Review Article

Controlled trials of vitamin D, causality and type 2 statistical error

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Abstract

Two recent studies published in *The Lancet* (Autier *et al.* (2013) *Lancet Diabetes Endocrinol* **2**, 76–89 and Bolland *et al.* (2014) *Lancet Diabetes Endocrinol* **2**, 307–320) have concluded that low levels of vitamin D are not a cause but a consequence of ill health brought about by reduced exposure to the sun, an association known as 'reverse causality'. The scientific evidence and reasoning for these conclusions are examined here and found to be faulty. A null result in a clinical trial of vitamin D in adults need not lead to a conclusion of reverse causation when low vitamin D is found in observational studies of the same disease earlier in life. To assume an explanation of reverse causality has close similarities with type 2 statistical error.

For example, a null result in providing vitamin D for treatment of adult bones that are deformed in the pattern of the rachitic rosary would not alter the observation that lack of vitamin D can cause rickets in childhood and may have lasting consequences if not cured with vitamin D. Other examples of diseases considered on a lifetime basis from conception to adulthood are used to further illustrate the issue, which is evidently not obvious and is far from trivial.

It is concluded that deficiency of vitamin D in cohort studies, especially at critical times such as pregnancy and early life, can be the cause of a number of important diseases. Denial of the possible benefits of vitamin D, as suggested by insistent interpretation of studies with reverse causation, may lead to serious harms, some of which are listed.

Two recent articles in *The Lancet* (Autier *et al.*[1] and Bolland *et al.*[2]) conclude that low levels of vitamin D in cohort and other observational studies are generally the result of reverse causation and that low vitamin D levels are not generally a cause but a consequence of ill health. They further conclude that evidence largely does not support vitamin D supplementation for prevention of disease. In an accompanying editorial *The Lancet* goes further and describes the extensive research on vitamin D as 'Chasing a myth?'[3]. This conclusion has close similarities to a type 2 statistical error. Clinical trials of vitamin D have shown the null hypothesis is often correct, or rather is not rejected, in adults treated over a limited period of time, but to generalise this to all ages is similar to a type 2 error. This is because a statistical test does not strictly accept a null hypothesis but rather fails to reject it. A statistical test has very precise conditions and can never prove the null hypothesis to be true for all or different conditions.

Autier *et al.*[1] insist that, to be reliable, claims of benefit from increases in 25-hydroxyvitamin D (25(OH)D, the usual proxy for vitamin D status) 'must be supported by evidence from randomised controlled trials' and not by evidence coming only from observational studies. In this submission I argue that clinical trials cannot be and are not in fact a reliable method of establishing causation of disease except in special circumstances. This is because disease may be a consequence of early pathology which is irreversible at a later date when a trial may take place. This can explain the apparent conflict between the findings of cohort and other observational studies of vitamin D with clinical trials.

Detailed evidence of many kinds must be considered in determining causation and any apparent conflict of evidence should not automatically be resolved to favour results of a clinical trial. Vitamin D deficiency experienced early in life may involve changes that have profound effects on biochemical, anatomical and immune systems

that are not reversible in a much later trial. The present review article considers interpretation of trial methodology with regard to causation and provides examples illustrating how false inferences on causation may be drawn from clinical trials. The vitamin D story is well known in outline and is not repeated here. Several recent reviews may be referred to for details[4].

The argument

Randomised controlled trials are the 'gold standard' design for establishing a causal relationship between an exposure and an outcome, according to *The Lancet*. Two *Lancet* press releases[5,6] promoting the Autier *et al.*[1] and Bolland *et al.*[2] articles emphasize the gold standard and state in a headline that 'New analysis suggests that further trials of vitamin D have little chance of showing health benefits'. Autier *et al.* are careful to point out that randomised trials of vitamin D supplementation might obtain null results as a result of a particular trial design. They consider a number of factors such as too low a dose of vitamin D in the trial or too short a treatment period.

However, Autier *et al.*[1] have omitted an important category of reason for null results in such trials. A deficiency of vitamin D in the fetus during pregnancy, in infancy or even adolescence may produce irreversible changes in biochemistry, immune status or organ structure that cannot be remedied by supplying the missing vitamin in adulthood. Causality may be proved in a gold standard trial when supplementation succeeds in correcting a defect, but not when it fails to do so. A null result may be obtained simply because the trial took place too long after an insult occurring at a much earlier time, possibly during a critical period, or alternatively over a long period of deprivation[7], when vitamin D could have prevented or cured the condition which has since become fixed and no longer remediable by supplementation. This scenario may also be viewed as a type 2 statistical error. A type 2 error mistakenly accepts the null hypothesis, when the alternative hypothesis is the true state of nature. It is sometimes summarised as 'absence of evidence' is wrongly taken to mean 'evidence of absence'.

Diseases caused by low vitamin D that may not be corrected in adulthood

The scientific reasoning used to establish or negate causality of disease associated with low levels of 25(OH)D is investigated here with examples. Diseases associated with low levels of 25(OH)D are examined through the lifespan, considering evidence linking deficiency of vitamin D at any stage with known outcomes.

Rickets is the classical example. It causes alteration of normal bone formation and deformation of limbs which may be corrected by supplementation with vitamin D in childhood[8]. If however the deformations, whether gross or minor, are not corrected by vitamin D while the bones are growing, they become set in a pathological form that cannot then be corrected by supplementation. A trial of vitamin D supplementation to correct the anatomical defects of rickets that may persist in adulthood would be a failure. But we cannot conclude from this that the deficiency of vitamin D associated with rickets in childhood is accounted for by 'reverse causation'.

Paget's disease of bone may also be associated with vitamin D deficiency[9]. It is a common disorder of middle-aged and elderly people in which normal bone formation is disrupted, causing affected bones to weaken, thicken and become deformed. The basic problem is increased bone absorption by osteoclasts[10,11]. A clinical trial of vitamin D supplementation in adulthood might or might not be beneficial. However, it could not be expected to tell us whether any change in osteoclast behaviour was or was not caused by vitamin D deficiency in early life and whether this might have resulted in lasting and irreversible changes in the osteoclast response.

Perthes' disease of bone involves disrupted blood supply to the head of the femur followed by distortion of the joint, pain and limping. The aetiology of Perthes' disease is not clearly understood but is known to involve a north–south gradient and a deprived environment which is consistent with possible vitamin D deficiency[12]. A trial of vitamin D for Perthes' disease might possibly be beneficial and if positive clarify the aetiology, but a null result would tell us nothing useful about causation because deficiency might have been caused by an earlier lesion that is irremediable by the time vitamin D therapy is started.

This same argument may be extended to other problems of bone caused by early vitamin D deficiency which may create weakness or reduced mineral density of bone followed by susceptibility to fracture in adulthood that is not readily reversible by vitamin D. Failure of trials in adulthood to show consistent prevention of fractures with vitamin D supplementation cannot tell us anything valuable about causation nor should it necessarily lead to a conclusion of 'reverse causality'.

Cardiac structure may also be associated causally with 25(OH)D levels. The Baltimore Longitudinal Study of Aging has found that 25(OH)D is positively correlated with left-ventricle wall thickness and there is a relationship between 25(OH)D and left-ventricle concentric remodelling[13]. Hypertension was also linked in the study to left-ventricle hypertrophy and low 25(OH)D. According to the argument of Autier *et al.*[1] and Bolland *et al.*[2], these low 25(OH)D levels are the consequence of ill health that is associated with heart pathology by reverse causality. However, experiments with young rats show that deprivation of vitamin D causes cardiac hypertrophy, left-chamber alterations and systolic dysfunction, which follow on from

cardiac inflammation, fibrosis and apoptosis[14]. This strongly suggests that the association of low 25(OH)D with cardiac pathology in the Baltimore study is causal and not the result of 'reverse causality'.

A trial providing a vitamin D supplement might possibly prove to be beneficial to some of those elderly people whose hearts have been remodelled in the Baltimore study. However, failure of such a trial to remodel the anatomy of the heart in a more normal direction might lead us to conclude misleadingly, if we follow the reasoning of Autier et al.[1] and Bolland et al.[2], that the low vitamin D associated with abnormal heart anatomy found in the study is the result of reverse causation. Bolland et al. argue in general that 'there is little justification for prescribing vitamin D supplements to *prevent* myocardial infarction or ischaemic heart disease' (my italics). They overlook the possibility of preventing these diseases in early life by sun exposure, supplements or diet, which might provide better or even optimal doses of vitamin D. The statement of Bolland et al. would be valid if they had referred to 'effective treatment' rather than 'prevention'. A meta-analysis of forty-two randomised controlled trials of vitamin D supplementation reported a 6 % reduction in overall mortality, but only in those taking it for more than 3 years, supporting the view that the effects of hypovitaminosis D can take time to become apparent[15].

Chronic lymphocytic leukaemia (CLL) was associated with a low level of 25(OH)D in a cohort study of lymphoid cancers undertaken by the European Prospective Investigation into Cancer and Nutrition[16]. Low 25(OH)D was further associated in CLL with a low dietary intake of vitamin D. These associations were linked consistently only to CLL and not to other lymphoid cancers in the study. The specificity of this association of low 25(OH)D with CLL suggests a possible causal relationship. A clinical trial of vitamin D for CLL might show benefits but benefits could not necessarily be expected at a late stage in the disease. This is because CLL may be caused many years before it is picked up by expansion of the white cell population with fixation of chromosome aberrations[17] and failure of regulation by apoptosis which may involve vitamin D metabolism[18]. These mutated white cells can not necessarily be expected to respond normally to vitamin D provided at a later stage.

Multiple sclerosis (MS) provides another example showing that Autier's reverse causation theory of low vitamin D levels is exaggerated. Robust evidence, ignored by Autier et al.[1], is considered by a number of senior researchers in the field as amounting to proof that vitamin D deficiency early in life is a primary cause of MS. This evidence has been summarised in many expert reviews and need not be revisited here[19,20]. Of special interest is the possible mechanism of an early insult to the body involving a critical step in the maturation of the thymus and the immune system. For example, it has been suggested that vitamin D insufficiency in early life may affect expression of HLA-DRB1 in the thymus, allowing autoreactive T cells to escape thymic deletion and remain to cause MS or other autoimmune disease in later years[21,22].

Indeed, this same mechanism may explain the association of low vitamin D levels with other nervous system diseases and with autoimmune diseases which Autier et al.[1] choose to explain by 'reverse causation'. DeLuca et al. concluded that vitamin D is a candidate in influencing susceptibility to several psychiatric and neurological diseases with the strength of evidence varying for schizophrenia, autism, Parkinson's disease, amyotrophic lateral sclerosis and Alzheimer's disease, while being especially strong for MS[20]. While trials of vitamin D in adult MS have produced conflicting or uncertain results, clinical trials providing vitamin D at an early critical period might still be able to prove causation in MS. One small trial of vitamin D supplementation in optic neuritis, an early symptom of MS, has already shown that the symptoms of optic neuritis may be postponed in more than half of patients given vitamin D[23], supporting the suggestion of a critical period when vitamin D is effective.

Autoimmune and other diseases are associated in significantly elevated rates with hospital admission for vitamin D deficiency, osteomalacia and rickets[24]. These diseases include: Addison's disease, ankylosing spondylitis, autoimmune haemolytic anaemia, chronic active hepatitis, coeliac disease, Crohn's disease, diabetes mellitus, pemphigoid, pernicious anaemia, primary biliary cirrhosis, rheumatoid arthritis, Sjogren's syndrome, systemic lupus erythematosus and thyrotoxicosis. While it is possible that these associations might be explained at least in part by reverse causation, alternative hypotheses such as failure of thymic deletion of T cells should logically be considered. Support for an explanation that does not involve reverse causation comes from certain autoimmune diseases that carry an increased risk in spring or early summer births and a reduced incidence in autumn births. This seasonal birthday observation is firmly established in MS and may be explained by low vitamin D levels in the mother's body after the winter and high vitamin D levels after the summer. Such increased risk with seasonal birth cannot easily be accounted for by reverse causation. Seasonal birth has been found in rheumatoid arthritis, ulcerative colitis, systematic lupus erythematosus, schizophrenia, autism and a number of other diseases[25–27]. Schizophrenia and autism, as classic mental diseases, might be thought to be least likely to be caused or influenced by insufficient vitamin D at a critical period, but recent understanding of these diseases finds that they frequently have autoimmune features[28].

There is no reason to believe that determination of the immune system by escape of T cells from thymic deletion should necessarily be reversible later in life by administration of a supplement in a 'gold standard' trial. Other important non-reversible changes may also occur at various stages of development.

Type 1 diabetes (T1D) is the result of the death of cells in the pancreas following an immune crisis associated with deficiency of vitamin D and a possible virus infection[29]. The risk of T1D is increased if there is a childhood diagnosis of rickets[30]. It is not surprising to find that subsequent provision of vitamin D in a trial cannot bring these pancreas cells back to life. But if vitamin D supplementation were to be given at an early enough stage on appearance of auto-antibodies predictive of T1D or during or shortly after an infection, then the organ might possibly be saved. Perhaps later, cell remnants might be rescued using vitamin D in combination with other immune therapy, doing something to restore a measure of pancreatic function. Such an experimental clinical approach has some promise that may be worth exploring but may be lost if low D levels in T1D cohorts are incorrectly dismissed as simply the result of reverse causation.

Discussion

The evidence reviewed above suggesting a link between deficiency of vitamin D in childhood or early life and later disease is observational and/or indirect. Therefore, although it may suggest causality of late-life effects resulting from deprivation of vitamin D in early life, it does not prove it. However, the extent of the evidence, derived by different methods, all pointing to the same conclusions, as in MS, may make a very strong case for causality. Nevertheless, with the exception of rickets, proven evidence of the link between early insults and later disease might be said to be lacking. However, it requires only one proven case, as in rickets, to make the point at issue; while other observational evidence suggests that rickets is far from being an isolated example.

Reverse causation may of course account for some cases of low vitamin D associated with disease in observational studies when a low 25(OH)D level cannot be corrected by provision of vitamin D in trials. However, other cases of low 25(OH)D may occur as a continuation of low levels of vitamin D associated with individual and family lifestyle, in particular habits of sun exposure and diet. At an earlier stage in life low levels of vitamin D may have caused irreversible damage that cannot be corrected by subsequent provision of vitamin D in a trial at whatever dosage or for whatever length of time. For optimum health, dietary items such as vitamins and other essential nutrients must be supplied at certain normal levels on a continuing basis. Interruption of supply followed later by replacement cannot necessarily be expected to reverse damage. In this respect clinical trials of a nutrient are different from clinical trials of a drug, although it is the drug trial model that many have in mind when assessing results.

Autier et al.[1] state that the randomised controlled trial is considered to be the 'gold standard design' for establishing a causal relationship between an exposure and an outcome and quote Byar et al.[31] as an authority for this. However, Byar et al. do not use the term 'gold standard' and only consider causality very briefly to say that some questions cannot be answered by randomised clinical trials for ethical reasons. In fact, the definition of clinical trials used by Byar et al. refers to the determination of 'effective treatment', not the determination of causality. They say that 'a comparative clinical trial may be defined as a medical experiment designed to evaluate which (if any) of two or more treatments is effective'. They have nothing to say about clinical trials as a method of investigating the causal relationship between a deficiency and later supplementation. So quotation of this reference by Autier et al. is misleading. However, Byar et al. end with wise counsel, recommending 'additional experimentation and observation as a means of understanding the nature of a disease and making the interpretation of trials more fruitful'.

How much vitamin D need a person take to achieve an optimum level? The US Institute of Medicine and the US Endocrine Society have released separate guidelines for vitamin D requirements. The Institute of Medicine defines 50 nmol/l (20 ng/ml) as the 'sufficient' level of 25(OH)D but this figure was determined by observations of bone health only – that is, calcium absorption, bone mineral density and osteomalacia/rickets[32]. However, the Endocrine Society using a medical model that considered other diseases recommended that a level of 25(OH)D above 75 nmol/l (30 ng/ml) should be attained[33]. The Endocrine Society suggested that in order to allow for day-to-day variation in intake of vitamin D, maintenance of a 25(OH)D level of 100 to 150 nmol/l (40 to 60 ng/ml) is ideal and that up to 250 nmol/l (100 ng/ml) is safe. The Endocrine Society recommends a daily dose of 10–25 μg (400–1000 IU) for children up to 1 year of age, 15–25 μg (600–1000 IU) for children aged 1–18 years and 37·7–50 μg (1500–2000 IU) for over 18s[33]. However, because of wide variation in sun exposure and absorption, some people will require larger daily doses if they are to achieve recommended blood levels of 25(OH)D.

Harms consequent upon false inference of reverse causation

An erroneous inference that low 25(OH)D levels are largely the result of reverse causation and that supplementation is ineffective may result in a number of harms which may be expected to include the following:

1. Several meta-analyses have found that higher levels or doses of vitamin D are associated with longer life[34–37] and one estimate found that doubling vitamin D intake would on average increase life by about 2 years[38]. Other evidence suggests that supplementation needs to be continued for 3 years or more[15] to gain extra life.

This possible opportunity will be lost if people are not encouraged to expose themselves to sunshine and take a vitamin D supplement.

2. People who do not supplement may expect to have greater susceptibility to infectious diseases such as tuberculosis[39] and viruses[40–42], which have been shown to respond to vitamin D supplementation in clinical trials through improved innate immunity, with increased production of cathelicidin and other natural bacteriocides.

3. Pregnant women will be discouraged from taking vitamin D, with adverse effects on babies which may include not only rickets but also MS[43], T1D[30] and an increased risk of gestational diabetes, pre-eclampsia and small-for-gestational-age infants[44].

4. Failure to undertake further trials or attempt treatment of T1D and other autoimmune conditions with vitamin D at an early stage[30] when effective treatment may be possible despite negative trials in established disease[43].

5. Failure to undertake further trials of vitamin D treating the earliest signs of MS in children and young adults, trials which have the possibility of halting the disease at an early stage[43].

6. Scientific evidence ignored[19] by Autier et al.[1] and Bolland et al.[2] suggests that the incidence of MS in the UK might be halved by a major programme of supplementation in pregnancy, childhood and early life that could lower the incidence to that in southern Europe. Virtual eradication may not be impossible if sufficiently comprehensive public health measures could be organised. This opportunity will be delayed or lost if the reverse causality hypothesis is accepted uncritically.

7. Future fortification of foods, which has done much to more or less eliminate rickets in the USA[8], will be seen as without value and so food companies and government policy makers in the UK will be discouraged from fortifying.

Conclusion

The reverse causality hypothesis of Autier et al.[1] and Bolland et al.[2] may account for low vitamin D in a number of disease states. But as a generalisation for the conflict of findings between cohort studies and clinical trials it is a false inference, similar to a type 2 statistical error, showing a deep misunderstanding of the way to perform informative trials. The error arises from conflation of two concepts which each require their own distinct reasoning: on the one hand 'effectiveness of intervention' and on the other 'inference of cause'. The Lancet has enthusiastically promoted its two vitamin D articles, suggesting highest quality by calling the research 'gold standard'. Thus it may be both symmetrical and appropriate to call their error of conflation a 'gold standard fallacy'.

The Lancet's representation of much vitamin D research as 'chasing a myth' cannot be justified and risks harms. These harms may occur as the result of failure to use supplementation and failure to further fortify foods, measures which have particularly important health benefits in early life as well as extending life in old age.

Acknowledgements

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