Vitamin D discovery outpaces FDA decision making

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Summary

The US FDA currently encourages the addition of vitamin D to milk and cereals, with the aim of reducing rickets in children and osteoporosis in adults. However, vitamin D not only regulates the expression of genes associated with calcium homeostasis, but also genes associated with cancers, autoimmune disease, and infection. It does this by controlling the activation of the vitamin D receptor (VDR), a type 1 nuclear receptor and DNA transcription factor. Molecular biology is rapidly coming to an understanding of the multiplicity of roles played by the VDR, but clinical medicine is having difficulty keeping up with the pace of change. For example, the FDA recently proposed a rule change that will encourage high levels of vitamin D to be added to even more foods, so that the manufacturers can claim those foods ''reduce the risk of osteoporosis''. The FDA docket does not review one single paper detailing the transcriptional activity of vitamin D, even though, on average, one new paper a day is being published on that topic. Nor do they review whether widespread supplementation with vitamin D, an immunomodulatory secosteroid, might predispose the population to immune dysfunction. This BioEssay explores how lifelong supplementation of the food chain with vitamin D might well be contributing to the current epidemics of obesity and chronic disease. ***BioEssays*** **30:173–182, 2008. © 2008 Wiley Periodicals, Inc.**

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Abbreviations: 1,25-D, 1,25-dihydroxyvitamin-D; 25-D, 25-hydroxyvitamin-D; AR, androgen receptor; CAR, constitutive androstane receptor; CASR, calcium sensing receptor; CDC, U.S. Centers for Disease Control; CFSAN, FDA Center for Food Safety and Applied Nutrition; COMP, cartilage oligomeric matrix protein; defB2, beta-Defensin 2; DHHS, U.S. Department of Health and Human Services; FDA, U.S. Food and Drug Administration; GR, glucocorticoid receptor; K_d, disassociation constant; LBP, ligand binding pocket; nVDRE, negative vitamin D response element; PCR, polymerase chain reaction; PDB, RCSB protein data bank; PKA, protein kinase A; PXR, pregnane X receptor; RR, risk ratio; RXR, retinoid X receptor; TGF-beta, transforming growth factor beta; ThRa, thyroid receptor alpha; TLR2, toll-like receptor 2; VDIR, VDR interacting repressor; VDP, vitamin D binding protein; VDR, vitamin D receptor; VDRE, vitamin D response element; WUSTL, Washington University St Louis.

Introduction

From time to time, the practice of clinical medicine gets out of step with the pace of discoveries in the biological sciences. The popular press is talking about vitamin D as ''The Sunshine Vitamin'', promoting it as capable of reducing the risk of cancers and autoimmune disease. Yet vitamin D is actually a secosteroid transcriptional activator, at the heart of innate immunity.[1–3] Vitamin D is immunomodulatory,[4,13] and molecular biologists are working as fast as they can to more fully elucidate all of its actions, and those of its nuclear receptor, the VDR, upon the human body, and upon human disease.

The knowledge that vitamin D activates the VDR to transcribe (or repress) 913 genes, and the possibility that it might affect expression of as many as 27,091,[5] portends a paradigm shift in the way that clinical medicine has visualized this ''Sunshine Vitamin''. Historically, it has been associated solely with bone formation and calcemia,[6] yet physicians are now being told that vitamin D closely regulates genes associated with diseases ranging from cancers to multiple sclerosis.

Although the FDA Center for Food Safety and Applied Nutrition (CFSAN) has struggled to understand the importance of measuring 1,25-dihydroxyvitamin-D (1,25-D) several times since 1994,[7–9] they have still not been able to produce a docket[7] reflecting any real comprehension of the underlying molecular biology. Two decades after 1,25-D was revealed as the active vitamin D metabolite, the sole D metabolite that activates gene transcription by the VDR, and 13 years after the FDA itself suggested that 1,25-D should be measured to support claims of a drug's osteoporotic activity,[8] the FDA is still accepting results from clinical studies that did not measure that active metabolite.

For half a century, medical science has been noting the *association* between vitamin D serum levels and disease. What developed has been a concept of 'vitamin D deficiency' based solely on the assumption that low vitamin D serum levels somehow **cause** disease processes. But this ignores the alternate hypothesis—that the disease processes themselves regulate the vitamin D metabolism—that the observed

low values of vitamin D in disease are a **result** of the disease process, and not the **cause**. Molecular biology has now taught us that the body is capable of making its vitamin D directly from 7-dehydro-cholesterol,[10,11] and that the generation of the vitamin D metabolites is modulated by inflammatory disease processes.[11,12] Not only does the whole concept of vitamin D deficiency need reconsideration, one should question whether it is misleading to even use the word 'vitamin' when discussing this secosteroid.

VDR—the vitamin D nuclear receptor and the transcriptional pathways that it controls

The VDR is a type 1 nuclear receptor, a transcription factor that forms homodimers and heterodimers active in the transcription and transrepression of genes. In their 2003 BioEssay, Lin and White[13] examined the structure of the VDR, endogenous vitamin D synthesis, and its subsequent hydroxylation to active metabolites in the liver and kidney. Their research group at McGill University has since identified 27,091 genes that might be transcribed or repressed by the VDR.[5] Several of these genes, and the resulting proteins, are known to be active in cancer.[5] Included are the beta-defensin[2] and cathelicidin[1–3] antimicrobial peptides, key to innate immunity and to the body's response to intracellular pathogens. It is thus becoming clear that the clinically accepted role of the vitamin D metabolites, that of regulating calcium homeostasis, is just a small subset of the functions actually performed by these hormones.

Most biologists understand that the murine model frequently fails to accurately model human immune disease and human cancers. Much of this is due to evolutionary divergence between the murine and human VDR. Wang et al.[5] found many differences between the genes targeted by the murine and human VDR. For example, the gene encoding cathelicidin antimicrobial peptide is not expressed by the murine VDR[3] at all. Further, we have described[14,15] how the relatively low structural homology between the murine and human VDR can help to explain murine modeling inaccuracy. Unraveling the intricacies of the human D metabolism is often made extremely difficult by the intermingling of murine and human biologies in the literature.

Fig. 1 shows key transcriptional pathways controlling the vitamin D metabolism. It would be nice if we were able to say that in the four years since Lin and White's BioEssay, molecular biology has fully fleshed out the metabolism that they described, but it would not be true. Some pathways have been well documented, while there is still a lot of work to be done on others. There is little doubt that additional transcription factors will be found as time goes by, adding additional layers of complexity to a metabolism once thought to follow a very simple 'vitamin in, benefit out' model.

This essay will focus on the transcriptional pathways that have already been elucidated, and particularly on the diverse feedback mechanisms that clarify why the **alternate** hypothesis for vitamin D activity—that the observed serum levels are modified by disease processes—is far more

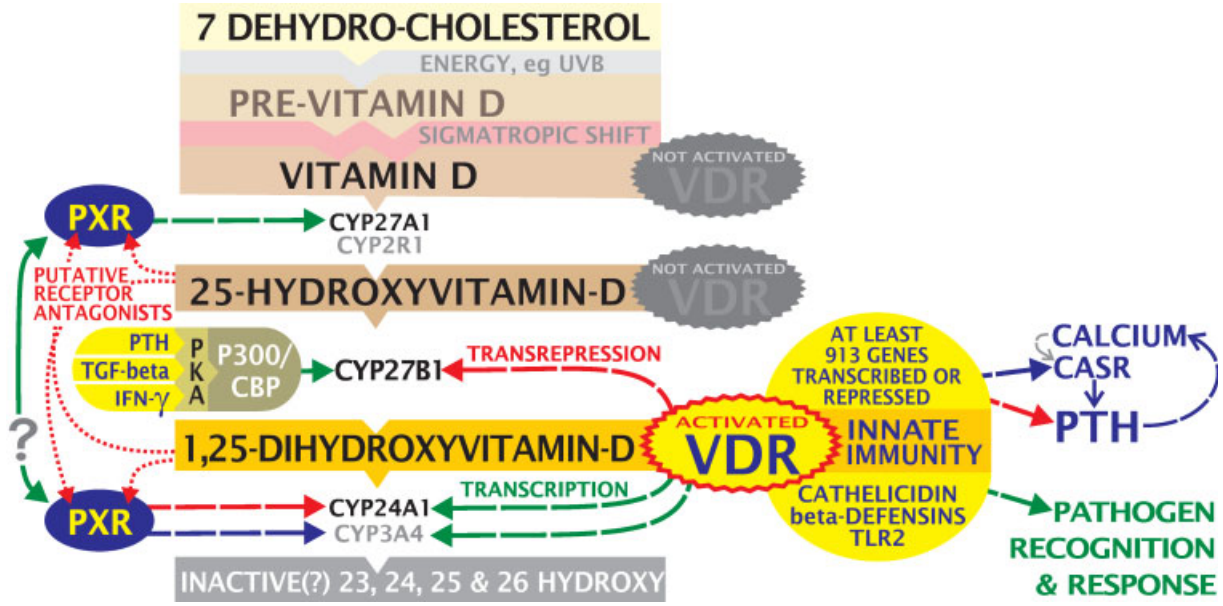

Figure 1. *Homo sapiens* vitamin D metabolism, with an emphasis on the transcriptional pathways. Red arrows designate downregulation, green arrows, upregulation, and blue arrows uncertainty. At the lower right can be seen the inter-relationship of calcium, the calcium-sensing receptor (CASR) and the parathyroid hormone.

plausible than the currently accepted 'vitamin' pragma—that the lower levels observed in sick individuals are indicative of a nutritional deficiency.

The metabolism starts at the top left of Fig. 1, with the substrate 7-dehydrocholesterol. Under the influence of energy, in keratinocytes,[10] macrophages,[16] intestinal,[16] and possibly other cell lines, the bond between carbons 9 and 10 of 7-dehdro-cholesterol is cleaved, to form vitamin D3.[13,16,17] Hydroxylation at the carbon 25 position takes place under the action of several P450 enzymes, most notably CYP27A1 [EC 1.14.13.15] and CYP2R1[18,19] [EC 1.14.14.-], resulting in 25-hydroxyvitamin-D3 (25-D).

Although 25-D has some physiologic activity, for example, binding to the vitamin D-binding protein (VDP), and the cartilage oligomeric matrix protein (COMP, see later in this review), it cannot activate the transcriptional activity of the VDR. Another hydroxylation, at the 1-alpha position, is necessary before the ligand-binding pocket (LBP) of the VDR can be constrained (by 1,25-D) into the configuration[14,15] needed for binding the coactivator complexes,[20] allowing the subsequent dimerization to facilitate gene transcription (and repression).

When the VDR is activated, it transcribes the gene for the P450 enzyme CYP24A1 [EC 1.14.-.-], an enzyme that inactivates 1,25-D. This is the best documented of the feedback control systems used by the body to limit the concentration of 1,25-D to just that amount needed for proper transcriptional activation of the VDR.

Almost as well documented is the trans-repression implemented by the activated VDR in order to limit the amount of transcribed CYP27B1 [EC:1.14.13.13] gene and, in turn, the amount of 25-D that is 1-alpha hydroxylated into 1,25-D. Kato et al.[21] have described how p300 is recruited to a VDR-interacting repressor (VDIR), which binds to the negative vitamin D response element (nVDRE) in the CYP27B1 gene promoter, thereby activating transcription of CYP27B1. An activated VDR–RXR heterodimer can displace p300 from VDIR by interacting with a chromatin-remodeling complex, yielding a second feedback mechanism capable of limiting the generated level of 1,25-D to precisely that which is required to induce VDR transcription.

Generation of CYP27B1, 1,25-D generation, and thereby VDR activation, occurs when p300 is activated by protein kinase A (PKA). PKA is in turn activated by a number of biochemicals, most notably the immune response mediators TGF-beta and interferon-gamma, as well as the parathyroid hormone (PTH).

When the immune system is challenged by injury or pathogens,[1] TGF-beta and/or interferon-gamma are released, additional CYP27B1 is generated, and thus additional 1,25-D. In turn, the VDR is activated to express more cathelicidin and beta-defensin-2 (defB2) antimicrobial peptides. Additionally, when the VDR is activated, TLR2 is expressed,[1] allowing the immune system to recognize gram-positive bacteria, including *Staphylococcus aureus*,[22,23] *Chlamydia pneumoniae*[24] and *Mycoplasma pneumoniae*.[25]

Further complications

The rest of the D metabolism is less well defined. Nevertheless, the aim of this essay is to explore the challenges that we face as we try to translate the molecular complexities of the D metabolism into a clinical environment. It is therefore important to identify a consensus model from the jumble of published murine, human and in silico studies.

The observation has been made that the enzyme CYP3A4[26] [EC:1.14.14.1] is more active than CYP24A1 in the human intestine and liver.[27] This enzyme was therefore added to the model (Fig. 1).

The VDR is a member of the NR1I subfamily of type 1 nuclear receptors.[28] For *Homo sapiens,* the other members of this family are the pregnane X receptor (PXR, SXR) and the constitutive androstane receptor (CAR).

PXR has been shown to induce transcription of CYP27A1.[29] PXR has also been reported to competitively downregulate the VDR-induced expression of CYP24A1.[27,30] PXR is an unusual transcription factor, with an LBP that enlarges to allow activation by large molecules, such as rifampicin, or shrinks to accommodate smaller molecules, such as the steroids. It is not yet known which endogenous ligand(s) modulate PXR expression of either CYP27A1 or CYP24A1. Rifampicin was used as the agonist during the CYP27A1 study,[29] but it is clear there must also be endogenous ligands.

A structural model of the PXR with the smaller LBP has just been published,[31] as [PDB:2o9i], and we have used our nuclear receptor modeling methodology[32,33] to show that 1,25-D binds into this PXR structure, as an antagonist, with very high affinity (unpublished work). It almost certainly will competitively displace the native ligand(s) at physiologic concentrations. Fig. 2 shows the minimum energy conformation of 1,25-D superimposed upon the T1317 agonist from [PDB:2o9i].

The mathematical modeling also shows that 25-D and 1,25-D have similar affinities for the PXR LBP, as their antagonistic potential is largely independent of the 1-alpha hydroxylation. This would suggest that feedback mechanisms dependent upon the **concentrations** of 25-D and 1,25-D, in addition to those from VDR **activation**, can modulate the expression of CYP27A1 and CYP24A1, and therefore regulate the concentrations of both 25-D and 1,25-D.

Finally, Wang et al. have located a possible VDR response element (VDRE) near the gene for CYP27A1.[5] This element is discrete and separate from the putative PXR response elements located by Li et al.[29] and suggests a role for the activated VDR in the expression of CYP27A1. However, this

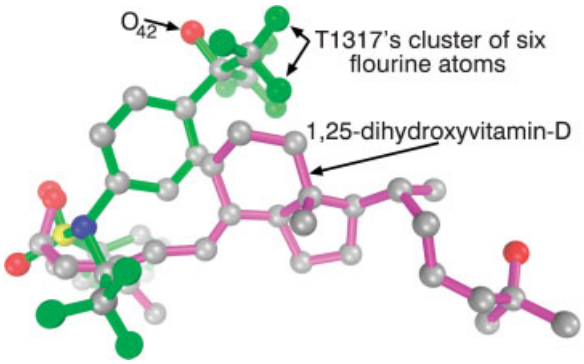

Figure 2. T1317 (green backbone) is shown activating the PXR structure of [PDB:2o9i]. Superimposed is 1,25-D (pink backbone) docked into the PXR with $K_d = 8.27$, almost certainly acting as an antagonist. There are no contacts with to match those near T1317's cluster of fluorine atoms, especially the hydrogen bond between T1317's O_{42} and PXR's His407.

final feedback pathway has been omitted from Fig. 1, as we currently have no evidence that it is expressed, and no clinical observations that would support its presence.

So why cling to the 'vitamin' pragma?

The model that we have elucidated for the D metabolism is complex, but not particularly so. At this point, there are two main feedback pathways subsequent to VDR activation, and two that are based on metabolite concentrations have putatively been added.

A good model can provide insight into infectious and immune disease processes that epidemiological observations alone could never provide. It is important for molecular biologists to explain the transcriptional and feedback processes to their clinical epidemiological colleagues. Armed just with the 'vitamin in, benefit out' model, clinicians are still being baffled by the contradictions that they see in their study data, which often cannot be analyzed to a useful conclusion.

For example, we earlier cited Vigano et al.[4] with their excellent study of the expression and regulation of the D metabolism in the pregnant endometrium. Just a few months ago, a commentary in Journal of Nutrition[34] was uncertain how to explain the results from a comprehensive clinical study[35] showing that at the end of their pregnancies, even though 90% were taking prenatal vitamins, "vitamin D deficiency" was still common in the cohort of pregnant women. The commentary suggested that maybe this might be due to lack of compliance on the part of women in the cohort, or perhaps they just needed even more supplementation than twice the daily reference intake (DRI), the amount they were being given.

Surely, when the model fails to describe the data, it is time to question the model, not the data. This study collected only the transcriptionally inactive metabolite, 25-D, as is still common in so many clinical studies. Consequently the "vitamin D deficiency" being observed may well be downregulation of the 25-D metabolite under the influence of the elevated levels of 1,25-D during pregnancy. In the absence of definitive 1,25-D data, it is not possible to draw valid conclusions from the lowered serum levels of 25-D that were observed.

Yet the knowledge that 1,25-D is overexpressed in pregnancy is not new. Placental conversion was demonstrated in-vitro in 1979,[36] overexpression of 1,25-D in vivo during 1980,[37] and the dysregulated D metabolism was described in 1981.[38] 25 years later, clinical researchers are still not measuring more than one of the D metabolites, and still do not comprehend that they are dealing with an expressed hormone, and not a vitamin.

What is a 'natural' homeostasis of vitamin D synthesis?

In addition to endogenously produced vitamin D, a little is ingested from naturally occurring dietary sources such as fish products and egg yolks. However, in the USA, the addition of synthetic vitamin D to foods became widespread as early as the 1930s, when peanut butter, hotdogs, soda pop and bread were all "fortified" with "The Sunshine Vitamin".[39] Patent protection on the process for manufacture of synthetic vitamin D expired in 1945, and during the latter half of the 20th century, steadily increasing concentrations of synthetic vitamin D have been added to the food chain. Nowadays, FDA regulates the addition of synthetic vitamin D to milk in the USA, and it is very difficult to buy milk which has not been 'fortified'.

Vitamin D supplementation of food and baby formula has spread throughout the world, even to the less economically developed countries. It is thus very difficult to find a population which can be studied in order to ascertain what the level of natural metabolic homeostasis for 25-D might actually be.

Two studies do provide a glimpse, however. The first found a "high prevalence of vitamin D deficiency in Chilean healthy postmenopausal women".[40] The average level of serum 25-D sampled from 90 "healthy ambulatory women" showed that 27% of premenopausal, and 60% of postmenopausal women, had 25-D levels under 50 nmol/L. A study[41] showing "Hypovitaminosis D is common in both veiled and nonveiled Bangladeshi women" found a 25-D level less than 40 nmol/L in approximately 80% of the healthy young women.

These studies show a wide variation in levels of 25-D being generated by populations whose diets have probably not yet been significantly altered by 'The Sunshine Vitamin,' indicating that the unsupplemented metabolic homeostasis is probably in the range 23–60 nmol/L, and that it falls with advancing age.

Another estimate of natural homeostasis is provided by the level at which 25-D begins to affect expression of the parathyroid hormone, PTH. Aloia et al.[42] found that break-point to be around 44 nmol/L in African American women who had adequate calcium intake. Their subsequent systematic

review of previous studies where the calcium intake had also been adequate, confirmed this breakpoint.

What is a safe level of vitamin D supplementation?

The rigorous response is to note that any quantity of exogenous vitamin D which does not affect the body's metabolic homeostasis, is safe. Levels of supplementation which do not increase the measured vitamin D metabolites to levels above those observed in the un-supplemented populations can definitely be regarded as 'safe'. When people ask this question, however, they usually mean ''how much vitamin D should I take in order to ensure optimal health?'' And that is an entirely different problem.

FDA staff currently suggest[43] a 25-D level in the 75–80 nmol/L range as ''adequate.'' This is a level well above the means of the Chilean and Bangladeshi women, and is based on a risk-benefit analysis.

The new FDA regulation[7] proposes to allow additional supplementation of the nation's food chain. Indeed, manufacturers who supplement Orange Juice with vitamin D will be able to make the claim that their product ''reduces osteoporosis''.

Tang et al. for the Cochrane Group,[44] performed a meta-analysis of the use of calcium in combination with vitamin D to prevent fractures and bone loss, and found that there was no evidence of benefit at the vitamin D dosage currently available from foods, or even at the levels contemplated by the new regulation. Further, even though their study was suffering from ''scarcity of data'' at higher dosages, Tang et al. suggested that ''if vitamin D is to be used as an adjunct supplementation to calcium, its dose should be at least 800 i.u. or more''. It would seem that, if supplementation with vitamin D is to be effective in the reduction of osteoporosis, it should be administered by a physician, and not via the food chain.

It has also been suggested[39] that 'The Sunshine Vitamin' can reduce the risks of cancers, rheumatoid arthritis (RA), lupus (SLE), and multiple sclerosis (MS). Merlino et al.[45] have reported that vitamin D intake does indeed modestly reduce the risk of Rheumatoid Arthritis. More recently, however, Costenbader et al.[46] demonstrated that among 186,389 nurses followed between 1980 and 2002, there was no evidence that increasing vitamin D intake had any relationship to the relative risk of developing either SLE or RA. A meta-analysis by Autier and Gandini[47] was able to demonstrate a decrease in total mortality with an increase in vitamin D supplementation, but the relationship was not statistically significant. Freedman et al.[48] the National Cancer Institute prospective study of vitamin D and Cancer mortality, studied 146,578 person-years of data, and did not find an association between levels of 25-D and total cancer mortality, with the exception of colorectal cancer. It is troubling that the highest observed levels of 25-D seemed to increase overall cancer mortality, although the effect was still not statistically significant (80 to $<$100 nmol/L, RR $=$ 1.21 and for $>$100 nmol/L, RR $=$ 1.35).

Neither Kimball et al.[49] nor Barnes et al.[50] were able to show a relationship between vitamin D supplementation and remission of multiple sclerosis, but neither were their studies designed to do so. Munger et al.[51] concluded: ''a broad recommendation for a several-fold increase in vitamin D intake among adolescents and young adults requires stronger evidence than that provided by observational studies alone. Meanwhile, use of vitamin D supplements for MS prevention should not be undertaken until efficacy is proven''.

At the level of the more basic science, Arnson et al.[52] noted that ''on the whole, vitamin D confers an immunosuppressive effect'' in autoimmune disease. That immunosuppression was confirmed by Waterhouse et al.[12] They joined Barnes et al.[50] in noting that correlation between the 25-D and active 1,25-D metabolites seemed strongest in disease, and weakest in health. Arnson et al. further remarked ''vitamin D affects the immune system at many levels and by a number of mechanisms''. And, indeed, that is the nub of the matter. Surely we are being naive if we expect the exogenous modulation of a metabolism that is responsible for the expression of over a thousand genes to provide a simple go/no-go result?

Are gut bacteria a factor in the obesity epidemic?

This question was posed by Bajzer and Seeley[53] when commenting on two recent papers from Gordon and colleagues at WUSTL[54,55] showing that the balance between Firmicutes and Bacteroidetes in the human and murine gut was correlated with the subject's tendency towards obesity. The WUSTL group was additionally able to transplant microbes from obese to 'germ-free' mice and show that obesity followed the microbes.

The US Centers for Disease Control (CDC) recently published data[58] showing increased obesity in US children and adolescents aged 2–19 years. During the decade between the study periods 1988–1994 and 1999–2004, waist circumference increased in 65.4% of boys, and in 69.4% of girls, an increase the authors described as ''epidemic''.

It has been assumed that the obesity epidemic is due to unhealthy lifestyle choices. Surprisingly, however, several carefully controlled studies have failed to confirm that assumed causal link between lifestyle and childhood obesity.[59,60] Additionally, other studies are showing an association between lowered levels of 25-D and obesity,[61,62] indicating the alternative hypothesis of metabolic homeostasis should be considered alongside the customary assumption of deficiency.

The VDR is responsible for expression of key antimicrobial peptides. Both cathelicidin and defensin antimicrobial peptides are active in the GI tract, and are known to regulate the

composition of bacterial flora,[56] in addition to their role of responding to known pathogens. The activity of, particularly, cathelicidin, is important in the neonatal gut.[57] Cathelicidin and defB2 are both expressed[2] by the VDR when it is activated by 1,25-D.

Is it possible that the chronic addition of immunomodulatory 'vitamin D' to the diet of *Homo sapiens* has disturbed the historic composition of gut microbiota, and thus is at least partly responsible for the current epidemic of obesity? Physicians know that chronic administration of **corticosteroids** encourages obesity. More research is needed to better define the immunomodulatory activities of this **seco** steroid, before encouraging even more of it to be added to the food chain.

Persister pathogens, chronic disease

O'Conner et al.[63] writing in the CDC's 'Emerging Infectious Diseases', mused that if just 5% of idiopathic chronic disease is attributable to infectious agents, then 4.5 million of the 90 million chronically ill Americans would be able to benefit from anti-infective strategies.

Biofilms,[64] metagenomic communities of microbes, represent an emerging, still largely unknown, class of pathogens. Cultures of blood and tissue, and even PCR analysis, can fail to detect[63] bacteria in biofilm communities. Many research teams are trying to elucidate potential disease mechanisms, and are producing tentative, but tantalizing, results.[63]

Waterhouse et al.[12] have documented a dysregulated D metabolism accompanying chronic diseases ranging from rheumatoid arthritis to multiple sclerosis. Our own work has shown that restoring VDR competence, with a VDR agonist,[65] induces an immunopathologic response when patients suffering from chronic inflammatory diseases are challenged with bacterial protein synthesis inhibitors. Chronically ill subjects, whose conditions have not previously responded to antibiotics, sometimes experience unrestrained immunopathology when a VDR agonist is administered concurrently with the antibacterials. An initial uncontrolled, observational study has shown that recovery often accompanies reduction of the putative bacterial load.[65,66] In the absence of definitive clinical data, why do we ignore warnings presaged by the molecular biology?

Tuberculosis

Tuberculosis remains a major problem in the USA, and globally it affects 9 million people each year. As early as 1985, it was noted that low serum concentrations of 25-D may be a consequence of the disease process.[67] But the recent demonstration that the time taken for an individual to convert to sputum negativity can be directly predicted by VDR genotype[68] should sound a warning bell throughout our Public Health agencies. Why is the prognosis of this deadly disease so closely tied to the vitamin D metabolism? Is vitamin D supplementation helpful in slowing the resurgence of tuberculosis, or might it make the disease worse? The answers to these questions are urgently needed.

Disabling the VDR delivers a knockout blow

Think about this for a minute—if you were a persistent pathogen, wouldn't it seem a good idea to disable your host's ability to produce antimicrobial peptides? And if you discovered that disabling just one receptor, the VDR, would get rid of both cathelicidin and defB2, wouldn't you try to evolve a mechanism for doing that?

During our molecular modeling of the actions of ARBs upon the nuclear receptors,[32] and our subsequent presentation to the FDA,[33] we were struck by the symmetry with which endogenous ligands exhibited very similar affinities across several members of the type 1 nuclear receptor family. For example, 1,25-D docked into the VDR with a (nanomolar) K_d of 8.48, but also exhibited a K_d of 8.12 into the glucocorticoid receptor (GR), 8.41 into the thyroid-alpha-1 receptor (ThRa) and 8.05 into the androgen receptor (AR) (all K_d values were computed using XSCORE[69,70]). Similar high affinities were found with 25-D, which yielded K_d values of 8.36, 8.17, 8.32 and 8.07, respectively. It would seem that activation of this subset of receptors is achieved by a delicate balance between the concentrations of a number of endogenous hormones.

Fig. 3A shows the minimum energy (docked) configurations for T3 and 1,25-D in the ThRa, and Fig. 3B for dexamethasone and 1,25-D in the GR. After examining the residues contacted by each ligand, it appears likely that 1,25-D is an antagonist of activation for each of these receptors, but so little is known about the active receptor residues that all one can say definitively at this point is that the vitamin D metabolites will competitively displace cortisol and T3 from these nuclear receptors. Waterhouse et al.[12] noted patients with hormonal abnormalities concomitant with dysregulated vitamin D metabolites.

Brahmachary et al.[71] have just completed an in silico analysis of the manner with which the type 1 nuclear receptors cooperate to express families of antimicrobial peptides. Any microbe that manages to block transcription by the VDR, will of course modify expression of the 16 families expressed by that receptor, but the secondary effects of elevated 1,25-D upon the GR, AR and ThRa, will alter expression of a "bonus" 67 transcriptional promoters. With reference to our model (Fig. 1), if a bacterial ligand could disable VDR transcription, then the concentration of 1,25-D will rise, since the VDR can no longer exert feedback via CYP24A1 or CYP27B1. Ultimately, these elevated levels of 1,25-D would displace the endogenous ligands from GR, AR and ThRa. The increasing concentration of 1,25-D would also depress generation of 25-D, leading to a lower concentration of 25-D in serum, an effect that might mistakenly be reported as "vitamin D deficiency".

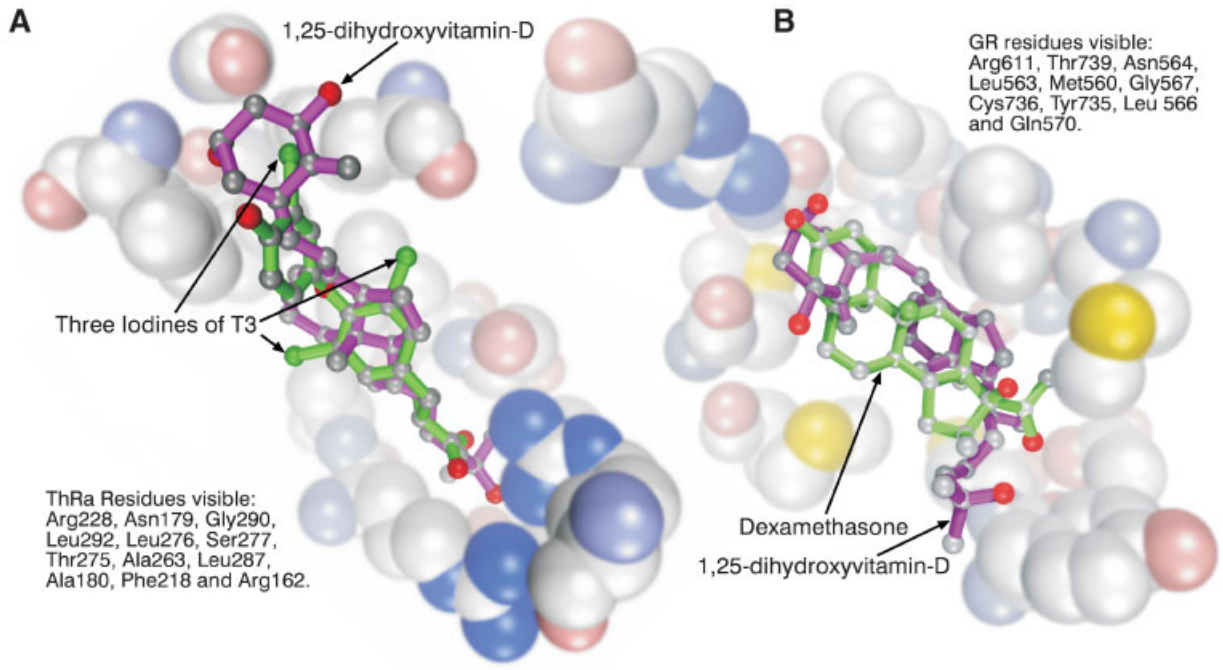

Figure 3. A: The Thyroid alpha receptor and its activator, T3 [PDB:2H77], with 1,25-D superimposed in the LBP, docked with est. $K_d = 8.41$. **B:** The glucocorticoid receptor and its activator, dexamethasone [PDB:1P93], with 1,25-D superimposed in the LBP, docked with est. $K_d = 8.12$ (David Goodsell's DG color scheme is used to depict the receptor residues).

We have recently identified a bacterial product which is, in fact, a high-affinity antagonist of VDR transcription.[72,73] It is the sulfonolipid Capnine, part of the motive mechanism for some gliding bacteria. Similar gliding species were recently isolated from biofilm deposits on surgically removed human prosthetic hip joints.[74] It is too soon to state that capnine is indeed active as a VDR antagonist in persistent infection, but there is now proof-of-concept that bacterial genomes are capable of producing at least one ligand that acts as a strong VDR antagonist.

Cartilage oligomeric matrix protein and arthritis

Cartilage oligomeric matrix protein (COMP) is a pentamer that is found in the synovium. It is implicated in impaired cartilage

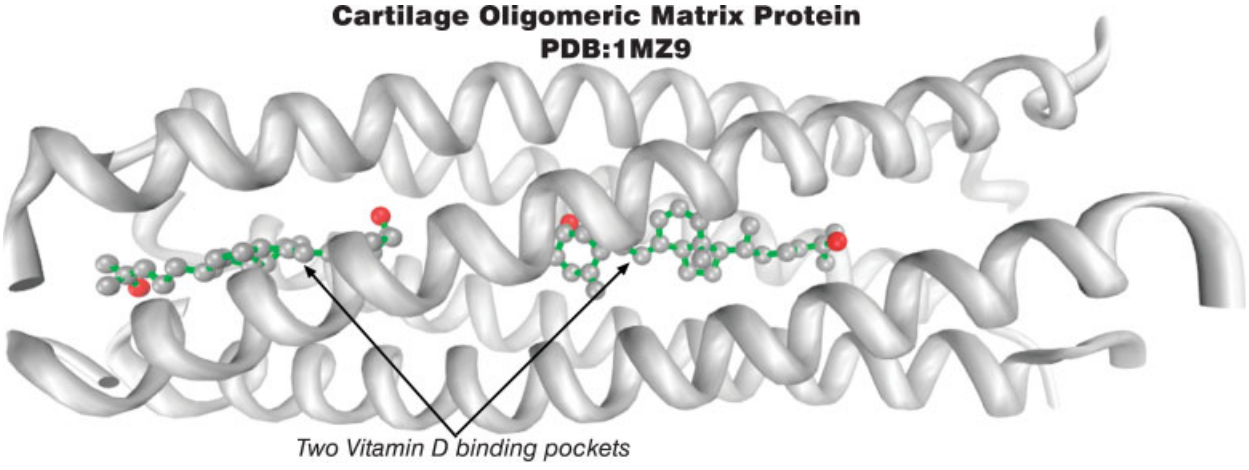

Figure 4. The structure of the pentamer 'cartilage oligomeric matrix protein' from [PDB:1MZ9]. The two binding pockets are both occupied by molecules of 25-hydroxyvitamin-D.

growth and maintenance.[75] Veterinary medicine has found that the level of urine COMP is a good indicator of the level of arthritis in a racehorse.[76] Momohara et al. confirmed that the serum level of COMP was similarly a good indicator of cartilage destruction in human rheumatoid arthritis.[77]

Five years ago, in 2002, Ozbek et al. published[78] the crystal structure of human COMP in complex with two molecules of 25-D [PDB:1MZ9], as shown in Fig. 4. Our modeling has subsequently verified the presence of 1MZ9's two distinct binding pockets, with equal affinities for 25-D and 1,25-D, and with residue contacts that would tend to stabilize the pentamer's structure.

Even though arthritis is projected to cripple 67 million Americans by 2030,[79] there has been little subsequent study of this interaction between COMP and vitamin D in humans, and it is not known whether vitamin D is harmful or beneficial to the arthritic synovium. In particular, the FDA safety review did not even contemplate the possibility that vitamin D supplementation might be a factor in arthritic cartilage degradation.

Conclusions

This BioEssay has examined a number of ways in which, while the widespread use of vitamin D as a food supplement may be providing short-term benefits to a subset of the population, epidemic expansion of obesity and chronic disease are quite possibly the legacies to be bestowed upon future generations.

The concept that ''The Sunshine Vitamin'' really is just a vitamin, with the consequent implication of a linear 'vitamin in, benefit out' model, is clearly no longer tenable. At any level of molecular analysis, the vitamin D metabolites are part of the delicate homeostasis that allows our bodies to express genes, and to express them when the need arises.

The conviction that one can, with impunity, continue to add higher and higher concentrations of this secosteroid to the food chain is still firmly held by many of our clinical colleagues. This is a recipe which could easily lead to a public health disaster. Yet the 'vitamin' model has a seductive simplicity, a simplicity that offers a welcome escape from the complex world of modern molecular medicine.

Biologists have a duty to share their new-found genomic knowledge with their clinical colleagues. They need to help them understand the steroidal nature of vitamin D. To help them understand that this substance is intimately involved in the transcription of hundreds, probably thousands, of genes that determine the course of immune disease and cancers. In particular, we must ensure that every researcher understands the importance of measuring the concentration of the actual transcriptional activator, 1,25-dihydroxyvitamin-D.

Biologists need to raise their voices and help Federal regulators understand what is being discovered about the wonderful genetic tapestry that has historically allowed *Homo sapiens* to thrive and to control its environment.

While it is true that molecular biology can still only precisely describe a very small fraction of the human experience, what it can describe it does so in exquisite detail. It is critical that Medicine revisits the role that has been assigned to ''The Sunshine Vitamin'', properly recognizing its function as a secosteroid, a transcriptional activator, key to the proper operation of the innate immune system.

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