Research Article ISSN 2639-944X

Journal of Medical - Clinical Research & Reviews

Complex Interaction between Iron, Vitamin B2, Vitamin B12 and Vitamin D During the Activation of Vitamin D

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Received: 30 Apr 2024; **Accepted:** 29 May 2024; **Published:** 04 Jun 2024

Citation: Gregory John Russell-Jones. Complex Interaction between Iron, Vitamin B2, Vitamin B12 and Vitamin D During the Activation of Vitamin D. J Med - Clin Res & Rev. 2024; 8(6): 1-5.

ABSTRACT

Functional Vitamin D is required for absorption of calcium from the intestine and for regulating calcium and phosphorus deposition to ensure healthy bone density. More recently it has been found that vitamin D is also essential for maturation of neuronal stem cells. Activation of vitamin D is a multi-enzyme process, which requires a contribution of several co-factors including iron, vitamin B2 and vitamin B12. We have used urinary phosphoric acid as a marker of functional vitamin D deficiency, and have compared various urinary metabolic markers with phosphoric acid levels to follow the essential elements in vitamin D activation. Levels of phosphoric acid were increased in each of iron, vitamin B2 and vitamin B12 deficiency, with the greatest increase seen in low functional vitamin B12.

Keywords

Vitamin B2, Vitamin B12, Iron, Vitamin D, Adrenodoxin, Adrenodoxin reductase, 25-hydroxylase, 1-alpha-hydroxylase.

Abbreviations

MMA: Methylmalonic acid, QA: Quinolinic acid, KA: Kynurenic acid.

Introduction

Normal activation of vitamin D is a multi-step process in which light from the sun, or more specifically UV light from the sun shines on the skin and causes the conversion of the precursor 7-dehydrocholesterol to vitamin D3 - cholecalciferol. This molecule travels to the liver, where the haem-containing enzyme 25-hydroxylase (CYP2R1) converts the cholecalciferol to calcidiol (25-hydroxy-vitamin D) (Figure 1). Finally the 25-hydroxyvitamin D (Calcidiol) is activated in the kidney by the haem-containing1-α-hydroxylase (CYP27B1) to form the active form of vitamin D, 1,25-di-hydroxyvitamin D (Calcitriol). This reaction, though involves a multi-enzyme complex of CYP27B1, adrenodoxin (an iron-sulphur protein) and adrenodoxin reductase (an FAD-dependent enzyme). The brain is unique amongst the other organs in that it has its own enzyme, 1α-hydroxylase, that activates 25-hydroxyvitamin-D to the active form 1,25-dihydroxy-vitamin D [1-3]. The active vitamin D so produced, then binds to specific vitamin D receptors in the brain, particularly in the hypothalamus, and dopaminergic neurons of the substantia nigra. High levels of expression of the 1-α-hydroxylase has been in the Purkinje cells in the cerebellum [4].

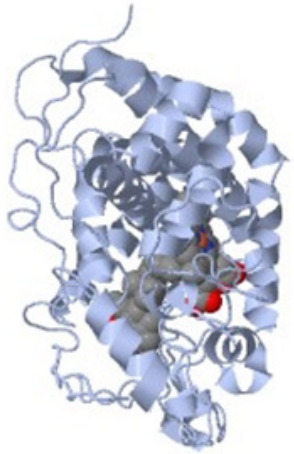

Figure 1: Structure of CYP27B1 - 25-hydroxyvitamin D3 1-alpha-

hydroxylase Note the Haem Structure in the centre of the enzyme.

Adrenodoxin acts in concert with CYP27B1 in the activation of vitamin D. The activity of the enzyme (Adrenodoxin) is helped by, and as such is dependent upon, the iron-sulphur co-factor in the active site of the enzyme (Figure 2). Dissociation of iron-sulphur processes, such as those found in Adrenodoxin, has been observed when serum ferritin levels drop below 70 ug/L [5-8]. Generation of the sulphur atom in the iron-sulphur protein comes with the movement of the sulphur, originally occurring in dietary methionine into the sulphation cycle. Such movement, though, is dependent upon generation of high levels of S-Adenosylmethionine, and as such, will be lower as levels of active vitamin B12 decrease [9-11].

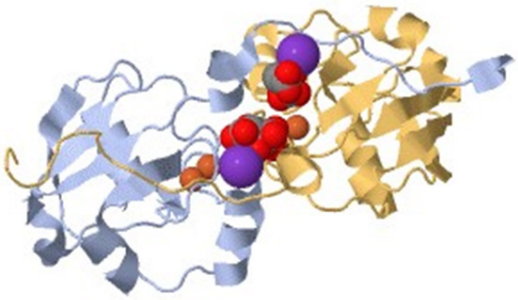

Figure 2: Structure of Adrenodoxin (also called Ferredoxin). Note the Iron-sulphur cluster in the centre of the molecule.

Adrenodoxin acts together with Adrenodoxin reductase in "helping" CYP27B1 to hydroxylate 25-hydroxy vitamin D and so form 1,25 dihydroxyD (Calcitriol). Its activity is critically dependent upon two co-factors, one derived from vitamin B3 (NAPDH) and the other from activation of vitamin B2 (FAD) (Figure 3) [12-16]. Mutations in CYP27B1 have been described which affect binding of Adrenodoxin and can cause rickets [17]. Reduced levels of FAD, would result in reduction in activity of Adrenodoxin reductase, and thereby lower production of 1,25 Di-hydroxy vitamin D.

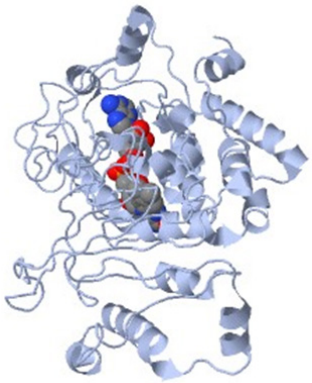

Figure 3: Structure of Adrenodoxin Reductase (also called Ferredoxin reductase). Note the FAD molecule in the centre of the enzyme.

Apart from its known function in calcium metabolism and bone metabolism, vitamin D (as 1,25-diOHD) has been shown to be a potent "neurosteroid", which plays a crucial role in the developing brain [18-21], and mutations in these enzymes have been associated with the development of autism [22,23]. Both CYP27B1 and CYP24A1 have been found in neural cells of the fetal brain indicating that the brain has the potential to process "incoming" 25-hydroxy vitamin D, which has been formed by the mother. Much lower levels of vitamin B12, which have been found in the brains of children with autism, plus the lack of functional vitamin B2, would mean that the ability of CYP27B1 to activate what little 25-hydroxy vitamin D is present would be severely restricted, thus leading to the poor neuronal development typical of autism. Using the vitamin D deficiency marker, phosphoric acid, we have compared markers of iron, vitamin B2 and vitamin B12 deficiency, in order to gain further insight into the importance of these molecules in vitamin D activation.

Experimental Procedures

A retrospective analysis was performed upon data submitted to us for analysis from a cohort of 1750 children and adults from countries including USA, Canada, United Kingdom, Ireland, Germany, Spain, France, Italy, Bulgaria, India, Sweden, Bulgaria, Serbia, Dubai, Croatia and Australia. No selection was made in the acceptance of data, with no data being rejected. Data is presented regardless of sex, or age. Ages varied from 1 year old to seventy-four years old. Organic Acid Test Data (1750 sets, Great Plains Laboratories, Lenexa, KS, USA), which had been submitted to us for interpretation, from a variety of groups including parents of children with autism spectrum disorder, individuals with chronic fatigue syndrome and from persons who were healthy, and who had no previously identified health condition. Individual data is plotted as Scattergrams (see figures 4 to 8). Data is presented as mmol/mol creatinine. Data was collected as per guidelines set out in the Declaration of the Helsinki principles.

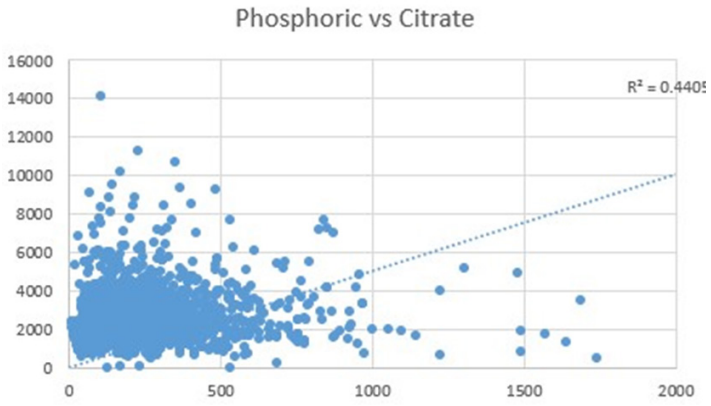

Figure 4: Comparison of urinary citrate (horizontal axis) with Phosphoric Acid (vertical axis).

Results

Measurement of Vitamin D activation and relationship to iron, vitamin B2 and vitamin B12

In the Organic Acid Test analysis (OAT), there are various markers that correlate with functional vitamin D sufficiency (Phosphoric Acid), iron sufficiency (citric acid), vitamin B2 sufficiency (glutaric

acid) and vitamin B12 sufficiency (MMA, Quinolinic Acid (QA), Homovanillic Acid (HVA), CoQ10 (3-hydroxyglutaric acid), and Pyroglutamic acid. Correlation graphs for each are plotted below,

Reduced activity of the iron-sulphur protein, aconitase, results in increased secretion of citrate (citric acid) into urine. Such reduction in activity is found when ferritin levels drop below 60 ug/litre (Figure 4), which is when various iron-sulphur proteins, such as aconitase and adrenodoxin begin to uncouple. This drop in serum ferritin precedes the drop in Haem proteins such as Haemoglobin. Hence, iron deficiency, such as is common in conditions such as autism, would be accompanied by a reduction in the conversion of 25OHD, to 1,25diOHD, which would result in functional vitamin D deficiency, and would correlate with an increase in urinary phosphoric acid ($R^2 = 0.4405$, Figure 4). Reduced serum ferritin, is also associated with the reduced activity of thyroid peroxidase, and has been associated with hypothyroxinemia in pregnancy [22,23].

Urinary glutaric acid, is one of the surrogate markers of functional vitamin B2 deficiency. Increases in glutaric acid correlated with increases in phosphoric acid (r^2=0.3794, Figure 5). Reduction of intracellular iron, requires intracellular glutathione (GSH), which requires the action of the FAD-dependent enzyme glutathione reductase, hence in low functional B2 (as FAD), the activity of glutathione reductase is compromised, as too maintenance of functional B12 activity. In addition, Adrenodoxin reductase (the oxidation/reduction partner to Adrenodoxin), requires FAD for activity. The metabolism of glutaric acid requires the FAD-dependent enzyme glutaryl-CoA-dehydrogenase, and as FAD levels decrease glutaric acid in urine increases. Hence, FAD deficiency, such as is common in conditions such as autism, would be accompanied by an increase in the amount of glutaric acid, and the reduced activity of Adrenodoxin reductions and a reduction in the conversion of 25OHD, to 1,25diOHD, with functional vitamin D deficiency, and would correlate with an increase in urinary phosphoric acid ($R^2 = 0.3794$, Figure 5).

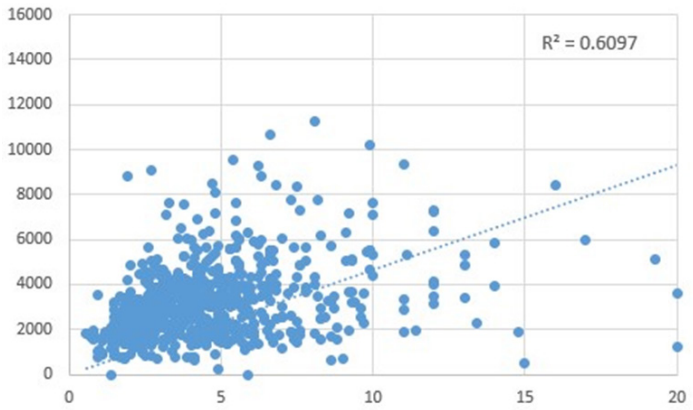

Figure 5: Comparison of urinary glutaric acid (horizontal axis) with Phosphoric Acid (vertical axis).

There are several markers of functional B12 deficiency in the OAT. MMA, a traditional marker of Adenosyl cobalamin deficiency, HVA – a breakdown product of dopamine, which becomes elevated in methyl cobalamin deficiency, as too is the tryptophan metabolite, QA. All three markers of functional vitamin B12 sufficiency correlated with increased phosphoric acid, MMA ($R^2 = 0.4274$), HVA ($R^2 = 0.4274$), and QA ($R^2 = 0.6102$) (Figure 6). Potentially the correlation is due to the need for functional vitamin B12 for homocysteine to be processed by cystathionine beta synthase, and to generate free cysteine, and free sulphur, which is used in the formation of iron-sulphur clusters, such as those in adrenodoxin. Lack of iron-sulphur clusters would in turn mean that the activity of adrenodoxin, and hence the formation of 1, 25-Dihydroxy vitamin D would be reduced.

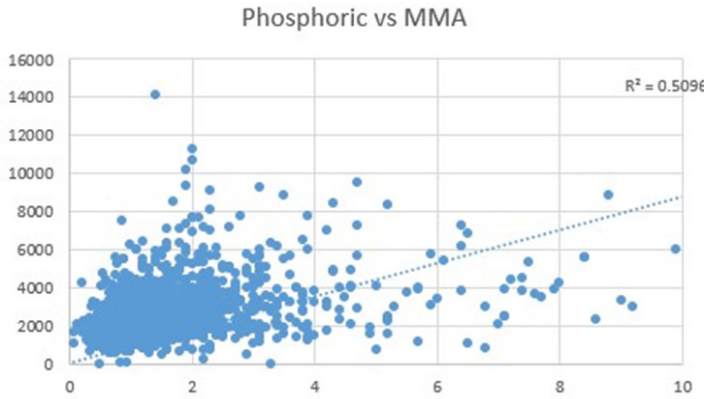

Figure 6: Comparison of urinary Phosphoric Acid (vertical axis) with MMA acid (horizontal axis) (top panel), HVA (middle panel) and QA (lower panel).

Formation of CoQ10 requires 3 methylation steps, and in methyl B12 deficiency, there is an increase in the CoQ10 precursor, 3-hydroxymethylglutaric acid, with increased phosphoric acid levels ($R^2 = 0.5602$, Figure 7).

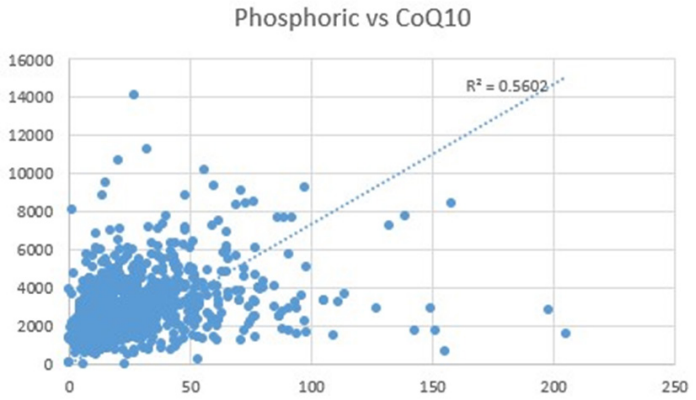

Figure 7: Comparison of urinary Phosphoric Acid (vertical axis) with the CoQ10 deficiency marker 3-hydroxymethylglutaric acid (horizontal axis).

Formation of intracellular glutathione is dependent upon intracellular cysteine levels, which come from processing of dietary methionine to homocysteine, which is then converted to cystathionine, and then cysteine. In the absence of sufficient intracellular cysteine, glutamate (used in the formation of glutathione) is rapidly converted to pyroglutamic acid. Urinary glutamic acid levels strongly correlated with urinary phosphoric

acid (0.7558, Figure 8).

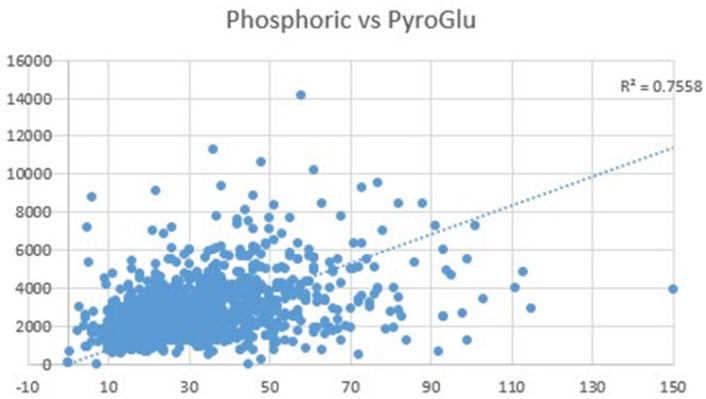

Figure 8: Comparison of urinary Phosphoric Acid (vertical axis) with Pyroglutamic acid (horizontal axis).

Discussion

Comparison of urinary phosphoric acid with several metabolic markers showed that there was an increasing rate of correlation between levels of phosphoric acid in urine, and increasing levels of urinary Citrate (iron deficiency), Glutaric acid (Vitamin B2 deficiency), MMA (AdenosylB12 deficiency) and HVA, QA, 3-hydroxy-3-methylglutaric acid, and PyroGlutamate (Methyl vitamin B12 deficiency). The higher correlation with pyroglutamate levels suggests that intracellular processing of cysteine and formation of glutathione are critical to the function of adrenodoxin and hence to the final step in activation of vitamin D. The data strongly supports the notion of dietary sufficiency in essential nutrients such as iron, vitamin B2 and vitamin B12 for maintenance of active vitamin D. Thus, there is a processing Nexus, where 3 enzymes, whose activities depend upon sufficiency if iron, B2, and B12, are involved in the ultimate conversion of 25-OH-vitamin D, to the active from 1, 25-diOH-vitamin D. The data is also commensurate with the known associations of iron and vitamin B12 deficiency and osteoporosis [24-32], and as such provide a mechanism for this association. The results have important ramifications for the treatment of conditions such as osteoporosis, dementia, diabetes, and depression, in which low vitamin D has been implicated.

Summary

The formation of biologically active vitamin D requires a complex series of reactions involving the haem-containing enzyme 25-hydroxylase (CYP2R1) and the multi-enzyme complex consisting of the haem-containing1-α-hydroxylase (CYP27B1), adrenodoxin (an iron-sulphur protein) and adrenodoxin reductase (an FAD-dependent enzyme) to form the active form of vitamin D, 1,25-di-hydroxyvitamin D (Calcitriol). Deficiencies in any of the co-factors, haem, vitamin B2 and vitamin B12 have been shown to significantly reduce the amount of functional vitamin D. These findings have important applications in the treatment of conditions such as autism and Alzheimer's disease as well as osteoporosis and dementia, which have been associated with functional vitamin D defciency.

Acknowledgements

Special thanks go to all the individuals who have supplied their data for free analysis, and the collation of which, has contributed to a substantial increase in the understanding of the importance of vitamin B2, vitamin B12, and iron to the activation of vitamin D.

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