Research Article ISSN 2639-944X

Journal of Medical - Clinical Research & Reviews

Altered Metabolism of Iron, Vitamin B, and Vitamin B12 Potentially Interfere with Vitamin D- Activation in Children with Autism

Gregory John Russell-Jones*

B12 Oils Pty Ltd.

***Correspondence:**
Gregory John Russell-Jones, B12 Oils Pty Ltd, E-mail: russelljonesg@gmail.com.

Received: 01 Apr 2024; **Accepted:** 05 May 2024; **Published:** 11 May 2024

Citation: Gregory John Russell-Jones. Altered Metabolism of Iron, Vitamin B, and Vitamin B12 Potentially Interfere with Vitamin D- Activation in Children with Autism. J Med - Clin Res & Rev. 2024; 8(5): 1-6.

ABSTRACT

Studies have shown that deficiencies in iron, Iodine, vitamin B12 and vitamin D are independently associated with developmental delay in children. Whilst there is the possibility that these operate in independent fashion, there is also the possibility that these nutrients come together in some sort of Nexus, which is causative for developmental delay. Activation of vitamin D is a multi-enzyme process, which requires the 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 urinary phosphoric acid levels to follow the essential elements in vitamin D activation. Activation of vitamin D was dependent upon sufficient levels of iron, vitamin B2 and vitamin B12, which are required during the activation of vitamin D by a multi-enzyme complex of CYP27B1, adrenodoxin, and adrenodoxin reductase. The findings bring together the various causes of developmental delay into a central Nexus, which can potentially be used in the treatment and prevention of the condition.

Abbreviations

MMA: methylmalonic acid, QA: quinolinic acid, KA: kynurenic acid.

Keywords

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

Introduction

Vitamin D is unique in that the activation of vitamin D to function as a “neurosteroid” involves many sequential steps. Hence, the initial step in activation requires UV light to initiate the conversion of the precursor 7-dehydrocholesterol to vitamin D3 - cholecalciferol. Cholecalciferol is transported to the liver, where the haem-containing enzyme 25-hydroxylase (CYP2R1) converts the cholecalciferol to calcidiol (25-hydroxy-vitamin D). This form, though is a pro-vitamin and must be 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 three-enzyme complex of CYP27B1, adrenodoxin (an iron-sulphur protein) and adrenodoxin reductase (an FAD-dependent enzyme). Apart from its known function in calcium metabolism and bone metabolism, vitamin D has a unique role in brain development, including homeostasis, embryogenesis, neural differentiation, neurodevelopment, gene regulation and immunological modulation [1]. Vitamin D also has a role in neurotropism, neuroprotection, and neuroplasticity [2], and neuroregeneration [3] and vitamin D deficiency has been associated with developmental disorders and abnormal brain development in conditions such as autism [4-6]. Both of the two vitamin D processing enzymes, CYP27B1 and CYP24A1 have been found in neural cells of the foetal brain indicating that the brain has the potential to process "incoming" transplacentally acquired 25-hydroxy vitamin D, formed by the mother.

Recently melatonin has been shown to work in conjunction with vitamin D to induce neuronal stem cell differentiation into myelin producing oligodendrocytes and to stimulate neuronal proliferation [7]. Melatonin is a methylation product of N-acetylserotonin, hence in methyl B12 deficiency, there is a reduced production of melatonin, which would then reduce the level of stem cell differentiation and result in reduced myelination of the neonatal brain, thereby resulting in a reduction in the number of immature neurons in the amygdala region of the developing brain [8]. The

major role of myelination is to increase the "conduction" speed of nerves, and to control how they interact. During development, myelination of appropriate areas of the brain has been correlated with the development of specific functions in the brain, such as the development of vocabulary, reading, cognitive function, and speech.

Studies on the metabolism of over 750 children with autism have shown that there are four readily identifiable metabolic deficiencies that potentially are causative for autism, and every child that we have data for fits with the first two.

i. They were all functionally deficient in vitamin B2. Generally this was due to either a dietary lack of dairy, the major source of B2, or a dietary lack of Iodine, Selenium and/or Molybdenum, all of which are required for the activation of vitamin B2. In this regard, Iodine deficiency is the single most preventable cause of delayed mental and physical development in the world [9-17],
ii. All children analysed were functionally deficient in vitamin B12. Vitamin B12 deficiency has been known for over 40 years to cause global developmental and physical delay [10-22]. The vitamin B12 deficiency is the result of low vitamin B12 in the mother, or due to functional vitamin B2 deficiency [23], which may be absolute or may be paradoxically high [24],
iii. Over 90% of the kids are iron deficient, the second most preventable cause of developmental delay. Iron levels are known to be low in the brains of autistic children [25-29],
iv. Nearly all are vitamin D deficient, which is also a known cause of developmental delay [4,5,30-37].

The observed diversity of deficiencies that have been associated with developmental delay, could potentially influence some central pathway, which may be affected by each of iron, B2, B12 and vitamin D levels in the foetus and neonate. 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.

Methods

A retrospective analysis was performed upon data submitted to us for analysis from a cohort of 845 children and adults with autism 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 32 years old. Organic Acid Test Data (845 sets, Great Plains Laboratories, Lenexa, KS, USA), which had been submitted to us for interpretation, from parents of children with autism spectrum disorder. Individual data is plotted as Scattergrams (see figures 1 to 7). Data is presented as mmol/mol creatinine. Data was collected as per guidelines set out in the Declaration of Helsinki.

Results

There are various markers in the Organic Acid Test analysis (OAT), 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.

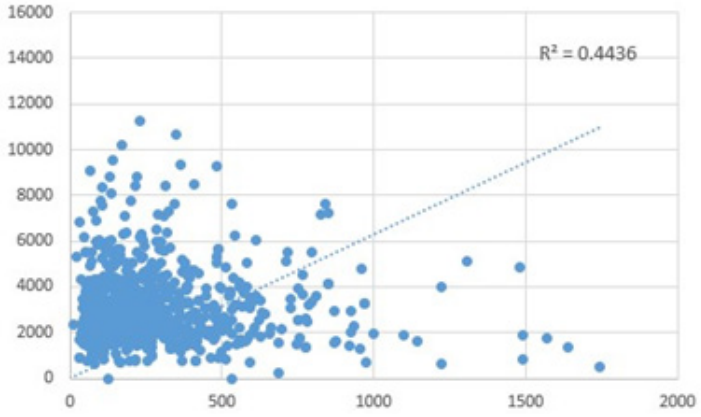

Figure 1: Comparison of urinary citrate (horizontal axis) with Phosphoric Acid (vertical axis).

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 1), 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 1). Reduced serum ferritin, is also associated with the reduced activity of thyroid peroxidase, and has been associated with hypothyroxinemia in pregnancy [38,39].

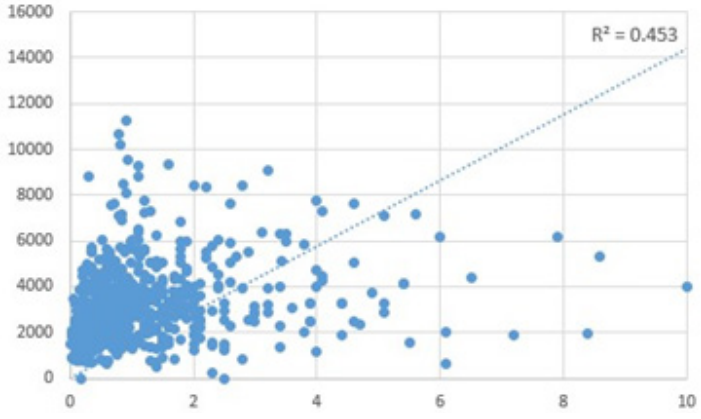

Figure 2: Comparison of urinary glutaric acid (horizontal axis) with Phosphoric Acid (vertical axis).

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 2). 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 2).

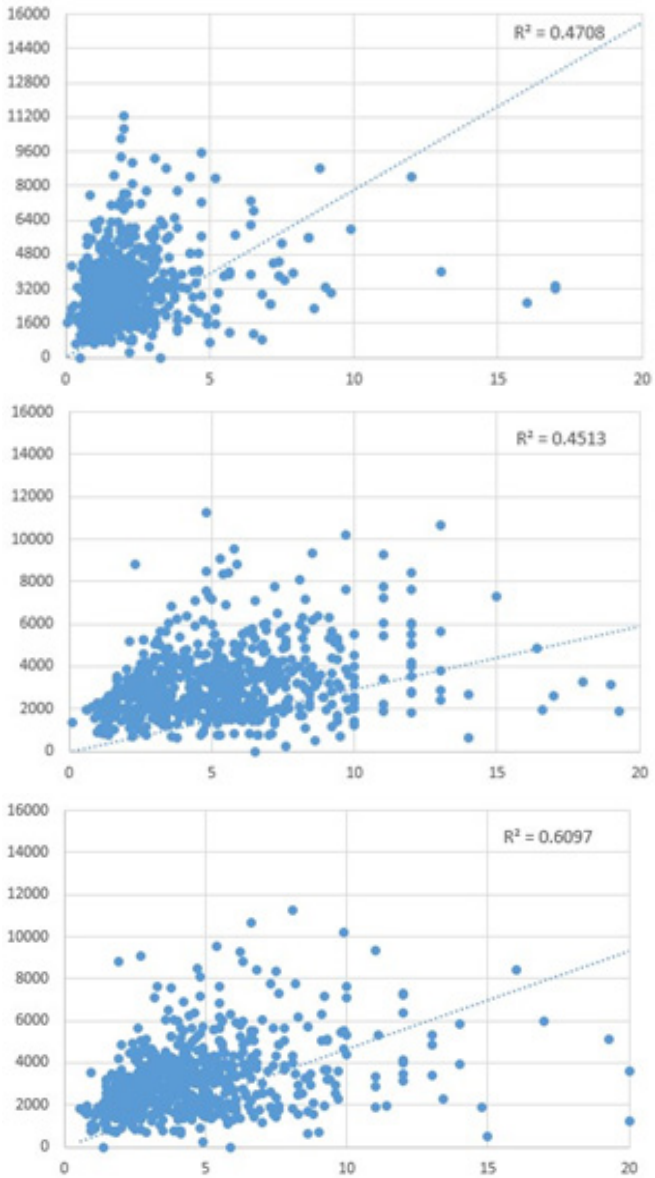

Figure 3: Comparison of urinary Phosphoric Acid (vertical axis) with MMA acid (horizontal axis)(top panel), HVA (middle panel) and QA (lower panel).

There are several markers of functional B12 deficiency in the OAT. MMA, a traditional marker of Adenosylcobalamin deficiency, HVA – a breakdown product of dopamine, which becomes elevated in methylcoblamin deficiency, as too is the tryptophan metabolite, QA. All three markers of functional vitamin B12 sufficiency correlated with increased phosphoric acid, MMA (R^2 = 0.4708), HVA (R^2 = 0.4513), and QA (R^2 = 0.6097) (Figure 3). 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.

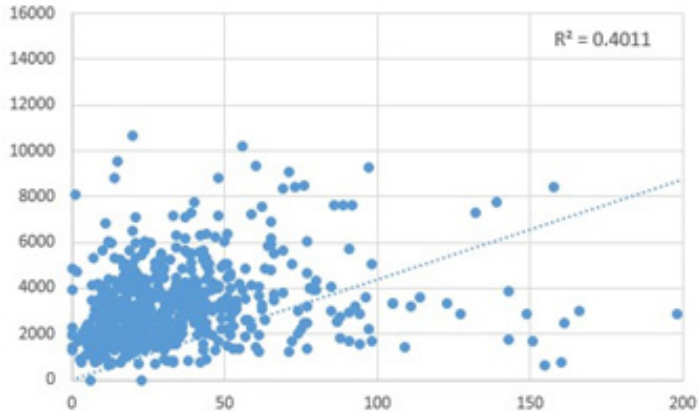

Figure 4: Comparison of urinary Phosphoric Acid (vertical axis) with the CoQ10 deficiency marker 3-hydroxymethylglutaric acid (horizontal axis).

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 = 0.4011, Figure 4).

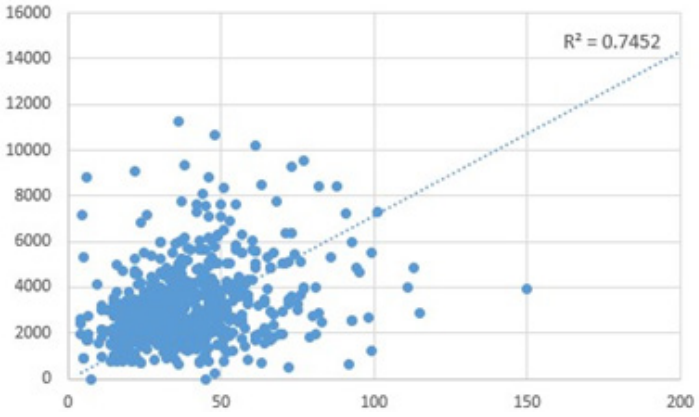

Figure 5: Comparison of urinary Phosphoric Acid (vertical axis) with Pyroglutamic acid (horizontal axis).

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 and hydrogen sulphide. In the absence of sufficient intracellular cysteine, glutamate (used in the formation of glutathione) is rapidly converted to pyroglutamic acid. Urinary pyroglutamic acid levels strongly correlated with urinary phosphoric acid (0.7452, Figure 5), suggesting that there is a lack of intracellular cysteine, of the formation of glutathione, and may also indicate a deficiency of cysteine, and hydrogen sulphide, which are essential for the formation of iron sulphur complexes, such as those that are found in Adrenodoxin.

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. Activation of vitamin B2 (riboflavin) is dependent upon Iodine, Selenium and Molybdenum, which possibly explains the association of Iodine deficiency with developmental delay and Selenium deficiency with intellectual decline in the elderly [40-43]. 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 [44-53], and as such provide a mechanism for this association.

In addition to its direct role as a "neurosteroid" the combination of low 1,25 dihydroxyvitamin D, and the reduced capacity for myelination that would occur due to lack of iron, and functional vitamin B12 and vitamin B2, combined with reduced production of the neurotransmitters hydrogen sulphide, acetyl choline, and the reduced mitochondrial energy production which accompanies Iron/B12 and B2 deficiency, would more than readily explain the delayed development that typifies autism. The results have important ramifications for the treatment of conditions such as osteoporosis, dementia, diabetes, and depression, in which low vitamin D has also been implicated.

Summary

In summary, the "Nexus Theory™" of Autism unites the various observations of the delayed mental development seen in Autism and encompasses the data on low Vitamin D, low iron, low vitamin B12, low vitamin B2, low Iodine, low selenium and low molybdenum commonly seen in autism. A real nature and nurture combination. As such the condition would appear to be entirely preventable by adequate nutrition in the mothers, particularly iron, vitamin B12 and Iodine, Selenium and Molybdenum and vitamin B2, and by maintaining sufficient vitamin D levels during pregnancy. Potentially, by addressing the known metabolic deficiencies present in these children, the condition could also be largely resolvable.

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.

Author Statement

Dr Gregory Russell-Jones: Solely responsible for conceptualization, methodology, writing of the original draft, performing the investigation, and writing, reviewing and editing the manuscript

Ethics

Compliance with Ethical Standards

We declare that there are no potential conflicts of interest.

Research did not involve human participants and/or animals.

No Informed consent is required, all data is “blinded” and as such is anonymous.

Data analysis was carried out under the Australian National Health and Medical Research Council guidelines (NHMRC). Under these guidelines, all data was deidentified and steps were taken to ensure the anonymity and confidentiality of the data. Deidentification has consisted of absolute anonymity and confidentiality of the data, such that no specifics such as gender, ethnicity, Country of Origin, etc. are associated with any data point in the study.

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