

TRANSCRIPT OF 18 JUN 2006 PRESENTATION

## Dr. Joyce Waterhouse Vitamins D in Chronic Disease

### Title Slide

Hi. I hope your coffee break has you all alert enough that I won't put you to sleep. Actually, I think there is some exciting new information here, and I hope it will interest you.

The title here refers to vitamins D, no that isn't a typo, we use vitamins D since there are many forms of vitamin D. Actually, it is not really a true vitamin, since our bodies produce it, with the aid of the sun. It is actually better, in our view, to think in terms of a steroid hormone and it's precursors.

You might ask, why is vitamin D important? As you probably know, the Marshall Protocol or MP that we have been discussing at this conference includes a reduction of vitamin D to help improve immune function and bacterial killing. We need to discuss this in detail because there are widespread misunderstandings about vitamin D among people who are not aware of the true importance of some recent discoveries.

There are some researchers that seem to be saying we all need to take more vitamin D and we think this is wrong and this presentation will explain why.

### Slide 2

#### Book Chapter

I'm going to talk mostly today about the material covered in a book chapter, “High levels of active 1,25D despite low levels of the 25D precursor — implications of dysregulated vitamin D for diagnosis and treatment of chronic disease.” It should be coming out in a month or so.

I will present research to back our view that Vitamin D dysregulation due to Th1 inflammation is widespread and is a result of macrophages becoming infected with cell wall deficient or CWD forms of bacteria. I will present evidence and arguments to counter many of the studies behind the push for increasing vitamin D in certain diseases and show the potential harm from excess vitamin D. I will also discuss the controversy over different views of Vitamin D and some very new molecular modeling results.

I won't have time for questions at the end of the talk, but you can ask me questions afterwards individually or email me at [jcw@autoimmunityresearch.org](mailto:jcw@autoimmunityresearch.org). By the way, you should all have handouts of the slides to make it a little easier.

#### Book Chapter:

“High levels of active 1,25-dihydroxyvitamin D despite low levels of the 25-hydroxyvitamin D precursor - implications of dysregulated vitamin D for diagnosis and treatment of chronic disease”

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In: Stolzt, Veronica D. (ed). Vitamin D: New Research. New York, Nova Science Publishers. 2006

#### Aims:

##### 1. To present research that:

- supports our view that Vitamin D dysregulation due to Th1 inflammation is widespread
- Th1 inflammation is due to bacteria-infected macrophages
- argues against the push for increasing vitamin D in certain diseases
- shows the potential harm from excess vitamin D

##### 2. Discuss controversy over different views of vitamin D and some new molecular modeling research

## Th1 Disease: Infected Macrophages lead to Excessive Inflammation and Increased 1,25D Production

- Th1 cells produce the cytokine Interferon Gamma and promote cell mediated inflammation
- Th1 cells stimulate macrophage activity and these activated macrophages also produce Interferon Gamma
- Macrophages surround and internalize bacteria through phagocytosis to destroy them
- In Th1 disease, the macrophages are unable to destroy the CWD bacteria and the bacteria thrive inside the very macrophages that are supposed to kill them leading to chronic disease
- 1,25D produced at increased levels by the activated macrophages, can be more easily detected in the blood than Interferon Gamma
- 1,25D can be used as a marker for Th1 activation in chronic disease

### Slide 3

#### Th1 Disease: Infected Macrophages lead to Excessive Inflammation and Increased 1,25D Production

Now, I'll briefly go into some background information in simplified form. In our view, the key to Th1 disease is bacteria-infected macrophages leading to excessive inflammation and increased 1,25D production. Th1 cells, a type of T helper lymphocyte, or immune cell, produce the cytokine Interferon Gamma and promote cell mediated inflammation.

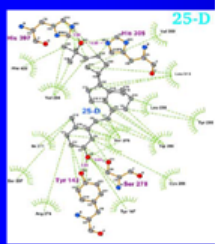
Th1 cells can stimulate macrophage activity and these activated macrophages also produce Interferon Gamma. The macrophages are supposed to surround and internalize the bacteria in order to destroy them, a process called phagocytosis.

Our understanding of Th1 disease is that the macrophages are unable to destroy the CWD bacteria during phagocytosis and instead the bacteria actually thrive inside the very macrophages that are supposed to kill them, leading to chronic disease.

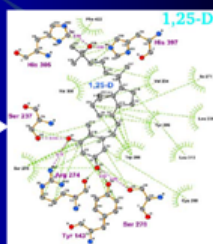
Although elevated Interferon Gamma can indicate Th1 disease, it stays mainly in the tissue, and only circulates in the blood to a limited degree. However, the 1,25D produced by the activated macrophages, can be more easily detected in the blood than Interferon Gamma. Thus, 1,25D, can serve as a useful marker or indicator of Th1 disease and that is why we measure it, along with 25D.

## REGULATED VITAMIN D METABOLISM

**FORMS OF VITAMIN D: 25D**  
precursor derived from sun & food



**1,25D**  
active hormone tightly regulated by kidneys



- Doctors usually only measure 25D because of assumed 1,25D regulation by kidneys
- This logic makes sense if 1,25D levels are always regulated by kidneys

### Slide 4

#### Regulated Vitamin D Metabolism

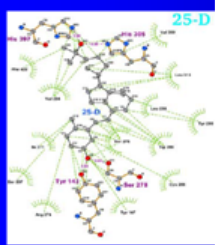
Now, on to vitamin D regulation.

This first part of the slide shows a simplified view of the vitamin D situation in a healthy person. The precursor form of vitamin D, 25D, is the form that most healthy people derive from the diet, supplements and sun exposure. By the way, this shows the situation without the inflammatory cytokines that cause the 25D from the sun to convert directly to 1,25D in the skin.

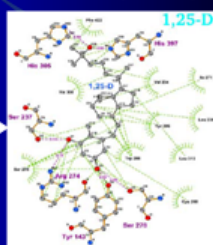
The main point to emphasize here, is that in healthy people, the kidneys tightly regulate the conversion of 25D to keep the 1,25D active steroid hormone in a fairly narrow range. There are parts of other molecules shown here — the vitamin D receptor around here, but this is the vitamin D part.

## DYSREGULATED VITAMIN D METABOLISM

**FORMS OF VITAMIN D: 25D**  
precursor derived from sun & food



**1,25D**  
active hormone NOT controlled by kidneys



- Dysregulation is caused by activated macrophages, which can convert 25D to 1,25D, without the kidneys



But the only difference is where you add the hydroxyl group, which is this little red oxygen. It's actually an OH and this is added and it makes all the difference. And here we have a nice picture of a kidney. O.K. So we have the molecule is transformed by adding the hydroxyl group so it can now activate the vitamin D receptor.

Most doctors rely on the 25D precursor to decide how much vitamin D is needed, without paying enough attention to the active hormonal form. This only makes sense if one assumes 1,25D levels are regulated by the kidneys with the aid of parathyroid hormone.

### Slide 5

#### Dysregulated Vitamin D Metabolism

O.K., Now for the vitamin D dysregulation. Dysregulation is caused by activated macrophages, which can convert 25D to

1,25D without the kidneys, at a high rate — there you can see the macrophage.

**So, the macrophages are the source of vitamin D dysregulation in Th1 diseases, aka vitamin D hypersensitivity.**

This extrarenal, meaning outside of the kidneys, production is well-known in sarcoidosis and other diseases where aggregations of macrophages form granulomas. And as I said before, in Th1 disease, the 25D from the sun goes immediately to 1,25D in the skin.

### **Vitamin D Dysregulation in Th1 Disease : Role of Enzymes and Inflammatory Cytokines (e.g., Interferon Gamma)**

- Activated macrophages contain the enzyme 1 alpha hydroxylase that causes the conversion from 25D to 1,25D
- Abundant Interferon Gamma in inflamed tissue suppresses the normal feedback inhibition of 1-hydroxylase
- Interferon Gamma also inhibits the enzyme 24-hydroxylase, which is involved in 1,25D inactivation
- Interferon Gamma effect leads to inability of kidney to compensate for the unregulated 1,25 D production by macrophages at least in some circumstances
- Inflammatory cytokines also cause increased conversion of 7-dehydrocholesterol in the skin into 1,25D
- Results of Elevated 1,25D in Vitamin D Dysregulation: When severely elevated, hypercalcemia may result. But even when moderately elevated, with normal calcium levels, bone loss and a wide range of negative consequences may occur.

#### **Slide 6**

### **Vitamin D Dysregulation in Th1 Disease — Role of Enzymes and Inflammatory Cytokines (e.g., Interferon Gamma)**

Now, you might ask, how does Vitamin D regulation break down? Normally, there are feedback controls that would compensate for overproduction.

Well, it turns out we know the enzymes and cytokines involved in this failure to compensate for the macrophage's production of 1,25D.

Activated macrophages contain the enzyme 1 alpha hydroxylase that causes the conversion of 25D to 1,25D. Abundant Interferon Gamma, an inflammatory cytokine, can suppress the normal feedback inhibition of 1 alpha hydroxylase that would otherwise help regulate 1,25D production. The Interferon Gamma in the region of inflamed tissue inhibits the enzyme 24-hydroxylase, which is involved in 1,25D inactivation.

This effect of Interferon Gamma seems to extend to the kidneys, at least in many granulomatous diseases, like sarcoidosis, so that the kidney can not effectively compensate for the unregulated 1,25D production by macrophages.

Also, inflammatory cytokines cause increased conversion of 7-dehydrocholesterol into 1,25D in the skin, thus providing additional 1,25D that contributes to dysregulation.

And what is the result? Well when 1,25D is severely elevated, high blood calcium levels can occur, which may be quite dangerous. But even when 1,25D is moderately elevated, with normal serum calcium levels, bone loss and a wide range of negative consequences may occur.

### **Vitamin D Measurement Issues Relating to Detecting Th1 Disease**

- Reference ranges often inaccurate for 25D or 1,25D due to undiagnosed Th1 disease in control populations
- We prefer Merck Manual's upper limit for 1,25D of 45 pg/ml as more accurate
- Blood should be frozen for transport of samples for 1,25D, since it degrades easily (largest lab in U.S., Quest Laboratories, does this)
- Low 25D, high 1,25D and/or a higher ratio of 1,25D to 25D (D ratio: normal=1.3) can be used as an indicator of the level of Th1 inflammation
- FDA recommends measuring both 25D and 1,25D in osteoporosis treatment studies
- Due to their importance, we think 25D and 1,25D should be widely measured

#### **Slide 7**

### **Vitamin D Measurement Issues Relating to Detecting Th1 Disease**

Now, we'll briefly cover some issues relating to measurement. We think many labs reference ranges are inaccurate because they include undiagnosed Th1 disease patients in their control populations.

For this reason, we prefer Merck Manual's upper limit of 45 pg/ml for 1,25D, to the higher upper limits some labs use.

**Blood serum for 1,25D tests should be frozen since 1,25D degrades easily. The largest U.S. lab does it this way.**

In D dysregulation, the 25D may become depleted through conversion to the active hormone. So 25D may be low while

1,25D may be high, thus one can see the need to measure both.

Low 25D, high 1,25D and/or a higher ratio of 1,25D to 25D, known as the D ratio, can be used as an indicator of the level of Th1 inflammation. The D-ratio from a large control population that we use as "normal" is 1.3.

The FDA recommends measuring both types of vitamin D when evaluating osteoporosis treatments. We think both tests should also be much more widely done in chronic disease.

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#### Inflammatory Bowel Disease: Crohn's Disease and Ulcerative Colitis...

Now, I am going to review literature that supports our view that D dysregulation due to activated macrophages is actually widespread in inflammatory diseases.

In most of these diseases there is also some evidence of bacterial involvement and/or responsiveness to antibiotics. The first example is Inflammatory Bowel Diseases.

Abreu et al found 1,25D levels were elevated above 60 pg/ml in 40% of Crohn's Disease and 7% of Ulcerative Colitis patients. The elevated 1,25D was related to reduced bone mineral density that was independent of glucocorticoid use. They also found elevated levels of 1 alpha hydroxylase from colonic biopsies of Crohn's patients, indicating extrarenal synthesis, as in sarcoidosis.

The percentages are much higher if the Merck Manual cutoff for elevated 1,25D, of 45 pg/ml is used — 68% for Crohn's and 45% for Ulcerative colitis.

For the role of bacteria, you can see Colombel et al and Hulten et al.

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#### Rheumatoid Arthritis (RA): Key Experiments Confirming Extrarenal Synthesis of Vitamin D...

Now, I will discuss a key experiment supporting extrarenal synthesis of 1,25D in rheumatoid arthritis.

Mawer et al challenged 19 RA patients with a large dose of the precursor, 25D, and found that patients generated peak serum levels of 1,25D significantly higher than controls.

The 1,25D levels were particularly elevated in the joint fluid in patients. This provides strong evidence for extrarenal synthesis of 1,25D in patients with RA.

Another important point is that the median serum 1,25D at baseline was not elevated in the RA patients — only 24 pg/ml. Thus the extrarenal synthesis of 1,25D was not obvious from the routine blood test.

So, although we do find Vitamin D tests helpful in diagnosis, this study shows that they are not always enough. One may need to look deeper to detect extrarenal synthesis in Th1 disease.

It is also the reason why a therapeutic probe with the Marshall Protocol may be needed, when the clinical picture suggests Th1

#### Inflammatory Bowel Disease: Crohn's Disease and Ulcerative Colitis [Abreu et al. 2004 Gut 53]

- 1,25D levels elevated above 60 pg/ml in 40% of Crohn's Disease and 7% of Ulcerative Colitis
- Elevated 1,25 D was related to a negative effect on bone mineral density independent of glucocorticoid use
- Elevated levels of 1 alpha hydroxylase (CYP27B1), the enzyme that converts 25D to 1,25D, from colonic biopsies of Crohn's patients (same enzyme as in macrophages in sarcoidosis)
- 68% of Crohn's Disease patients and 45% of ulcerative colitis patients had 1,25D levels above 45 pg/ml (our estimate from Abreu et al, Fig. 2).
- Role of bacteria and antibiotics: Colombel et al. 2001 Gut 49(6); Hulten et al. 2000 Dig Dis Sci. 45(3)

#### Rheumatoid Arthritis (RA): Key Experiments for Confirming Extrarenal Synthesis of Vitamin D [Mawer et al. 1991 J Bone Miner Res. 6(7)]

- Mawer et al challenged patients with large oral dose of the precursor form, 25D, and found that RA patients generated peak serum levels of 1,25D significantly higher than controls
- 1,25D levels were particularly elevated in the synovial (joint) fluid providing strong evidence for extrarenal synthesis of 1,25D in patients with RA
- Shows excess conversion of 25D to 1,25D in areas of inflammation in RA
- Median serum 1,25D before challenge not elevated — only 24 pg/ml, thus extrarenal synthesis not obvious
- May need similar experiments to uncover extrarenal synthesis in other diseases
- Shows why a therapeutic probe with the Marshall Protocol may be needed to clarify diagnosis when vitamin D results unclear

disease, but the vitamin D test results are unclear. A therapeutic trial is when one uses the MP, and assess symptom changes — like bacterial die off reactions — to determine if the protocol is appropriate.

### Additional Studies Supporting Vitamin D Dysregulation and Role of Bacteria in RA

- In vitro studies of macrophages from synovial fluid in RA also revealed synthesis of 1,25D [Hayes *et al.* 1992 *Ann Rheum Dis.* 51(2)] and elevated D-ratios [Inaba *et al.* 1997 *Life Sci* 61(10)]
- Inaba *et al.* found elevated 1,25D to be related to elevation of the inflammatory cytokines IL-1 and IL-2, which are related to disease activity. IL-1 has also been implicated in increased bone loss.
- Sambrook *et al.* found little, if any bone loss near the wrist joint in patients with RA with low 1,25D [Sambrook *et al.* 1990 *Arthritis Rheum.* 33(5)]
- Role of bacteria and antibiotics: [Tilley *et al.* 1995 *Ann Intern Med.* 122(2); O'Dell *et al.* 1997 *Arthritis Rheum.* 40(5); Clark *et al.* 1988 *Ann of Allergy.* 60(5)]

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#### Additional Studies Supporting Vitamin D Dysregulation and Role of Bacteria in RA

Some additional independent studies in RA are also relevant here.

In vitro studies of macrophages from synovial fluid or joint fluid in RA also revealed synthesis of 1,25D and elevated D-ratios. Inaba found elevated 1,25D to be related to elevation of IL-1 and IL-2. These 2 inflammatory cytokines are correlated with disease activity. IL-1 has also been implicated in increased bone loss.

Sambrook *et al.* found little, if any bone loss near the wrist joint in patients with RA with the lowest 1,25D. Those with higher 1,25D had significant bone loss near their wrist joints.

In our view, these various findings argue against vitamin D supplementation to prevent inflammatory damage or bone loss in RA. The role of bacteria and antibiotics shown in several studies further support our view that RA is similar to sarcoidosis in its underlying bacterial cause.

### Systemic Lupus Erythematosus (SLE): Evidence for it Also Being a Th1 Disease

- In systemic lupus erythematosus (SLE), studies consistent with Vitamin D dysregulation:
  - D-ratio of 2.1, 1,25D: 27 pg/ml, 25D: 13 ng/ml [Muller *et al.* 1995 *Rheumatol.* 14(4)]
  - D-ratio of 2.2 [for patients not taking hydroxychloroquine, Huisman *et al.* 2001 *J Rheumatol.* 28(11)]
- Increased lupus mortality associated with UVB radiation in a correlational study [Grant *et al.* 2004 *Lupus* 13]
- Lupus patients have flares of symptoms when exposed to UVB, and some report sensitivity to fluorescent light or the UVA light
- Bacteria reference: [Ginsburg *et al.* 1992 *Arthritis Rheum.* 35(4)]

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#### Systemic Lupus Erythematosus (SLE): Evidence for it Also Being a Th1 Disease

O.K., as for lupus, two studies measuring vitamin D levels are consistent with Vitamin D dysregulation, giving D-ratios of 2.1 and 2.2, well above the 1.3 average for the healthy.

Interestingly, increased lupus mortality has been found to be associated with UVB solar radiation in a correlational study and we believe this effect probably occurs through increasing 1,25D.

It is known that lupus patients have flares of symptoms in response to UVB light, and sometimes even to fluorescent or UVA light, something we observe in sarcoidosis. There was at least one study that seems to support the role of bacteria. From all of this, we conclude lupus (SLE) is a Th1 disease.

### Fibromyalgia, Generalized Chronic Pain, Chronic Fatigue Syndrome

- Only one study in fibromyalgia measured both vitamin D metabolites and the D ratio was slightly elevated: 1,25D: 34.6 pg/ml, 25D: 20.6 ng/ml, D-ratio: 1.7 [Huisman *et al.*, *ibid*]
- Several other studies showed a tendency to low levels of 25D and this is consistent with our hypothesis of extrarenal conversion by activated macrophages depleting 25D
- Linked to bacteria: [Vodjani *et al.* 1999 *J of Chronic Fatigue Syndrome* 5(3/4); Nicholson *et al.* 2003 *J Chronic Fatigue Syndrome* 11(2); Wright 2005 *Presentation, ARF's Chicago Conference*, March 12-13, 2005]

### Sjogren's Syndrome

- Mean 1,25D: 33 pg/ml, mean 25D: 12.4 ng/ml D-ratio of 2.7 [Muller *et al.* 1990 *Ann Rheum Dis.* 49(9)]

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#### Fibromyalgia, Generalized Chronic Pain, Chronic Fatigue Syndrome

Only one study in fibromyalgia measured both vitamin D forms and the D ratio was elevated at 1.7.

Several other studies showed a tendency to low levels of 25D in these several chronic pain and fatigue states and this is consistent with our hypothesis of extrarenal conversion by activated macrophages depleting 25D.

Several researchers have linked chronic fatigue syndrome and fibromyalgia with bacteria, including *Borrelia* and *Mycoplasma*. Now, in Sjogren's Syndrome, the D-ratio was 2.7 and the study authors mentioned this indicated a disturbed vitamin D metabolism.

## Multiple Sclerosis (MS) and Vitamin D -- Explaining Geographical Patterns

A higher rate of MS is found in higher latitudes where there is less sun exposure -- some claim vitamin D link

Alternative Explanation -- Pathogen Distribution:

- A study related the geographical and seasonal pattern of MS to that of the tick that carries *Borrelia burgdorferi* [Fritzsche 2005 *Med Hypoth.* 64(3)]
- *Chlamydiae pneumoniae* are known to be more commonly acquired in the winter and this may relate to geographical pattern due to the greater amount of time spent indoors at higher latitudes. *Chlamydiae pneumoniae* has been linked to progressive MS [Munger et al. 2003 *Epidemiology.* 14(2)]
- Our view: Multiple infections are likely to be the cause because the immune dysregulation initiated by the first pathogen would facilitate infection with other species.
- Other independent evidence for role of bacteria — [Brorson et al. 2001 *Infection.* 29(6); for review, see Mattman 2000 *Cell Wall Deficient Forms: Stealth Pathogens* and ARF Chicago Conference]

### Slide 13

#### Multiple Sclerosis (MS) and Vitamin D: Explaining Geographical Patterns

O.K. Multiple Sclerosis. We have shown some vitamin D patterns and how they relate to our view that bacterial infection causes vitamin D dysregulation. In Multiple sclerosis or MS, vitamin D data is lacking, but there are other types of studies that have been used to try to link MS with a lack of vitamin D. And I will discuss some of the problems we see with these studies.

The initial reason for the interest in vitamin D in MS is that a higher rate of disease had been observed in higher latitudes — and it has been claimed that this is due to less sun exposure producing less vitamin D.

We will focus on one of the alternative explanations — that the pattern is caused by the geographic distribution of bacterial pathogens not solar radiation. An analysis by Fritzsche related the geographical and seasonal pattern of MS to that of the tick that carries the Lyme spirochete *Borrelia burgdorferi*.

Also, *Chlamydiae pneumoniae* is known to be more commonly acquired in the winter and this may relate to geographical patterns due to the greater amount of time spent indoors at higher latitudes. *Chlamydiae pneumoniae* has been linked to progressive MS by Munger et al.

Our view is that multiple bacterial pathogens are probably the cause of MS and other Th1 diseases. This seems likely because immune dysregulation initiated by the first pathogen would tend to promote infection with other species.

There is also abundant direct evidence for the role of bacteria in MS, for example, see Brorson et al and Mattman. Mattman has some reviews of numerous studies.

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#### Multiple Sclerosis — Studies on Vitamin D Consumption and Serum 25D Patterns

Some studies have been done linking low vitamin D consumption and serum 25D patterns to a greater rate of MS. One reason we think low 25D might be observed to precede MS is increased conversion of 25D to 1,25D by macrophages thus depleting 25D in early stages of illness prior to being diagnosed — the low 25D is thus not a cause but an early effect of MS.

There are other factors that may bias results and you can see the book chapter for some examples. Since we know that correlation does not imply causation, these types of observational studies, by their very nature, can not prove a benefit of Vitamin D in preventing MS.

But, if future randomized controlled trials were to show a preventative effect of vitamin D for MS, this might be due to enhancement of immune response to the initial phase of bacterial infection through correction of very low levels of vitamin D in certain individuals. There is some limited evidence for this in Tuberculosis. But even if this preventative effect were proven, this would not mean vitamin D supplementation would be beneficial in patients already ill with MS, since, in our view, increased 1,25D synthesis would be occurring once the illness is established.

## Multiple Sclerosis -- Studies on Vitamin D Consumption and Serum 25D Patterns

- Another type of study relates MS incidence rates to lower vitamin D consumption or lower 25D blood levels
- 25D might be lowered by increased conversion to 1,25D due to activated macrophages
- Other factors can bias observational studies like these [see book chapter]
- Since we know that correlation does not imply causation, these types of studies can not prove a benefit of Vitamin D in preventing MS
- If future randomized controlled trials were to prove a preventative effect of vitamin D for MS, this might be due to enhancement of immune response to the initial phase of bacterial infection through correction of very low levels of vitamin D in some individuals, as has been suggested in tuberculosis
- But this would not mean vitamin D supplementation would be beneficial for patients already ill with MS due to increased extrarenal synthesis of 1,25D

### Multiple Sclerosis Treatment-- Short Term Experiments With 1,25D Treatment in Mice and Humans

- Studies show a benefit from elevated 1,25D in prevention and treatment of experimental allergic encephalomyelitis (EAE) in mice

#### Pitfalls in EAE studies and similar studies in other animal models

- Differences in physiology between humans and mice and the short time span of the experiments
- Likely difference in disease causation in EAE, where myelin basic protein and adjuvant are injected into the mice to try to mimic MS
- Even if the EAE model did apply to human MS, giving large amounts of 1,25D might be able to halt the disease process in EAE due to immunosuppression, but the short time span of the experiment would fail to detect a long-term worsening due to bacterial increase
- In studies in humans with MS, the short term nature of the studies are the likely reason for seasonal reductions in lesions and some benefit being reported from vitamin D supplementation.

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### Multiple Sclerosis Treatment — Short Term Experiments with 1,25D Treatment in Mice and Humans

Another type of study that has been done in MS involves short term experiments in which some form of vitamin D is given.

Several studies have shown a benefit from elevated 1,25D in prevention and treatment of experimental allergic encephalomyelitis, EAE, in mice. This is an animal model used to try to approximate MS.

Some of the pitfalls of studies of this type include differences in the physiology of humans and mice and the short time span of the experiment. There is also likely to be different disease causation involved in EAE. Even if a given animal model, such as this one, did apply to human MS, giving large amounts of 1,25D might be able to halt the disease process in EAE only due to immunosuppression. But the short time span of the experiment would fail to detect a long-term worsening that might occur due to bacterial increase occurring as a result of the immunosuppression.

Likewise, in studies in humans with MS, we view the short term nature of the few studies that have been done as the likely reason that benefit has sometimes been linked to vitamin D.

### New Findings on Role of 25D in Displacing 1,25D and Blocking Innate Immunity

- Molecular modeling shows that 1-alpha-hydroxy position is key to activation of the Vitamin D Receptor [Marshall *et al.* 2006, Karolinska Conference, Sweden]
- 25D can bind to the vitamin D receptor (VDR), but since it lacks the 1-alpha-hydroxy found in 1,25D, it will bind to the VDR but fail to activate it
- Affinity constants show that 25D above approximately 20 ng/ml can displace 1,25D, blocking VDR activation and thus blocking innate immunity
- This is supported by our finding that bacterial killing increases for patients on the Marshall Protocol when 25D goes below 20 to 25 ng/ml.

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### New Findings on Role of 25D in Displacing 1,25D and Blocking Innate Immunity

Now, I will discuss some new research on the mechanisms by which we think too much of either form of Vitamin D may suppress bacterial killing and harm long term health in Th1 disease.

Recent molecular modeling research indicates that high 25D levels block innate immunity. Sophisticated computer modeling of the molecules shows that the 1-alpha-hydroxy position is key to activation of the Vitamin D Receptor or VDR. 25D can bind to the VDR, but since it lacks the 1-alpha-hydroxy found in 1,25D, it will bind to the VDR but fail to activate it.

The affinity constants show that 25D above approximately 20 ng/ml can thus displace 1,25D, blocking VDR (Vitamin D Receptor) activation and thus blocking innate immunity. This is supported by our finding that bacterial killing increases for patients on the Marshall Protocol when 25D goes below 20 to 25 ng/ml.

### Effects of Elevated 1,25D: Decreased Bacterial Killing and Increased Hormonal Disruption

- 1,25D aids the immune response by stimulating the vitamin D receptor (VDR), but too much 1,25D suppresses the immune system, inhibits bacterial killing and causes hormonal disruption.
- Newer Research: New evidence indicates these negative effects of high 1,25D do not occur through its binding to the VDR, but through its excessive binding to thyroid, glucocorticoid and other receptors [Marshall 2006. FDA CDER Visiting Professor presentation, FDA Biosciences Library, Accession QH447.M27]
- If a bacterial cause underlies these Th1 diseases, then immunosuppression from high 1,25D is harmful in the long term despite short term anti-inflammatory effects that may sometimes give the appearance of benefit

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### Effects of Elevated 1,25D: Decreased Bacterial Killing and Increased Hormonal Disruption

As for 1,25D, although it aids the immune response by stimulating the vitamin D receptor, too much 1,25D suppresses the immune system, inhibits bacterial killing and causes hormonal disruption.

New evidence indicates these negative effects of high 1,25D do not occur through its binding to the VDR, but through its excessive binding to thyroid, glucocorticoid and other receptors. Dr. Marshall has recently presented molecular modeling evidence for this at an FDA Visiting Professor presentation.

If, as we believe, a bacterial cause underlies these Th1 diseases, then immunosuppression from high 1,25D is harmful in the long

term, despite short term anti inflammatory effects that may sometimes give the appearance of benefit.

### Slide 18

#### Some Examples of 1,25D, 25D and D-Ratio in a Variety of Illnesses

O.K. These are not real portraits of the people — so, you don't need to go around looking for the silhouettes to match it.

Most of the data collected on hundreds of patients — prior to starting the MP — is fitting the pattern of vitamin D dysregulation that we described above, indicating increased production of 1,25D by macrophages. Dr. Marshall presented slides of some of this data on sarcoidosis, RA, Lyme, CFS and fibromyalgia at the Chicago Conference and elsewhere.

Today, I will just talk briefly about 4 of the 12 patients we discuss in the book chapter to illustrate the pattern of vitamin D values in a variety of illnesses.

In some cases, the 25D is somewhat elevated. Usually this is due to vitamin D in food or supplements, and it tends to bias the D-ratio downward. Patient 9 has MS, diagnosed 9 years ago. One can see her 1,25D of 53 is above the Merck upper limit and her 25D is rather high at 35 ng/ml and her ratio is a little elevated at 1.5. She has a history of worsening on daily doses of 2000-4000 IU vitamin D. And one symptom that had worsened while on the vitamin D improved after stopping it prior to the Marshall Protocol.

Patient 10 has amyotrophic lateral sclerosis or ALS. He has an elevated 1,25D of 58.9, a 25D of 36 ng/ml and a D ratio of 1.6. Both patients 9 & 10 have had bacterial die-off reactions, also called Jarisch Herxheimer reactions, as expected on the Marshall Protocol, but it is too soon to know if they have improved.

Patient 11 has rheumatoid arthritis and had an initial 1,25D of 65, 25D of 32, and a D ratio of 2. During 2 years on vitamin D supplementation prior to the Marshall Protocol, her condition worsened. Also, she failed to improve during several years on an antibiotic-only protocol, but improved significantly on the Marshall Protocol in less than 18 months.

Patient 12 has been disabled for 20 years by chronic fatigue syndrome, fibromyalgia and Lyme disease. With her 1,25D at 64 and her 25D at only 11, giving a ratio of 5.8, this patient is a good example of 25D being depleted by conversion to 1,25D. Sun exposure elevates her heart rate and worsens a number of other symptoms. She has improved considerably on the MP.

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#### Resolving Some Misunderstandings on Vitamin D: Secondary Hyperparathyroidism and Osteoporosis

O.K., as to the controversy on vitamin D. Why do some advocate taking so much vitamin D and why do we think they are wrong?

To begin with, many vitamin D advocates focus on the issue of secondary hyperparathyroidism, which occurs when serum calcium is low, causing parathyroid hormone to increase, which can lead to bone resorption and osteoporosis. The hyperparathyroidism leads to a greater production of 1,25D by the kidneys, to try to compensate for the low calcium.

**Some Examples of Levels of 1,25D, 25D and D-Ratio in a Variety of Illnesses**

PATIENT 9	PATIENT 10	PATIENT 11	PATIENT 12
39 yrs FEMALE* diagnosed Multiple Sclerosis 9 years ago	43 yrs MALE diagnosed Amyotrophic Lateral Sclerosis 2 years ago	54 YRS FEMALE* diagnosed Rheumatoid Arthritis 9 years ago	46 yrs FEMALE disabled 20 yrs CFS, FMS, Lyme disease and IBS
1,25D: 53 pg/ml 25D: 35 ng/ml D-ratio: 1.5	1,25D: 58.9 pg/ml 25D: 36ng/ml D-ratio: 1.6	1,25D: 65 pg/ml 25D: 32 ng/ml D-ratio: 2.0	1,25D: 64 pg/ml 25D: 11 ng/ml D-ratio: 5.8

\*History of worsening associated with high levels of Vitamin D supplementation.

#### Resolving Some Misunderstandings on Vitamin D: Secondary Hyperparathyroidism and Osteoporosis

- **Secondary Hyperparathyroidism:** when calcium is low, parathyroid hormone is increased, which can lead to bone resorption and osteoporosis. The hyperparathyroidism leads to a greater production of 1,25D by the kidneys, to try to compensate for the low calcium.
- This is why some think it wrong to look at 1,25D levels when considering whether a patient is deficient in vitamin D. The claim is that the 1,25D is meaningless since it may be compensating for a low 25D.
- However, in sarcoidosis, RA and inflammatory bowel disease, hyperparathyroidism was specifically ruled out as an explanation for the 25D and 1,25D levels, rather they were clearly related to inflammation
- Based on MP treatment responses, vitamin D data, molecular modeling and other evidence, we think inflammation also explains the 25D and 1,25D patterns in the other Th1 diseases, as well.

This is why some researchers think it wrong to look at 1,25D levels when considering whether a patient is deficient in vitamin D. Their claim is that the 1,25D is meaningless, since it may be compensating for a low 25D.

Of course, we agree that secondary hyperparathyroidism certainly can lead to bone loss. However, in several Th1 diseases we have discussed, sarcoidosis, RA and Inflammatory Bowel Disease, any hyperparathyroidism was specifically ruled out as an explanation for the patterns. Rather, the Vitamin D results were clearly related to inflammation. We think that evidence points to inflammation as the explanation of the vitamin D patterns in the other Th1 diseases, as well.

### More on Calcium, Vitamin D and the Marshall Protocol

- Actually, secondary hyperparathyroidism, which can raise 1,25D, occurs to compensate for low calcium in the blood, not low 25D
- 1,25D can increase the percentage of calcium absorbed, but is not even necessary for its absorption. Most calcium absorption is passive and not dependent on vitamin D, when calcium intake is adequate [Bronner *et al.* 1999, *J. of Nutr.* 129(1); Bronner *et al.* 2003 *J. Nutr.* 133]
- In a study of patients who had abundant calcium in their diet (averaging 1269 mg), only 7% of the variation in parathyroid levels was related to 25D (based on the coefficient of determination). And there was no link found between bone mineral density and 25D [Sigurdsson *et al.* 2000 *Osteoporosis* 11].
- Calcium intake below the recommended level is widespread according to the NIH (44% to 87% depending on sex and age). In our view, providing adequate calcium is a safer way to avoid secondary hyperparathyroidism than vitamin D supplementation
- MP patients maintain bone health by avoiding elevated 1,25D that stimulates bone loss (through osteoclast activity), having adequate calcium and 1,25D
- No problems are found from low 25D among MP patients

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#### More on Calcium, Vitamin D and the Marshall Protocol

To clarify a little further, secondary hyperparathyroidism occurs in order to compensate for low calcium, not low 25D. The 1,25D can increase the percentage of calcium absorbed, but is not even necessary for its absorption, since when calcium is adequate, most of the absorption is passive.

The role of calcium is shown in a study of patients who had abundant calcium in their diet, and it was found that only 7% of the variation in parathyroid levels was related to 25D. And there was no link between bone mineral density and 25D. It turns out that calcium intake below the recommended level is widespread according to the NIH, varying from 44% to 87% depending on sex and age.

And in our view providing adequate calcium is a safer way to avoid secondary hyperparathyroidism than vitamin D supplementation. MP patients maintain bone health by first avoiding elevated 1,25D that stimulates bone loss through osteoclast activity and by having adequate calcium and just generally keeping 1,25D in the normal range. We have observed no problems from even quite low 25D levels among MP patients.

### Another Area to Clarify: Is Extrarenal Production of 1,25D Good or Bad?

- There is now a theory that extrarenal production of 1,25D in many tissues is good and must be fueled with a large amount of 25D
- There are normally low levels of 1,25D production in many tissues, but we have been referring to the excessive production by activated macrophages due to a Th1 inflammatory disease.
- Some say that even the increased extrarenal production by macrophages is protective, that it is an attempt to protect the body from too much inflammation by immunosuppression.
- But, we have presented evidence for several ways in which high 25D and 1,25D can negatively affect a patient who is infected with intracellular bacteria and thus lead to long term harm.
- The success of the MP in treating patients is further evidence for our view, since lowering 25D and 1,25D has been found to be beneficial for bacterial killing.

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#### Another Area to Clarify: Is Extrarenal Production of 1,25D Good or Bad?

Another area that needs to be clarified is the question of whether extrarenal production of 1,25D is good or bad. The answer is it depends on how much and what is the source.

There is now a theory that a lot of extrarenal production of 1,25D in many tissues is important and must be fueled with a large level of 25D. Now there are normally low levels of 1,25D production by many types of cells and that is fine and normal. But we have been referring to something different — to the excessive production of 1,25D by macrophages in a Th1 disease.

Well, some say that even this increased 1,25D production by macrophages is good — that it is an attempt to protect the body from too much inflammation by suppressing the immune system.

But, we think this idea falls apart if we are dealing with a chronic infection. We have presented evidence for several ways in which high 25D and 1,25D can negatively affect the immune system's ability to fight CWD bacteria and thus lead to long term harm.

The success of the MP in treating patients is further evidence for our view, since lowering vitamin D has been found to be beneficial for bacterial killing.

### Why Such High Levels of Vitamin D Consumption are Promoted: Hidden Th1 Disease May Be Underlying Answer

- Some researchers believe high levels of vitamin D are needed, however serum levels of 25D recommended by some vitamin D advocates exceed levels that have been found to cause long term harm in other studies [Adams *et al.* 1997 *Ann Intern Med.* 127; Rajasree *et al.* 2001 *Eur J Epidemiol* 17(6)]

It makes more sense to us that the reason they find a need for high intakes of vitamin D are:

- An unwitting reliance on the immunosuppressive effect of high 25D, which may temporarily correct problems with kidney, parathyroid function, or other problems (e.g., muscle weakness in the elderly) that are really associated with bacteria-induced inflammation (the high 25D suppresses the bacterial killing that causes Jarisch Herxheimer Reactions and symptoms, but leads to long term harm)
- And/or energetic conversion of 25D to 1,25D by activated macrophages, which continually depletes the 25D, making it harder to keep serum 25D levels as high as they want them

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#### Why Such High Levels of Vitamin D Consumption are Promoted: Hidden Th1 Disease May be Underlying Answer

Some vitamin D proponents believe that very high levels of vitamin D are needed, levels that may exceed those found to cause harm in some studies. How could it be that some seem to find a benefit? In the light of what we know, it makes the most sense to us that the reason they find a need for high intakes of vitamin D are two fold.

They may be unwittingly be relying on the immunosuppressive or anti-inflammatory effect of high 25D, which may temporarily correct problems with kidney, parathyroid function, or other problems that are really associated with bacteria-induced inflammation. The high 25D they achieve suppresses the bacterial killing and associated die off reactions and may cause some short term benefit, but long term harm. This explanation may even apply to some elderly patients who seem to have improved muscle strength when given vitamin D.

Another reason some vitamin D advocates may find they require such high vitamin D intakes is that conversion to 1,25D by macrophages continually depletes the 25D in Th1 disease, making it harder to keep serum levels of 25D as high as they want them.

### Future Research Directions

- Need more data on vitamin D metabolite levels, including 1,25D, in chronic diseases
- Role of 1,25D production by macrophages not always as obvious as in sarcoidosis
- Like in RA, 1,25D elevation in blood may be at a lower level and elevated 1,25D restricted to areas of inflamed tissue in many diseases.
- Our data on response to MP is strong and warrants a trial with the MP for many diseases
- But to convince the skeptical, further experiments could be done on vitamin D dysregulation in a variety of illnesses similar to Mawer's study in RA to detect increased synthesis of 1,25D by macrophages (e.g. by challenging with a dose of 25D and comparing response with controls, measuring 1,25D levels in inflamed tissues etc...)

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#### Future Research Directions

As for future research directions, there is a need for more data on 25D and 1,25D levels.

But, as we have shown, the role of 1,25D production by macrophages is not always obvious from simple blood tests. In the less clear cut cases, as in rheumatoid arthritis, 1,25D elevation may be at a lower level and more restricted to areas of inflamed tissue and thus not show up in the blood test.

Despite our belief that the response so far to the MP is strong enough to warrant immediate trials for many diseases, further experiments on vitamin D might be useful. Experiments could be done to detect increased synthesis of 1,25D by macrophages by challenging with a dose of 25D and comparing responses with controls — and then looking at levels of 25D and 1,25D in inflamed tissues and so on, like the experiments discussed earlier in RA.

### Some Diseases that Might Be Investigated (References Suggest a Bacterial Role and/or Vitamin D Dysregulation)

- Heart Disease/Stroke: Miller *et al.* 2004. *Am J Physiol Heart Circ Physiol.* 287(3); Maniscalco *et al.* 2004 *Pathophysiology* 11(2); Mattman 2005. ARF's Chicago Conference.
- Psychiatric illnesses, like bipolar, depression, schizophrenia: Gloth *et al.* 1999. *J Nutr Health Aging.* 3(1); Carney *et al.* 1988. *Br J Psychiatry.* 152; Fallon 1994 *Am J Psychiatry.* 151(11); Fritzsche, 2002 *Int J Health Geogr.* 2002 1(1)
- Parkinson's disease: Goldsmith *et al.* 1990 *Arch Environ Health.* 45(2); Kumar *et al.* 2004 *Arch Neurol.* 61(7).
- Alzheimer's [Loeb 2004 *J Am Geriatr Soc.* 52(3); Miklossy *et al.* 2006 *Neurobiol. Aging* 27(2)]
- Autism: Bolte 1998 *Med Hypoth.* 51(2).
- Cancer: Tuohimaa *et al.* 2004 *Int J Cancer.* 108(1); Mawer *et al.* 1997 *J Clin Endocrinol Metab.* 1997 82(1); Cohen *et al.* 2005 *J. Urol.* 173; Cantwell 2004 *JOIMR* 2004; Huan 2001 *World J Gastroenterol.* 7(2); Broxmeyer 2004 *Med Hypoth.* 63(6).

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#### Some Diseases that Might Be Investigated (References Suggest a Bacterial Role and/or Vitamin D Dysregulation)

What other diseases might be good candidates for this type of investigation? The answer is, basically, any chronic disease with unknown cause.

But especially diseases where some link to vitamin D has been found — either a positive or negative effect, since this might be a sign of increased synthesis of 1,25D by macrophages.

Examples of some of the diseases that might have Vitamin D dysregulation include: heart disease and stroke; psychiatric illnesses, like bipolar, depression and schizophrenia; Parkinson's Disease; Alzheimer's; autism and cancer.

Listed on the slide are studies that find evidence suggesting a role for bacterial pathogens or a beneficial effect of antibiotics or suggestions of vitamin D having a positive or negative effect.

I should mention that there has been research, mostly observational studies, that support a positive role for Vitamin D in cancer prevention. We think many of the problems with these types of studies in cancer are similar to the problems we discussed in the section on MS. And one must also be careful to distinguish the effects of calcium from that of vitamin D and oftentimes it's not clear whether it's the calcium.

### Cancer Treatment and Progression in Relation to Vitamin D

- Many studies support anti-tumor effects of 1,25D, but the 1,25D must be at high levels that produce risk of side effects and none of these drugs have yet been approved for cancer
- Mayo clinic's web site concludes the data on vitamin D's role in cancer prevention and treatment is unclear
- We have seen no evidence for an increase in cancer rates in patients on the MP who have lowered their 25D and 1,25D levels
- Research discussed above indicates high 1,25D may be reducing the killing of intracellular bacteria
- If bacteria and associated inflammation are the underlying cause of a cancer, effectively treating the bacteria may outweigh any anti-tumor effect of increasing 1,25D.

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#### Cancer Treatment and Progression in Relation to Vitamin D

With regard to cancer treatment, there are many studies supporting 1,25D's anti-tumor effects. But for this effect to be useful in treatment, the 1,25D must be at high levels that produce a high risk of side effects. Neither 1,25D, nor any of the synthetic analogs created to mimic it, has yet been approved to treat cancer. The Mayo clinic, on their web site, concludes the data on vitamin D's role in cancer is still unclear.

We have seen no evidence for an increase in cancer rates in patients on the MP who have lowered their vitamin D levels. Research discussed above, shows high 1,25D may be reducing the killing of intracellular bacteria. If bacteria, and the inflammation that accompanies the bacterial infection, are the underlying cause of a cancer, then we think effectively treating the bacteria may outweigh any anti-tumor effect of increasing 1,25D.

And as was mentioned, H. pylori has been linked to ulcers, but it's also been linked to stomach cancer. So there is a clear example where the bacteria is the carcinogen.

### Breast Cancer, Vitamin D and Antibiotic Use

- A study found a tendency for 1,25D to become very low in late stage breast cancer [Mawer *et al.* 1997. *J Clin Endocrinol Metab.* 82(1)]
- A possible parallel situation: In a few of the very sickest patients with a very high bacterial load, 1,25D has become quite low through an unknown mechanism [tuberculosis: Palmieri 1997 *JCE & M.* 82; Th1 patients: Greg Blaney, M.D., pers. comm.] but then the 1,25D increased with appropriate antibacterial treatment, pointing to a bacterial cause for the very low 1,25D
- This suggests that the progression of a bacterial infection to a very severe level could account for the very low 1,25D in late stage breast cancer and thus bacteria could be the underlying cause of the cancer
- Some of the early breast cancer patients [Mawer *et al. ibid*] had an unexplained elevation in 1,25D, which may reflect the more typical situation of a Th1 disease before bacterial loads become too extreme and 1,25D drops

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#### Breast Cancer, Vitamin D and Antibiotic Use

There is some interesting work on breast cancer and Vitamin D that may suggest bacterial involvement. There was a study that found a tendency for 1,25D to become very low in late stage breast cancer. In a possibly parallel situation, in some of the very sickest patients with a very high bacterial load, 1,25D has become quite low through an unknown mechanism.

In tuberculosis, Palmieri found this and in some Th1 patients, Dr. Blaney has found this also — this very low 1,25D also. But then, remarkably, the 1,25D increased with appropriate antibacterial treatment.

This may suggest that the progression of a bacterial infection to a very severe level could account for the very low 1,25D in late stage breast cancer and thus bacteria could be the underlying cause of the cancer. Perhaps effective antibiotic treatment for CWD bacteria could reverse the process in breast cancer and restore the 1,25D levels as it did in the tuberculosis and the very sick Th1 patients I just mentioned.

As you know from what I have said before, the 1,25D is usually elevated in Th1 disease. So it is interesting that some of the early breast cancer patients had an unexplained elevation in 1,25D, which may reflect the more typical situation of a Th1 disease before bacterial loads become too extreme and 1,25D drops.

### More on Antibiotic Use and Breast Cancer

- Recent research showing breast cancer rates correlate with more frequent antibiotic use [Velicer et al. 2004 JAMA 291] may suggest a bacterial cause
- Antibiotic use reflects the susceptibility to and frequency of bacterial infections
- Many of the bacteria may transform into cell wall deficient (CWD) forms leading to a high bacterial load and later Th1 disease
- CWD bacteria are resistant to antibiotics in the usual ways they are used
- Treatment with many common antibiotics even promotes the transformation of bacteria into their CWD forms [Mattman, *ibid*] that can then persist inside macrophages and other cells and increase over time

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#### More on Antibiotic Use and Breast Cancer

A recent article showed breast cancer rates correlate with more frequent antibiotic use. We think this may suggest a bacterial cause for breast cancer.

In our view, antibiotic use can be thought of as reflecting the susceptibility to and frequency of bacterial infections. Many species of bacteria have been shown to be able to transform into cell wall deficient forms when under attack in order to escape destruction.

CWD bacteria are resistant to antibiotics in the usual ways they are used, so taking antibiotics will not generally eliminate them. And treatment with the most commonly used antibiotics even promotes the transformation of bacteria into their CWD forms, which can then persist inside the body inside macrophages and other cells and increase over time.

Thus, in our interpretation, the more exposure to bacteria indicated by the greater antibiotic use in the study, the more opportunities for the creation of CWD bacterial forms, which may then lead to Th1 disease and possibly to cancer.

### Prostate Cancer Rate Greater at Low and High Serum 25D Levels: A "U" Shaped Curve

- A large study found the highest rates of prostate cancer occurred when serum 25D was <8 ng/ml (equivalent to <19 nmol/l) and > 33 ng/ml (>80 nmol), with the lowest rate when the 25D was 16-24 ng/ml. [Tuohimaa et al. 2004 *Int J Cancer* 108(1)].

Explanation consistent with prostate cancer being a Th1 disease with an underlying bacterial cause:

- Low level of 25D could be linked to more cancer because 25D's low level might be due to its rapid depletion (through conversion to 1,25D) and thus be a marker of Th1 disease. Resulting high 1,25D could suppress the immune system's ability to fight bacteria (through non VDR receptors) and help lead to cancer
- High level of 25D could help cause a higher rate of cancer due to high 25D suppressing innate immunity, reducing ability to combat bacteria
- Lowest cancer rate would be in the middle region, where the above factors are less prominent

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#### Prostate Cancer Rate Greater at Low and High Serum 25D Levels: A "U" Shaped Curve

A particularly interesting, large new study on vitamin D and prostate cancer went beyond the geographical correlational studies and actually measured the 25D levels. They found that the highest rates of cancer occurred when serum 25D was low (<8 ng/ml) and when it was high (>33 ng/ml), giving a U shaped curve.

We think these patterns may indicate Th1 disease due to bacteria. Here is our hypothesis to explain this pattern. The low 25D link to cancer, rather than indicating a deficiency, could be a sign of depletion of 25D through conversion to 1,25D. Thus, it may be simply a marker for a Th1 process. As we have discussed, the resulting high 1,25D in Th1 disease suppresses the immune system's ability to fight bacteria and thus may help lead to cancer.

On the other hand, a very high level of 25D could help cause a higher rate of cancer due to the high 25D suppressing the immune system, as previously discussed. The result would then be that innate immunity is less functional and less able to combat bacteria, which may then lead to cancer.

The lowest cancer rate was in the middle region, 16-24 ng/ml, where the above two factors are least prominent. But it should be emphasized, that we do not think this data shows that the optimal level of 25D is in the middle region. In our view, the low 25D is linked to cancer merely because it is a marker of Th1 disease, not a causal factor.

The authors of the prostate cancer study concluded that too high a level of 25D might increase the risk of prostate cancer. As you saw

from our patients slide, it isn't that hard to exceed 30 ng/ml of 25D, especially with vitamin D supplements — 3 of the 4 patients had levels above 30.

## Conclusion

- Vitamin D dysregulation may produce elevated 1,25D, depleted 25D, or an elevated D-ratio and we believe it is widespread in chronic disease
- Elevated 25D or 1,25D might make the patient feel better or worse in the short run, but in either case, make them worse in the long run through immunosuppression promoting bacterial increase and this requires reevaluation of many vitamin D studies
- Vitamin D blood tests do not always accurately reveal Th1 disease. Thus, if the clinical picture suggests Th1 disease, we find a therapeutic probe using the Marshall Protocol is the “gold standard” test.
- Further study of vitamin D dysregulation appears likely to provide a window onto the immune system, improving diagnosis and treatment for many chronic diseases

## Slide 29 Conclusions

In conclusion, we have shown that Vitamin D dysregulation may produce patterns of elevated 1,25D, depleted 25D or an elevated D-ratio, as we show for a variety of chronic Th1 diseases.

Elevated 25D or 1,25D might make the patient feel better or worse in the short run, but in either case, make them worse in the long run. This worsening occurs through immunosuppression promoting bacterial increase.

**We think the evidence for this new view of vitamin D requires re-evaluation of many previous studies and calls for new types of studies in many chronic diseases.**

Although usually quite helpful, Vitamin D blood tests do not always accurately reveal Th1 disease. Thus, if the clinical picture suggests Th1 disease, we find a therapeutic probe using the Marshall Protocol as the “gold standard” test for Th1 disease and bacterial involvement.

If you think about it, it seems little wonder that vitamin D has become so popular. It's basically an over-the-counter steroid — but its effects are more subtle and insidious than something like prednisone since it blocks only innate immunity, leaving adaptive immunity intact.

We think that further study of vitamin D dysregulation appears likely to provide a window onto the immune system, improving diagnosis and treatment for many chronic diseases.

Thank you for your attention.

