vascular Dysfunction. *Int J Mol Sci*. 2022 Nov 19;23(22):14377. doi: 10.3390/ijms232214377.
51. Zhou A, Hyppönen E. Vitamin D deficiency and C-reactive protein: a bidirectional Mendelian randomization study. *Int J Epidemiol*. 2023 Feb 8;52(1):260-271. doi: 10.1093/ije/dyac087.
52. Timms PM, Mannan N, Hitman GA, Noonan K, Mills PG, Syndercombe-Court D, et al. Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? *QJM*. 2002 Dec;95(12):787-96. doi: 10.1093/qjmed/95.12.787.
53. Liu Q, Pan J, Bao L, Xu C, Qi Y, Jiang B, et al. Major Vault Protein Prevents Atherosclerotic Plaque Destabilization by Suppressing Macrophage ASK1-JNK Signaling. *Arterioscler Thromb Vasc Biol*. 2022 May;42(5):580-596. doi: 10.1161/ATVBAHA.121.316662
54. Sutherland JP, Zhou A, Hyppönen E. Vitamin D Deficiency Increases Mortality Risk in the UK Biobank: A Nonlinear Mendelian Randomization Study. *Ann Intern Med*. 2022 Nov;175(11):1552-1559. doi: 10.7326/M21-3324.
55. Zhou A, Selvanayagam JB, Hyppönen E. Non-linear Mendelian randomization analyses support a role for vitamin D deficiency in cardiovascular disease risk. *Eur Heart J*. 2022 May 7;43(18):1731-1739. doi: 10.1093/eurheartj/ehab809.
56. Scully T, Ettela A, LeRoith D, Gallagher EJ. Obesity, Type 2 Diabetes, and Cancer Risk. *Front Oncol*. 2021 Feb 2;10:615375. doi: 10.3389/fonc.2020.615375.
57. Ekwaru JP, Zwicker JD, Holick MF, Giovannucci E, Veugelers PJ. The importance of body weight for the dose response relationship of oral vitamin D supplementation and serum 25-hydroxyvitamin D in healthy volunteers. *PLoS One*. 2014 Nov 5;9(11):e111265. doi: 10.1371/journal.pone.0111265.
58. Drincic A, Fuller E, Heaney RP, Armas LA. 25-Hydroxyvitamin D response to graded vitamin D_3 supplementation among obese adults. *J Clin Endocrinol Metab*.2013 Dec;98(12):4845-51. doi: 10.1210/jc.2012-4103.
59. Hyppönen E, Boucher BJ. Adiposity, vitamin D requirements, and clinical implications for

obesity-related metabolic abnormalities. *Nutr Rev*. 2018 Sep 1;76(9):678-692. doi: 10.1093/nutrit/nuy034.

60. Ma Y, Johnson CS, Trump DL. Mechanistic Insights of Vitamin D Anticancer Effects. *Vitam Horm*. 2016;100:395-431. doi: 10.1016/bs.vh.2015.11.003.
61. Muñoz A, Grant WB. Vitamin D and Cancer: An Historical Overview of the Epidemiology and Mechanisms. *Nutrients*. 2022 Mar 30;14(7):1448. doi: 10.3390/nu14071448.
62. Chandler PD, Chen WY, Ajala ON, Hazra A, Cook N, Bubes V, et al. VITAL Research Group. Effect of Vitamin D3 Supplements on Development of Advanced Cancer: A Secondary Analysis of the VITAL Randomized Clinical Trial. *JAMA Netw Open*. 2020 Nov 2;3(11):e2025850. doi: 10.1001/jamanetworkopen.2020.25850. Erratum in: JAMA Netw Open. 2020 Dec 1;3(12):e2032460. PMID: 33206192
63. Bener A, Al-Ali M, Hoffmann GF. High prevalence of vitamin D deficiency in young children in a highly sunny humid country: a global health problem. *Minerva Pediatr*. 2009 Feb;61(1):15-22. PMID: 19179999.
64. Roth DE, Abrams SA, Aloia J, Bergeron G, Bourassa MW, Brown KH, et al. Global prevalence and disease burden of vitamin D deficiency: a roadmap for action in low- and middle-income countries. *Ann N Y Acad Sci*. 2018 Oct;1430(1):44-79. doi: 10.1111/nyas.13968.
65. Sutherland JP, Zhou A, Leach MJ, Hyppönen E. Differences and determinants of vitamin D deficiency among UK biobank participants: A cross-ethnic and socioeconomic study. *Clin Nutr*. 2021 May;40(5):3436-3447. doi: 10.1016/j.clnu.2020.11.019.
66. Barragry JM, France MW, Corless D, Gupta SP, Switala S, Boucher BJ, et al. Intestinal cholecalciferol absorption in the elderly and in younger adults. *Clin Sci Mol Med*. 1978 Aug;55(2):213-20. doi: 10.1042/cs0550213.
67. Vimaleswaran KS, Berry DJ, Lu C, Tikkanen E, Pilz S, Hiraki LT, et al. Genetic Investigation of Anthropometric Traits-GIANT Consortium; Streeten EA, Theodoratou E, Jula A, Wareham NJ, Ohlsson C, Frayling TM, et al.. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. *PLoS Med*. 2013;10(2):e1001383. doi: 10.1371/journal.pmed.1001383.
68. J, MacLaughlin JA, Holick MF. Sunscreens suppress cutaneous vitamin D3 synthesis. *J Clin Endocrinol Metab*. 1987 Jun;64(6):1165-8. doi: 10.1210/jcem-64-6-1165.
69. Itkonen ST, Andersen R, Björk AK, Brugård Konde Å, Eneroth H, Erkkola M, et al. Vitamin D status and current policies to achieve adequate vitamin D intake in the Nordic countries. *Scand J Public Health*. 2021 Aug;49(6):616-627. doi: 10.1177/1403494819896878.
70. Jääskeläinen T, Itkonen ST, Lundqvist A, Erkkola M, Koskela T, Lakkala K, et al. The positive impact of general vitamin D food fortification policy on vitamin D status in a representative adult Finnish population: evidence from an 11-y follow-up based on standardized 25-hydroxyvitamin D data. *Am J Clin Nutr*. 2017 Jun;105(6):1512-1520. doi: 10.3945/ajcn.116.151415.
71. Boucher BJ. Discrepancies between current guidance from NICE on the treatment of vitamin D deficiency and the recommended daily amounts [RDAs] for its prevention in the UK. *Expert Rev Endocrinol Metab*. 2022 May;17(3):201-203. doi: 10.1080/17446651.2022.2067143.
72. Moy RJ, McGee E, Debelle GD, Mather I, Shaw NJ. Successful public health action to reduce the incidence of symptomatic vitamin D deficiency. *Arch Dis Child*. 2012 Nov;97(11):952-4. doi: 10.1136/archdischild-2012-302287.
73. Spreadable Fats (Marketing Standards) and Milk and Milk Products (Protection of Designations) (England) Regulations 2008. Regulation 4 requires the fortification of margarine with vitamins A and D. [accessed March 7th 2023]
74. Ministry of Food and successors: Welfare Food Scheme, https://discovery.nationalarchives.gov.uk/details/r/C10953 [accessed March 7th 2023
75. Iacono JM, Dougherty RM, Puska P. Dietary fats and the management of hypertension. *Can J Physiol Pharmacol*. 1986 Jun;64(6):856-62. doi: 10.1139/y86-148.
76. Paalanen L, Härkänen T, Tolonen H. Protocol of a research project 'Projections of the burden of disease and disability in Finland - health policy prospects' using cross-sectional health surveys and register-based follow-up. *BMJ Open*. 2019 Jun 20;9(6):e029338. doi: 10.1136/bmjopen-2019-029338. PMID: 31227540
77. Dawson-Hughes B, Nelson J, Pittas AG. Intratrial Exposure to Vitamin D and New-Onset Diabetes Among Adults With Prediabetes: A Secondary Analysis From the Vitamin D and Type 2 Diabetes (D2d) Study. Diabetes Care

2020;43:2916-2922. *Diabetes Care*. 2021 May;44(5):e106. doi: 10.2337/dci21-0006.

78. Aguiar M, Andronis L, Pallan M, Högler W, Frew E. The economic case for prevention of population vitamin D deficiency: a modelling study using data from England and Wales. *Eur J Clin Nutr*. 2020 May;74(5):825-833. doi: 10.1038/s41430-019-0486-x.
79. Bejar CA, Goyal S, Afzal S, Mangino M, Zhou A, van der Most PJ, Bao Y, et al. A Bidirectional Mendelian Randomization Study to evaluate the causal role of reduced blood vitamin D levels with type 2 diabetes risk in South Asians and Europeans. *Nutr J*. 2021 Jul 27;20(1):71. doi: 10.1186/s12937-021-00725-1.
80. Hyppönen E, Vimaleswaran KS, Zhou A. Genetic Determinants of 25-Hydroxyvitamin D Concentrations and Their Relevance to Public Health. *Nutrients*. 2022 Oct 20;14(20):4408. doi: 10.3390/nu14204408.
81. Ang Zhou , Joseph B Selvanayagam , Elina Hyppönen . Mendelian randomization analyses support a role for vitamin D deficiency in cardiovascular disease risk *Eur Heart J* 2022 May 7;43(18):1731-1739. doi: 10.1093/eurheartj/ehab809. Non-linear DOI: 10.1093/eurheartj/ehab809
82. Emerging Risk Factors Collaboration/EPIC-CVD/Vitamin D Studies Collaboration. Estimating dose-response relationships for vitamin D with coronary heart disease, stroke, and all-cause mortality: observational and Mendelian randomisation analyses. *Lancet Diabetes Endocrinol* 2021 Dec;9(12):837-846. doi: 10.1016/S2213-8587(21)00263-1.