Vitamin D during pregnancy, lactation, and childhood

Vitamin D deficiency rickets in New Zealand children

And

Vitamin D supplementation during exclusive lactation to improve both infant and maternal 25-hydroxyvitamin D status

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Abstract

Background

Vitamin D is essential for calcium homeostasis and mineralisation of the skeleton. Vitamin D deficiency during pregnancy, infancy and childhood is common, and severe deficiency can lead to rickets. Rickets is a mineralisation defect at the epiphyseal growth plates specific to growing children. However, currently there are no national statistics on vitamin D deficiency rickets in New Zealand children, nor any data on longitudinal vitamin D status during pregnancy and lactation for the southern hemisphere. To prevent rickets and severe vitamin D deficiency, many countries use dietary fortification and recommend daily vitamin D supplementation to expectant mothers during pregnancy and infants during exclusive breastfeeding. This is not currently the case in New Zealand.

Aims

1) To prospectively determine the annual incidence and characteristics of vitamin D deficiency rickets in New Zealand children

2) To determine the effect of two different intermittent monthly doses of maternal cholecalciferol on infant and maternal vitamin D status

3) To describe longitudinal vitamin D status during pregnancy and lactation in healthy women and their infants in Dunedin, New Zealand (latitude 45°S)

Methods

The New Zealand Paediatric Surveillance Unit (NZPSU) was utilised to prospectively collect cases of vitamin D deficiency rickets (defined by serum 25-hydroxyvitamin D <50nmol/L and elevated alkaline phosphatase levels, and/or radiological rickets) in New Zealand for 36 months between July 2010 and June 2013 inclusive. Concurrently, a three arm, randomised, double-blind, placebo-controlled trial recruited women from Dunedin (45°S) who were planning to exclusively breastfed. Women (n=90) were randomly assigned to receive either cholecalciferol (50,000 IU or 100,000 IU) or placebo monthly from week 4 to week 20 postpartum. The treatment effects relative to placebo were then estimated using a linear fixed-effects regression model. In addition, longitudinal data (3 antenatal and 2 postnatal time points) on vitamin D status during pregnancy and lactation (out to week 20 postpartum) was analysed (total n=126). Participants were the n=90 from the aforementioned trial, in combination with a further n=36 similarly
recruited historical control mother-infant pairs. Data at the final postnatal time point week 20 was included only for those in the placebo and historical control groups.

Results

Fifty-eight children with confirmed vitamin D deficiency rickets were identified using the NZPSU. Median age was 1.4 years (range 0.3-11), and 95% were born in New Zealand; however the majority of mothers (68%) were not. The overall annual incidence of rickets in New Zealand children aged < 15 years was 2.2/100,000 (95%CI 1.4-3.5); with the incidence in those aged <3 years greater at 10.5/100,000 (95%CI 6.7-16.6). Key risk factors identified were: darker skin colour, Indian and African ethnicity, age <3 years, exclusive breastfeeding, southern latitude, and season (winter/spring).

In the intervention trial, after 16 weeks of supplementation, maternal vitamin D status was significantly higher in both the 50,000 IU/month and 100,000 IU/month groups. For infants, the unadjusted mean changes in vitamin D status were not significantly different from the placebo group. However, after adjustment for season of birth, vitamin D-fortified formula intake, and skin colour the mean effect size for the 100,000IU/month group was 19.1nmol/L (95%CI 2.5-35.6; \(P=0.025\)).

Analysis of the longitudinal pregnancy and lactation data for 126 maternal and infant pairs revealed high rates of both maternal and infant vitamin D deficiency during pregnancy and the first 20 weeks postnatal (71% still exclusively breastfeeding). Maternal vitamin D deficiency (25OHD < 50nmol/L) was seen at one or more time points (in those with data at all time points) during the full longitudinal study in 65% (52/80). Infant vitamin D deficiency was more common, seen in 76% at one or more time points. Deficiency at birth was found in 68% of infants, mean cord blood of 41nmol/L. At 20 weeks postpartum three infants with severe deficiency had developed secondary hyperparathyroidism (serum PTH values between 120 and 281pg/ml - Upper limit normal ≤ 65pg/ml). Season was the main variable affecting vitamin D status, with considerable variation in longitudinal status when examined by season of conception. In addition, stage of pregnancy also appears to exert an independent effect on vitamin D status once all other variables in the model were accounted for.

Conclusions

This thesis has demonstrated that in New Zealand, vitamin D deficiency during pregnancy, lactation and childhood remains an important health concern. In Dunedin, at 45°S, both infant
and maternal deficiency during pregnancy and lactation is very common, and sometimes severe. Vitamin D deficiency rickets, the most severe manifestation of vitamin D deficiency is still occurring in New Zealand, with a higher incidence in children with mothers from India and Africa, and in children younger than three years who are currently or previously breastfed. While infancy and breastfeeding are risk factors for rickets, high-dose monthly maternal supplementation may also hold promise as an alternative dual maternal and infant supplementation strategy. Further research is now required to expand on the findings of the randomised controlled trial at the heart of this thesis.
Acknowledgements

I would like to express my heartfelt thanks to everyone who helped and contributed to me completing this work.

Firstly, special thanks go to my supervisors, Professors Barry Taylor, and Lisa Houghton who provided this opportunity, and have supported me throughout (as well as been very patient with me for my propensity to take on other projects and students!). I am very fortunate to have supervisors with such a wealth of knowledge and experience, and I very much appreciate the support and guidance they have provided me. Concerning Professor Taylor, this is just one more phase of my career in which he has guided me, and for his past, and ongoing support, I am grateful.

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Finally, I would like to thank and mention my family, Zuzana, Charlie, and Sam – thank you for being you, and for your love, support, and distraction when required, and thank you to my parents, Richard and Margaret for your continual support, love, and encouragement.
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<tbody>
<tr>
<td>1,25(OH)₂D</td>
<td>1, 25-DiHydroxyvitamin D</td>
</tr>
<tr>
<td>25OHD</td>
<td>25-Hydroxyvitamin D</td>
</tr>
<tr>
<td>AAP</td>
<td>American Academy of Pediatrics</td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>APEG</td>
<td>Australasian Paediatric Endocrinology Group</td>
</tr>
<tr>
<td>APPES</td>
<td>Asia Pacific Paediatric Endocrinology Group</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>Ca-Cr</td>
<td>Calcium to Creatinine</td>
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<tr>
<td>Ca/Cr</td>
<td>Calcium/creatinine ratio</td>
</tr>
<tr>
<td>CaSR</td>
<td>Calcium Sensing Receptor</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>D₂</td>
<td>Ergocalciferol</td>
</tr>
<tr>
<td>D₃</td>
<td>Cholecalciferol</td>
</tr>
<tr>
<td>FGF23</td>
<td>Fibroblast growth Factor 23</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>IOM</td>
<td>Institute of Medicine</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Liquid chromatography-Tandem mass spectroscopy</td>
</tr>
<tr>
<td>LMC</td>
<td>Lead Maternity Carer</td>
</tr>
<tr>
<td>MOH</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>NHANES</td>
<td>The National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>NIWA</td>
<td>National Institute of Water &amp; Atmospheric Research</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>NM-BAPTA</td>
<td>Chromophore 5-nitro-5'-methyl-(1,2-bis(o-aminophenox)ethan-N,N',N'-tetraacetic acid</td>
</tr>
<tr>
<td>NZ</td>
<td>New Zealand</td>
</tr>
<tr>
<td>NZPSU</td>
<td>New Zealand Paediatric Surveillance Unit</td>
</tr>
<tr>
<td>PSNZ</td>
<td>Paediatric Society New Zealand</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid Hormone</td>
</tr>
<tr>
<td>PHIL</td>
<td>Public Health Image Library</td>
</tr>
<tr>
<td>RAI</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>QMMC</td>
<td>Queen Mary Maternity Centre</td>
</tr>
<tr>
<td>RANK</td>
<td>Receptor activator nuclear factor-κB</td>
</tr>
<tr>
<td>RANKL</td>
<td>Receptor activator nuclear factor-κB ligand</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised Controlled Trial</td>
</tr>
<tr>
<td>RDI</td>
<td>Recommended Daily Intake</td>
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<tr>
<td>US</td>
<td>United States</td>
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<tr>
<td>UVB</td>
<td>Ultraviolet B</td>
</tr>
<tr>
<td>UVR</td>
<td>Ultraviolet radiation</td>
</tr>
<tr>
<td>VDBP</td>
<td>Vitamin D binding protein</td>
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<tr>
<td>VDR</td>
<td>Vitamin D receptor</td>
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<tr>
<td>VDDR</td>
<td>Vitamin D Deficiency Rickets</td>
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Publications/presentations associated with this thesis:

Publications:


Published abstracts:


Presentations:

Oral


Asia Pacific Paediatric Endocrinology Society (APPES) - Tokyo – November 2016


Australasian Paediatric Endocrinology Society (APEG) – Alice Springs – August 2016

Poster


APEG/APPES joint meeting Darwin – Oct 2014
1 Introduction

The broad aim of this thesis is to investigate aspects of the role of vitamin D during pregnancy, lactation, and childhood. More specifically, my thesis contributes to describing the incidence and clinical characteristics of vitamin D deficiency rickets in New Zealand children, explores a potential option for dual maternal and neonatal supplementation during exclusive lactation, and examines vitamin D status longitudinally spanning pregnancy and the first 5 months of lactation. These are questions that, in the New Zealand setting, have hitherto scarcely been addressed.

The concept for this thesis developed out of my clinical experience in paediatrics and paediatric endocrinology, in particular through my patients over the past 15 years who I have diagnosed and treated for childhood vitamin D deficiency rickets. My Master’s thesis (which subsequently converted to this PhD thesis) commenced with the New Zealand Paediatric surveillance unit study detailed in chapter 2, which investigates the characteristics and incidence of vitamin D deficiency rickets in New Zealand children. This project gave me a deeper understanding of rickets and vitamin D deficiency in the New Zealand context, and it was therefore logical to progress to an intervention study, which at its core seeks to test an intervention aimed at improving infant vitamin D status and thus potentially preventing rickets. In doing so, I also intentionally commenced recruiting early in pregnancy to assemble a longitudinal pregnancy cohort from which detailed descriptive data on vitamin D status in pregnancy and lactation could be derived – a first for the southern hemisphere, and one of the largest such studies to date.

The following literature review covers the background to these studies, in particular with a focus on vitamin D, rickets, and factors in pregnancy, lactation, and childhood that may contribute to a subsequent risk for rickets.
1.1 Literature review search strategy

For the following section a review of the literature was undertaken using various search strategies. As this thesis spanned multiple years of part time work, this has been a fluid process and has required updating on a regular basis. PubMed (National Library of Medicine, Bethesda, MD, USA) was used to perform initial and ongoing literature searches. The initial search strategy was similar to that used for the excellent and very thorough Institute of Medicine review on this topic (IOM, now known as the National Academy of Medicine)(A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011), and was as follows: first general searches on vitamin D, bone health and calcium. Articles were then screened by title, followed by abstract and full text as needed. This helped establish the primary literature. Secondary searches of this literature were then conducted focusing separately on vitamin D status and epidemiology, rickets, pregnancy, and lactation. The Cochrane library was also used. Additional hand searches were performed on reference lists of relevant papers and systematic reviews such as the IOM report (above). A less structured literature search related to more general aspects of bone health and development was also performed. All searches were limited to English language material (although relevant historical non-English papers were identified in reference list searches), but no time limits were placed on publication date, in order to identify evolution in the nature of the field over time, this was particularly relevant for aspects of the thesis relating to historical perspectives.
1.2 Vitamin D

With currently over 50,000 Ovid® Medline articles mentioning vitamin D as a key word, Vitamin D remains one of the most talked about and controversial vitamins of the last 100 years. This interest stems from its importance to bone health, the ease of which it is measured clinically (and the expense generated from this), and the ongoing debate about possible roles outside of bone health.

Vitamin D is unique among vitamins in that its principle source in humans (and most other living plants and terrestrial animals (Wagner, Taylor, Dawodu, Johnson, & Hollis, 2012)) is not dietary but comes from endogenous production following ultraviolet B (UVB) exposure from sunlight. The Oxford English dictionary defines a vitamin as:

“Any of a diverse group of organic compounds of which small quantities are needed in the diet because they have a distinct biochemical role, often as coenzymes, and cannot be adequately synthesized by the body, so that in most cases a deficiency produces characteristic symptoms or disease.”

(Oxford English Dictionary)

Therefore, following this classic definition, vitamin D is not in fact a “vitamin”, and a more accurate name might be a steroid prohormone. On the other hand, given that many humans now struggle to achieve the appropriate UVB exposure to ensure adequate vitamin D status, dietary vitamin supplementation remains vital, and thus using the word vitamin – as I shall continue to do in this document – is not inappropriate, not to mention the weight of history in favour of its use.

1.2.1 Structure, Synthesis, and metabolism

There are two main forms of vitamin D of relevance to humans. These are Cholecalciferol (vitamin D₃) and ergocalciferol (vitamin D₂) (Figure 1). These combined with a number of other metabolites I will henceforth collectively refer to as vitamin D unless otherwise specified. Cholecalciferol, the most commonly used form of vitamin D supplement in New Zealand, is pharmaceutically manufactured by the ultraviolet irradiation of 7-dehydrocholesterol obtained from Lanolin (a wax obtained from the wool of sheep, and other wool producing animals), whereas vitamin D₂ is produced via irradiation of ergosterol from yeast (M. F. Holick, 2007, 2009).
Cholecalciferol is also the form produced endogenously in humans and other vertebrates. It is a secosteroid (based on four carbon rings like all steroids, but with the B ring broken) (Hochberg, 2003). This process occurs following skin exposure of 7-dehydrocholesterol (found in the epidermis and dermis) to UVB radiation from the sun (wavelength 290 – 315 nm) (Elder & Bishop, 2014; M. F. Holick, 2007). Pre-vitamin D₃ is first formed, and then when combined with heat is rapidly converted to cholecalciferol. Excess pre-vitamin D₃ is however broken down to inactive photoproducts (lumisterol and tachysterol) with ongoing UVB exposure (M. F. Holick, 1987), which is the explanation for why too much sunlight exposure does not causing vitamin D toxicity.

Cholecalciferol is biologically inert and must undergo two separate hydroxylation steps, similar to ergocalciferol, to reach the biologically active hormone 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D). Vitamin D is transported to the liver in the venous circulation bound to the vitamin D binding protein (DeLuca, 2004; Elder & Bishop, 2014). Here (in the liver) the first hydroxylation step [catalysed by the enzyme vitamin D 25-hydroxylase (CYP2R1)] occurs to form 25-hydroxyvitamin D (25OHD₃). 25OHD₃ is the major circulating form of vitamin D, and due to its half-life of one month, makes it the main form clinically measured to determine vitamin D status (DeLuca, 2004; Elder & Bishop, 2014; M. F. Holick, 2007, 2009; Vieth, 1999). The second hydroxylation step predominantly occurs in the kidney (but also in some tissues including macrophages and the placenta) and is under the control of the 1α-hydroxylase enzyme (CYP27B1). The end product of these sequential reactions is 1,25(OH)₂D, the active form of vitamin D, which exerts its biological effect by subsequent binding to the vitamin D receptor (VDR). See Figure 1 as an overview of the above process.

The final part of this metabolic process is inactivation of 1,25(OH)₂D. This occurs via the process of 24-hydroxylation (DeLuca, 2004; M. F. Holick, 2007) carried out by 24-hydroxylase (CYP24). 24-hydroxylase converts 1,25(OH)₂D firstly to calcitocic acid which is excreted in bile (Elder & Bishop, 2014; M. F. Holick, 2007) (See Figure 2). Of interest clinically, autosomal inherited loss of function mutations in CYP24A1 have recently been discovered as a cause of infantile hypercalcaemia (Schlingmann et al., 2011). These mutations lead to a prolonged activity of 1,25(OH)₂D, due to failure of inactivation; and in consequence, in spite of a normally non-toxic exposure to cholecalciferol or ergocalciferol, a potentially dangerous hypercalcaemia results.
Figure 1: The structure and metabolism of vitamin D (Hochberg, 2003). © Copyright S. Karger AG, Basel, used with permission.
Figure 2: The metabolism and function of vitamin D. Reproduced with permission from (M. F. Holick, 2007), Copyright Massachusetts Medical Society.
The primary role of vitamin D is in maintenance of calcium and bone homeostasis (DeLuca, 2004; M. F. Holick, 2007). This is complex process is illustrated in Figure 2. Extracellular calcium is of critical importance for normal muscle and nerve cell function, and is maintained within a constant and tight range (Cooper & Gittoes, 2008; DeLuca, 2004). Both hypo- and hypercalcaemia rapidly lead to symptoms and can be life threatening. Classic symptoms of these are shown in Table 1.

**Table 1: Clinical manifestations of hypo- and hypercalcaemia**

<table>
<thead>
<tr>
<th>Hypocalcaemia</th>
<th>Neuromuscular excitability:</th>
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<tbody>
<tr>
<td>(Cooper &amp; Gittoes, 2008)</td>
<td>Muscle twitching/spasm/tetany</td>
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<td></td>
<td>Tingling/Numbness</td>
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<td></td>
<td>Seizure</td>
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<td></td>
<td>Cardiac arrhythmia/prolonged QT interval</td>
</tr>
<tr>
<td>Other:</td>
<td>Neuropsychiatric symptoms</td>
</tr>
<tr>
<td></td>
<td>Cataract</td>
</tr>
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<td></td>
<td>Raised intracranial pressure</td>
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<table>
<thead>
<tr>
<th>Hypercalcaemia</th>
<th>General:</th>
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<tr>
<td>(Minisola, Pepe, Piemonte, &amp; Cipriani, 2015)</td>
<td>Flushing, fatigue, weight loss, anorexia</td>
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<tr>
<td></td>
<td>Cardiovascular:</td>
</tr>
<tr>
<td></td>
<td>Various ECG abnormalities including - Prolonged PR interval, widened QRS complex, shortened QT interval, bundle branch block, bradycardia</td>
</tr>
<tr>
<td></td>
<td>Arrhythmias and cardiac arrest</td>
</tr>
<tr>
<td></td>
<td>Valvular heart disease and vascular calcification</td>
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</table>
Hypertension

**Renal:**

Polyuria and dehydration

Polydipsia and thirst

Renal stones / nephrocalcinosis

**Neurological:**

Decreased concentration/confusion /delirium

Hypotonia/hyporeflexia/weakness

Dementia/memory loss

Sleep disturbance

**Psychiatric:**

Irritability, depression, anxiety, psychosis

**Gastrointestinal:**

Nausea/vomiting

Abdominal pain

Constipation

**Skeletal:**

Bone pain

Osteoporosis

Fracture

Joint calcification
As shown in Figure 2 and Table 2, both vitamin D and parathyroid hormone (PTH) are at play to regulate calcium. PTH is released from the four parathyroid glands in response to decreasing ionised calcium, detected by the calcium sensing receptor (CaSR) located on the surface of parathyroid cells (Hochberg, 2003). Firstly, PTH works on the kidney to activate 1α-hydroxylation to stimulate 1,25(OH)_{2}D synthesis. Secondly, also at the kidney, PTH acts at the proximal tubule to increase calcium absorption, and decrease phosphate resorption. PTH in concert with 1,25(OH)_{2}D also has direct bone effects (described below).

Vitamin D acts to increase calcium via three main mechanisms, all mediated via the VDR: 1) increasing intestinal calcium and phosphate absorption. This is done via 1,25(OH)_{2}D interaction with the intestinal VDR (DeLuca, 2004; M. F. Holick, 2007); 2) Mobilisation of calcium stores from bone. Acting in conjunction with parathyroid hormone (both must be present) 1,25(OH)_{2}D stimulates osteoblasts to produce Receptor Activator Nuclear Factor-κB ligand (RANKL). RANKL acts on Receptor Activator Nuclear Factor-κB (RANK) and these are essential regulators of osteoclast function (DeLuca, 2004; Wada, Nakashima, Hiroshi, & Penninger, 2006). This process stimulates osteoclastogenesis as well as activating inactive osteoclasts, both processes leading to bone resorption and thus liberation of stored calcium and phosphate. Of interest, sex steroids such as oestrogen and androgens inhibit RANKL induced osteoclast stimulation and their loss is one mechanism for increased post-menopausal osteoporosis (Wada et al., 2006); lastly 3) again in conjunction with PTH (highlighted above), 1,25(OH)_{2}D acts on the kidney to directly increase calcium absorption. Finally, Fibroblast growth Factor 23 (FGF23) briefly mentioned in Figure 2 (M. F. Holick, 2007), is important to mention as part of this complex system. FGF23 is produced in bone (by osteocysts and osteoblasts) and has a primary role to maintain serum phosphate levels within the normal range, which is achieved by its role as an important part of the negative feedback loop on both PTH and 1,25(OH)_{2}D (Pool & Wolf, 2017).
Table 2: Sites and actions for PTH and calcitriol (1,25(OH)_{2}D)

<table>
<thead>
<tr>
<th>Site:</th>
<th>Action: PTH</th>
<th>Action: Calcitriol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone (osteoblast)</td>
<td>In synergy with 1,25(OH)_{2}D stimulates osteoblasts to produce RANKL,</td>
<td>In synergy with PTH stimulates osteoblasts to produce RANKL, which stimulates</td>
</tr>
<tr>
<td></td>
<td>RANKL, which stimulates osteoclasts. Leads to bone resorption and liberation</td>
<td>osteoclasts. Leads to bone resorption and liberation of stored calcium and phosphate</td>
</tr>
<tr>
<td></td>
<td>of stored calcium and phosphate</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>Activates renal 1α-hydroxylation to stimulate 1,25(OH)_{2}D synthesis</td>
<td>Stimulation of calcium reabsorption at renal tubule.</td>
</tr>
<tr>
<td></td>
<td>Direct action at proximal renal tubule. Stimulates calcium reabsorption and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>inhibits phosphate reabsorption</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Stimulates production of 1,25(OH)_{2}D – which leads to subsequent increasing</td>
<td>Increasing intestinal calcium and phosphate absorption</td>
</tr>
<tr>
<td></td>
<td>increasing intestinal calcium and phosphate absorption</td>
<td></td>
</tr>
</tbody>
</table>

**PTH** - parathyroid hormone; **1,25(OH)_{2}D** – calcitriol/1,25 dihydroxyvitamin D\textsubscript{3}

1.2.1.1 Non-osseous functions

The widespread presence of the VDR at sites other than the kidney, bone, and gut has given rise to considerable speculation as to possible non-osseous effects of vitamin D. Of particular interest is the existence of the VDR on cells of the immune and vascular system, giving rise to considerable exploration of the role vitamin D may play in infection, malignancy, autoimmune disease, and cardiovascular health (among others). These discussions have been detailed in a
number of systematic reviews, including but not limited to an excellent and very detailed assessment by the Institute of Medicine (IOM, now known as the National Academy of Medicine) on behalf of the United States and Canadian governments (Autier, Boniol, Pizot, & Mullie, 2014; Martineau et al., 2017; A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011; Theodoratou, Tzoulaki, Zgaga, & Ioannidis, 2014).

The key limitation from these findings, including the IOM report, is the observational-nature of the studies, presenting associations as opposed to demonstrating true causality. These include studies showing associations between low vitamin D status and cancer (Garland & Garland, 1980; Wu et al., 2007), cardiovascular health (Giovannucci, Liu, Hollis, & Rimm, 2008; Wang et al., 2008), diabetes (Pittas, Lau, Hu, & Dawson-Hughes, 2007), multiple sclerosis (Munger et al., 2004), and psychological health (Anglin, Samaan, Walter, & McDonald, 2013). However, this evidence has been deemed to be inconclusive, and notably often provides inconsistent (Freedman, Looker, Abnet, Linet, & Graubard, 2010) and conflicting results, e.g. convincing negative data from the 36,000 post-menopausal participants of the Women's Health Initiative study published in the New England Journal of Medicine, which showed no difference in incidence for colon cancer after 7 years of calcium and vitamin D supplementation ($p=0.51$) (Wactawski-Wende et al., 2006). In addition, various important confounders have also not been adequately controlled for in these studies. Importantly, in those studies with positive findings, the non-osseous outcomes described were often not the primary outcomes of the available trial data, usually being secondary analyses of data collected in studies looking at vitamin D status or bone outcomes.

However, considerable debate exists, and dissent is illustrated nicely by the title of this paper in response to the IOM “The vitamin D requirement during human lactation: the facts and IOM's 'utter' failure” (Hollis & Wagner, 2011). In addition, in children some interesting data are emerging in the area of respiratory health and infection. An example of this is the finding of a recent systematic review and meta-analysis, which found after analysis of 25 eligible randomised controlled trials and individual patient data for 10,933 participants (aged 0-95) that Vitamin D supplementation reduced the risk of acute respiratory tract infection (adjusted odds ratio 0.88 (95% CI 0.81 - 0.96) ($P <0.001$) (Martineau et al., 2017). This included data from New Zealand children showing reduced primary care visits for acute respiratory infection after high dose vitamin D supplementation in pregnancy and infancy (Grant et al., 2015). Again of note,
as with much of the trial data on non-osseous vitamin D outcomes, this was not the primary outcome of this NZ study.

Clearly more information from trials specifically addressing these questions are needed, but in the meantime, as per the above section (1.1.2), and the conclusions of the IOM report (A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011), calcium homeostasis and bone health remain the key role of vitamin D; and notwithstanding the interest of other factors, and given the practicalities of the present studies, bone remains the focus of this thesis.

1.2.2 Summary
Vitamin D is a complex fat-soluble vitamin with an essential role in calcium and bone homeostasis. In addition, based on growing data and the widespread presence of the VDR throughout the body vitamin D is likely to have many other secondary roles. It is present in two main forms: cholecalciferol and ergocalciferol. These then undergo two hydroxylation steps, first in the liver and then the kidney, to become the active hormone 1,25-dihydroxyvitamin D. 1,25OHD in conjunction with PTH and FGF23 are then responsible for the maintenance of calcium and phosphate homeostasis, and associated bone health.

1.3 Vitamin D status
The following section aims to summarise and address the literature on vitamin D status. This includes the epidemiology of deficiency, including a summary of the literature for the general population, children and infants, as well as that pertaining to pregnancy. However, before this can be addressed, an understanding of how and what is measured when referring to vitamin D status is required, as well as a summary of the current debate around definitions of vitamin D deficiency/sufficiency.

1.3.1 Measurement of vitamin D
As discussed in section 1.1.1, serum or plasma 25OHD is widely accepted as being the primary marker of vitamin D status (DeLuca, 2004; Elder & Bishop, 2014; M. F. Holick, 2007, 2009; A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes
When measured, 25-hydroxyvitamin D$_3$ makes up most of this total measured 25OHD in the New Zealand context, with 25-hydroxyvitamin D$_2$ making up a much smaller component, which varies based on D$_2$ dietary intake and supplement use.

25OHD is the preferred marker of status for a number of reasons:

1) It is the best indicator of incoming vitamin D from the diet and UVB photosynthesis (A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011).

2) It is also the major circulating form of vitamin D, measured in nmol/L, whereas, for example, 1,25(OH)$_2$D is measured in pmol/L.

3) It has a stable and long half-life –approximately one month (Vieth, 1999), vs. 1,25(OH)$_2$D (the active hormone) which is tightly regulated with a half-life of only 4-6 hours (Datta et al., 2017).

4) Finally, serum 25OHD levels are inversely correlated with a range of clinically relevant measures including PTH levels (Houghton et al., 2010; M. K. Thomas et al., 1998) and rickets (Markestad, Halvorsen, Halvorsen, Aksnes, & Aarskog, 1984). In comparison, 1,25(OH)$_2$D levels are directly and closely regulated by factors such as PTH and calcium status, and in the presence of even severe deficiency/rickets levels may be low, normal, or elevated.

There are a number of assays available to measure 25OHD. Liquid chromatography mass spectrometry (LC-MS/MS) is currently the technique of choice (Elder & Bishop, 2014); particularly due to increased sensitivity, improved differentiation and detection of both 25-hydroxyvitamin D$_2$ and D$_3$, and the potential (not available with all systems) to simultaneously measure other important metabolites such as the C3-epi isomer. Another common assay used to measure 25OHD is via radioimmunoassay techniques (RIA), which until recently was the method most frequently used (Zerwekh, 2004). RIA techniques remain acceptable, but have a number of potential drawbacks, including underestimation of 25-hydroxyvitamin D$_2$ and cross-reactivity with 24,25 dihydroxyvitamin D (Maunsell, Wright, & Rainbow, 2005), as well as the use of radioactive tracers (Maunsell et al., 2005).

Finally, of critical importance to both clinical and research analysis, 25OHD is very stable in serum/plasma, with evidence confirming this stability for periods of two years or more when stored at -20 to -80 degrees, despite undergoing multiple freeze/thaw cycles (Zerwekh, 2004).
1.3.2 Defining deficiency / sufficiency

Once 25OHD has been measured, there remains a lack of consensus with regards to defining optimal vitamin D status. The following is an attempt to briefly summarise the reasons for this debate:

The ideal would be a 25OHD threshold that unequivocally correlates with optimum general health; however, this remains to be determined. The key factor here is that determining a 25OHD threshold regarded as “normal” or “sufficient” varies depending on what clinical or biochemical endpoint one chooses. The non-osseous endpoints are lacking clarity currently (as discussed in 1.2.1.1 above), and as such clearly give rise to much of this debate. Therefore, for the sake of this thesis, and as previously stated, bone health will be the primary focus of the review. Rickets is arguably the most important bone consequence of vitamin D deficiency, and this rarely occurs at levels >30nmol/L (Munns et al., 2016; Munns et al., 2012; Ward, Gaboury, Ladhani, & Zlotkin, 2007), although higher levels than this (<50nmol/L) have been implicated in some studies, making an absolute threshold hard to establish (A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011). PTH, a marker of bone turnover, is inversely related to 25OHD status, achieves a plateau at levels of 25OHD of 60-90 nmol/L (M. F. Holick et al., 2011; Houghton et al., 2010; A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011). This is useful in establishing 25OHD status, but limitations include the fact that PTH values increase during some life stages e.g. puberty (Abrams et al., 2005). Lastly, the efficiency of intestinal calcium absorption may continue to improve until a threshold of ≥75nmol/L is reached (Heaney, Dowell, Hale, & Bendich, 2003).

In conclusion, given the above data, both national and international guidelines, as well as most experts agree that a 25OHD level of ≤50nmol/L is considered deficient (M. F. Holick, 2007; M. F. Holick et al., 2011; Paxton et al., 2013; A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011). However, defining sufficiency or what is a “normal” level remains a challenge, with definitions varying between 50nmol/L (Munns et al., 2016; Paxton et al., 2013) and ≥75nmol/L (M. F. Holick, 2009; M. F. Holick et al., 2011). The reasons for this variation in guideline and expert opinion regarding sufficiency are attested by the various PTH and calcium absorption data presented above. Specific aspects of this issue are also discussed in subsequent chapters on rickets (1.3), pregnancy and lactation (1.4).
1.3.3 Main determinants of Vitamin D status

Multiple factors influence an individual’s vitamin D status, and therefore potentially have a bearing on their risk for vitamin D deficiency and rickets. For individuals who have multiple concurrent risk factors, this is particularly important (as is often the case clinically). Factors influencing status are generally well recognised and can be summarised as:

1) Factors limiting skin exposure to UVB radiation e.g. Latitude, obstruction by clothing / religious or cultural clothing such as veiling, modern lifestyle factors, disability, chronic illness and/or prolonged hospitalisation.

2) Skin pigment – dark skin increases the risk of deficiency/rickets, and in the rickets literature is most commonly associated with those of African or South Asian (e.g. Indian) ethnicity.

3) Dietary factors – e.g. Degree of exposure to food fortification, supplementation, and for infants, exclusive breast-feeding.

4) Medical conditions that affect vitamin D metabolism or storage e.g. obesity, gastrointestinal malabsorption (celiac disease or inflammatory bowel disease), severe liver and/or renal disease.

5) Medications that interfere with vitamin D metabolism e.g. anticonvulsants or antibiotics (rifampicin).

Literature highlighting these factors is addressed below.

1.3.3.1 Factors limiting skin exposure to UVB radiation

Latitude is an important factor for UVB exposure, particularly for a country like New Zealand, spanning latitudes 35 – 47 degrees south. Much of this is related to the interplay of latitude and season. Higher latitudes (more southern or northern) experience greater variations in UVB exposure with season (Webb, Kline, & Holick, 1988) – and thus potentially greater fluctuations in measured 25OHD (Rockell et al., 2005) and PTH (Rockell, Skeaff, Venn, Williams, & Green, 2008). This is potentially very important in Otago at 45-47 degrees latitude (Houghton et al., 2010). Due to earth’s elliptical orbital of the sun, New Zealand also experiences greater UVB intensity (~7% greater) in Southern latitude (Hemisphere) summers vs the Northern hemisphere (Diffey, 2002).
In addition, UVB exposure clearly changes with time of day (earth rotating on its own axis) as well as with season (the tilted axis earth orbiting the sun). These factors influence the angle of the sun, e.g. the more oblique the sun’s rays are to the earth (as seen at the start or end of a day, or in winter/spring), the further UVB radiation has to travel, and the more atmospheric attenuation of solar UVB radiation occurs (Diffey, 2002). The National Institute for Water and Atmospheric Research (NIWA) in New Zealand have produced some detailed reviews on this topic. These demonstrate the markedly differing sun exposure times in New Zealand for vitamin D production between summer and winter e.g. 1-10 minutes summer (depending on extent of skin exposure – whole body vs face and hands) vs 20 – 200 minutes in winter (McKenzie, Liley, & Bjorn, 2009). Clinically, season is clearly identified as a risk factor in the rickets literature (Munns et al., 2012; Ward et al., 2007).

Public health policy also limits UVB exposure, as ultraviolet radiation (UVR) is a cause of skin cancer, including melanoma (AAP, 1999). This is particularly the case for younger children and infants, with the American Academy of Pediatrics (AAP) and New Zealand Ministry of Health (MOH) recommending no direct sun exposure to infants less than six months of age, in order to reduce skin cancer risk in later years (AAP, 1999; Ministry of Health, 2013). A recent study from Germany highlights this low UVB exposure in infants, with very low UVB exposure seen in the 40 study infants irrespective of season (Siafarikas et al., 2011). Sunscreen use also blocks UVR including UVB (Matsuoka, Ide, Wortsman, MacLaughlin, & Holick, 1987), although some promising efforts are being made to optimise sunscreens to allow for improved vitamin D synthesis (Kockott, Herzog, Reichrath, Keane, & Holick, 2016).

7-Dehydrocholesterol in the skin also decreases substantially with age, therefore reducing the body’s ability to make cutaneous vitamin D even with appropriate UVB exposure (Malik, 2007). When combined with the fact that UVB can only act on exposed skin and is unable to penetrate clothing or glass, those who spend the majority of their time indoors due to lifestyle or health reasons are at risk, particularly the institutionalised elderly (Malik, 2007). Clothing has the same effect e.g. the cultural practice of veiling substantially decreases vitamin D status (Beck-Nielsen, Jensen, Gram, Brixen, & Brock-Jacobsen, 2009).

In contrast, the negative relationships mentioned above is that vitamin D status when living under sun-rich conditions is generally sufficient (Vieth, 1999), with the highest level ever published purely attributed to UVB being 225nmol/L in a South American farmer (Vieth, 1999).
1.3.3.2 Skin pigment
Skin colour has a considerable influence on vitamin status. Melanin competes with 7-dehydrocholesterol for UVB photons, and therefore more UVB exposure is required in dark skinned individuals to generate the same vitamin D as light skinned (Clemens, Adams, Henderson, & Holick, 1982). Thus dark skin pigment, particularly when combined with the impacts of season and latitude (above), is one of the greatest risk factor for vitamin D deficiency and rickets, predominating in those of African and South Asian (Indian) descent (seen in Table 3 – page 34).

1.3.3.3 Dietary factors
Dietary intake in the absence of fortification is not normally an important source of vitamin D (small quantities are naturally found in fatty fish, liver, eggs, shiitake mushrooms – in decreasing order of quantity) (Ministry of Health, 2013). This is particularly the case in New Zealand where comparatively little vitamin D food fortification occurs, as opposed for North America where there is universal supplementation of milk products (Moore, Murphy, & Holick, 2005; Vatanparast, Calvo, Green, & Whiting, 2010). However, even with near universal supplementation of dairy products (usually the biggest source of fortification) individuals often still do not achieve a recommended daily intake (RDI) of vitamin D in the diet (Moore et al., 2005; Vatanparast et al., 2010). This lack of fortification in NZ provides strong grounds for conducting vitamin D trials, and observational studies such as those described in Chapters 3 and 4.

Breastmilk, the ideal food source for infants, is naturally a poor source of vitamin D, a topic which will also be discussed in the subsequent section on vitamin D and lactation (section 1.4.2). For this reason, breastfeeding, particularly when prolonged is an insignificant contributor to vitamin D status compared to UVB (Specker, Tsang, & Hollis, 1985). It is also an independent risk factor for deficiency and rickets (Munns et al., 2012; A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011; Ward et al., 2007). However, in contrast, due to fortification, commercial infant formula when consumed at a volume of 1000ml daily, does reach the RDI (A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011).

In response to the low vitamin D state of breastmilk, many countries and guidelines recommend universal daily supplementation of exclusively breastfed infants (Wagner, Greer, American
While trials have demonstrated appropriate vitamin D status when used in doses of between 250-500IU/day (as per various RDI guidelines) (Siafarikas et al., 2011), rickets continues to be seen in countries recommending universal supplementation (Ward et al., 2007; Weisberg, Scanlon, Li, & Cogswell, 2004). This is in part due to the degree of adherence to guidelines, which has been found to be very variable (Crocker et al., 2011; Gallo, Jean-Philippe, Rodd, & Weiler, 2010; Lehtonen et al., 2014; Perrine, Sharma, Jefferds, Serdula, & Scanlon, 2010; Taylor, Geyer, & Feldman, 2010). For example, a recent study from the United States suggests that 80% of breastfed infants failed to achieve the recommended intake of 400IU/day (Ahrens, Rossen, & Simon, 2016). Closer to home, data from Australia suggests very few breastfed infants receive vitamin D supplementation (Lehtonen et al., 2014), which is likely to be very similar to the NZ situation.

Clearly, supplementation at all ages positively contributes to vitamin D status. For this thesis, more detail will be provided in the sections related to pregnancy and lactation. Of importance, there is often, as discussed in the previous paragraph, a poor uptake of supplement recommendations, even for instance in high risk Middle Eastern populations (Saadi et al., 2006). Interestingly, the relationship between 25OHD and intake does not appear to linear e.g. increasing status above 50nmol/L requires more intake than for levels <50nmol/L (A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011). This clearly has important clinical implications when considering supplementation and dosing regimens.

Of interest, but only rarely implicated in rickets, some dietary factors may also be detrimental to both calcium and vitamin D status e.g. the high phytic acid content in some south Asian foods (Ladhani, Srinivasan, Buchanan, & Allgrove, 2004), and some unfortified health food milk alternatives (Carvalho, Kenney, Carrington, & Hall, 2001).

1.3.3.4 Medical conditions that affect vitamin D metabolism or storage

Obesity, one of the commonest medical conditions, has a direct effect on vitamin D status, being associated with lower levels of serum 25OHD (A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011). As vitamin D is a fat-soluble vitamin, this is likely mostly due to sequestration of 25OHD into adipose tissue. Additional evidence for this is seen in the fact that 25OHD levels may actually
increase when weight loss is achieved (Reinehr, de Sousa, Alexy, Kersting, & Andler, 2007; Tzotzas et al., 2010).

Any disease process which chronically reduces liver or renal function is likely to have an adverse impact on 25OHD status (M. K. Thomas et al., 1998). This is due to the importance of the liver and kidney for hydroxylation of vitamin D to 1,25(OH)₂D. Again, as a fat-soluble vitamin, disease states associated with malabsorption are also implicated in low vitamin D status. Classically these include coeliac disease and inflammatory bowel disease. However, a recent study has suggested that when BMI (as a marker of adiposity) was controlled for, vitamin D status in fact did not differ between those with and without celiac disease (Villanueva, Maranda, & Nwosu, 2012).

1.3.3.5 Medications
A number of medications are well documented to interfere with vitamin D metabolism, and thus negatively influence vitamin D status. These include anticonvulsants (particularly phenytoin and carbamazepine); antibiotics (rifampicin); and potentially some anti-retroviral drugs and anti-hypertensives. The drugs all share a common mechanism for this interference, which is in the activation of the Pregnane X receptor. This is an intracellular receptor that plays a role in de-toxifying drugs. Activation of the Pregnane X receptor upregulates 24-hydroxylase and therefore increases catabolism of 1,25(OH)₂D and 25OHD, thus potentially decreasing vitamin D status (Grober & Kisters, 2012; Michael F. Holick, 2005).

1.3.3.6 Other factors
Finally, specific to newborn infants, not surprisingly, infant vitamin D status is strongly influenced by maternal status in pregnancy (Ladhani et al., 2004), with the two closely correlated (Bowyer et al., 2009; Grant et al., 2014; S. D. Thomas, Fudge, Whiting, & Coates, 2011), infant levels being approximately 75-80% of maternal levels. This will be discussed further in Section 1.4.1.
1.3.4 Epidemiology of Vitamin D deficiency

The epidemiology of vitamin D deficiency has varied over the past century. Previously heralded as a disappearing diagnosis (Harrison, 1966), by the 1990s numerous articles were appearing discussing deficiency in adults and rickets in children. Given that determinants of status have been discussed previously, this section will provide an overview of the prevalence of vitamin D deficiency in children, adults and pregnancy. The epidemiology of vitamin D deficiency rickets will be discussed in 1.3.3.

1.3.4.1 Prevalence of vitamin D deficiency in childhood

Vitamin D status in newborns has been examined both within New Zealand and abroad. Overall deficiency is relatively common at all ages. New Zealand provides an ideal place to investigate vitamin D deficiency due to our southern latitude, variable UVB levels with season, and minimal public health policy for supplementation and food fortification.

Camargo et al. have published data from Christchurch and Wellington on cord blood levels of newborns (Camargo et al., 2010). The median cord blood 25OHD from this population of 929 newborns was deficient at 44nmol/L, with 57% demonstrating levels <50nmol/L and only 27% reaching sufficiency defined as ≥75nmol/L. In this sample, the main determinants of deficiency were season and non-European ethnicity. Emphasising breastfeeding as an additional risk factor, a study in 2-3 month old exclusively breastfed infants in Auckland found a median 25OHD of 53nmol/L, with 24% <27.5 nmol/L. Season was again important. In slightly older children from Auckland (aged 6-23 months), deficiency was associated with season (winter), ethnicity (Pacific), age (younger), and no exposure to “follow-on” formula (Grant, Wall, Crengle, & Scragg, 2009). Local Dunedin data in children aged 12-22 months are very similar with the mean winter 25OHD value of 38.7nmol/L compared with summer values of 74.1nmol/L (a 35nmol/L difference between summer and winter at the Dunedin latitude of 45°S). In these data, in addition to season, breastfeeding and higher primary caregiver education attainment were negatively associated with status, and smoking and male gender positively associated (Houghton et al., 2010). In older children (aged 5-14 years), a snapshot of national data are provided by 1585 children in the 2002 NZ National Children’s Nutrition Survey (Rockell et al., 2005). Overall, the mean 25OHD level was 50nmol/L, season and ethnicity again were major determinants, with risk of deficiency more commonly reported in Maori and Pacifica children (mean 25OHD levels of 43 and 36nmol/L, respectively).
International data support these findings. With regards to the southern hemisphere, data from Sydney (latitude 34°S), using cord blood from 901 neonates found a median 25OHD of 60nmol/L (range 17-245) (Bowyer et al., 2009). Maternal levels were slightly lower, median 52nmol/L (17-174), however these were taken between 8 and 17 weeks prior to delivery (median 2.7 months).

In this study, a higher risk of low vitamin D status were associated with winter/early spring, veiling, dark skin colour, and younger maternal age. After adjusting for potential confounders, in this sample mean birth weight was 151 grams (95% CI 50-250) lower in vitamin D deficient mothers (Bowyer et al., 2009). Other Australian data provides similar rates of vitamin D deficiency in neonates and children (S. D. Thomas et al., 2011). Publications from around the world are similar, with variable rates of deficiency (depending on definitions) and similar determinants of status, including from large population based samples in the Northern Hemisphere (Lawson & Thomas, 1999; Prentice, 2008), and also sunny areas like the Middle East (Molla et al., 2005).

Finally, children of recent immigrants and refugees appear at particular risk. This is a worldwide phenomenon (Madar, Stene, & Meyer, 2009), but also seen in our region with deficiency found in 61% of children in a Sydney based study by Sheikh et al (Sheikh et al., 2009).

1.3.4.2 Prevalence of vitamin D deficiency in pregnancy

As with children and infants, vitamin D deficiency is common during pregnancy, and largely follows the same patterns with the same determinants. Specific longitudinal data and intervention trials (focusing on infant outcomes) in pregnancy will be discussed in 1.4.1, and as stated in 1.2.3.6 above, pregnancy is of great importance to subsequent infant status, as infant status is determined by maternal status in pregnancy with 25OHD levels being closely correlated.

Rates of antenatal deficiency vary from 10% in Queensland, Australia (latitude 12-28°S) (McLeod, Scott, Lust, & McIntyre, 2011) to 80-87% respectively in multi-ethnic high-risk samples from Melbourne (Grover & Morley, 2001) (38°S) and Wellington (41°S), New Zealand (Judkins & Eagleton, 2006). Latitude and ethnicity are clearly important as reflected in status from these studies. Median 25OHD in the mainly light-skinned Queensland sample was 92nmol/L (McLeod et al., 2011). This compares to the high risk (multi-ethnic, mostly dark skinned or covered) pregnancy sample from Wellington, where 100% of the Indian and Middle Eastern women had
levels below 50nmol/L, including 10 women who were discovered to have secondary hyperparathyroidism with elevated PTH levels (Judkins & Eagleton, 2006).

Veiled mothers are particularly at risk, with one study finding 71% of veiled mothers in a population based Sydney sample with levels <25nmol/L, and a risk of deficiency 21 times that of unveiled (Bowyer et al., 2009). This is confirmed in Middle Eastern data from Israel (Mukamel et al., 2001) and Saudi Arabia (Serenius, Elidrissy, & Dandona, 1984). However, as seen in the Wellington study above (where veiling was uncommon), deficiency and severe deficiency are not confined to those who are covered/veiled (Judkins & Eagleton, 2006).

Of great relevance to New Zealand, in a recent Auckland study, 43% of “low” risk light-skinned European women also had vitamin D deficiency (25OHD < 50 nmol/L) during winter and spring (Ekeroma et al., 2015). In addition, data from the New Zealand Adult Nutrition Survey highlights that one-third of New Zealand women of childbearing age (15 - 44 years) had vitamin D deficiency (Ministry of Health, 2012). This raises considerable concerns regarding New Zealand’s current targeted supplementation strategy for vitamin D in pregnancy (Ministry of Health, 2013), as many of the aforementioned women would not be offered supplementation under this strategy.

No data reporting vitamin D status in pregnancy for those living in the South Island latitudes are available, however given the latitude, and traditionally largely “low” risk European majority of the South, these data would also be of great importance to gaining a better understanding of whether a targeted vitamin D supplementation strategy to only those at “high” risk is suitable for southern New Zealand.

1.3.4.3 Prevalence of vitamin D deficiency in adults

Adult studies of prevalence also differ by definition of status and by the determinants of status, as highlighted above. Overall, as with children and pregnancy, deficiency is common. The National Health and Nutrition Examination Survey (NHANES) 2005/2006 examined 4495 US adults. Vitamin D deficiency as defined by 25OHD status <50nmol/L was found in 42% of this sample, and the major determinants of deficiency were non-white ethnicity, poor health, reduced diary intake (an important source of vitamin D in the US due to fortification). The effect of season was not examined. Similar population based NZ data from the New Zealand Adult Nutrition Survey 2008/09 found 32% of NZ adults have vitamin D deficiency (25OHD < 25
nmol/L (all seasons combined). Season, that is, late winter and early spring, was the major negative determinant of status, particularly for those living in the South Island (Ministry of Health, 2012). Those of Pacific ethnicity were also 2.3 times more likely to be deficient, as were those who were obese.

Additional risk factors (as seen in children and pregnancy), include recent immigration and refugee status, and include NZ experience (Wishart, Reeve, & Grant, 2007). In the Wishart NZ refugee data collected over 12 months in 2014/15, reproductive age women aged 17-45 years and men >46 years were at greatest risk. This risk with increasing age as discussed in 1.2.3.1, and is related to decreasing 7-dehydrocholesterol in the skin (Malik, 2007), and compounded by illness in the elderly, particularly those who are institutionalised (Malik, 2007).

1.3.5 Summary
Serum 25OHD is the primary biochemical marker of vitamin D status. While subject to some debate, vitamin D deficiency on the basis of bone health is defined as a 25OHD level <50nmol/L. Vitamin D deficiency remains common across the life cycle, including in childhood and in women of childbearing age and during pregnancy. There are multiple determinants of vitamin D status, the most important being season, and factors reducing skin UVB exposure such as dark pigment and clothing. Latitude (as a surrogate for solar radiation exposure) is also very important, and particularly relevant in countries like New Zealand that span multiple latitudes with associated large swings in 25OHD status with season. To date, there are minimal published studies from more southern NZ latitudes, and none focusing on vitamin D status during pregnancy.

1.4 Vitamin D Deficiency Rickets (VDDR)
Adequate supply of calcium and phosphate is required for successful mineralisation of bone osteoid tissue. Failure of this mineral supply (for any reason) results in a mineralisation defect. In adults this leads to osteomalacia (impaired mineralisation of bone matrix) and osteoporosis (overall low bone mass). In children, this failure leads to rickets. There are multiple causes of rickets, but as discussed above, vitamin D is vital for calcium and phosphate balance, and vitamin D deficiency is the leading cause of rickets in children. Rickets is a mineralisation defect, and as in adults, it causes generalised osteomalacia, but specific to children, due to their open growth plate and rapid growth rate, leads to a failure of mineralisation at the growth plate.
These two defects lead to the classic bony features of rickets, including: weakening of long bones, particularly those involved in weight bearing, hence the classic lower limb bowing seen in Figure 4; poor growth, delayed dentition, slowed motor development, and rarely, pathological fracture. The growth plate defect leads to the other stigmata of rickets, including swelling at the ends of long bones, particularly wrists and knees, costo-chondral abnormalities known as “rachitic rosary”, and frontal bossing of the skull. The most significant and dangerous feature of rickets is hypocalcaemia, particularly common during periods of rapid growth e.g. infancy and early childhood (Ladhani et al., 2004). As highlighted in Table 1 from section 1.1.2 hypocalcaemia is a leading cause of morbidity leading to neuromuscular irritability, tetany, paraesthesia, and eventually seizures (Munns et al., 2016).

1.4.1 Growth plate physiology and pathophysiology (rickets)

To understand rickets, one must first have a basic understanding of the growth plate. Rickets is an illness exclusive to childhood, due to the mineralisation defect that is present at the open growth plate or physis (Tiosano & Hochberg, 2009). The growth plate in the context of a long bone is illustrated in Figure 3. In children, longitudinal growth relies on proliferation and hypertrophy of growth plate chondrocytes (cells which produce cartilage, the flexible scaffold upon which bone is made) (Muir, 1995). This resultant cartilage then undergoes apoptosis (programmed cell death), vascular and bony invasion, and remodelling to form new bone tissue (Nilsson, Marino, De Luca, Phillip, & Baron, 2005). This whole process is referred to as endochondral ossification (Nilsson et al., 2005). The hardness and rigidity of final bone is due to the presence of hydroxyapatite (a crystalline complex of calcium and phosphate) in the osteoid matrix.

Longitudinal growth starts at the top of the histological image in Figure 3 and pushes down, with chondrocytes progressing through the stages from the relatively inactive initial germinal zone (often referred to as the resting zone), through the proliferating and hypertrophic zones. Once in the hypertrophic zone, cell division ceases, and at the bottom of the histological aspects of Figure 3, terminal chondrocyte maturation occurs with apoptosis, and vascular invasion. Mineralisation also occurs longitudinally between the columns of hypertrophic chondrocytes. This mixed tissue is subsequently remodelled in the metaphysis (zone incorporating the growth plate adjacent to the diaphysis) to become “bone” (Ballock & O’Keefe, 2003; Muir, 1995; Nilsson et al., 2005).
Resting Zone

Proliferating Zone

Hypertrophic Zone
- Type X Collagen
- Alkaline Phosphatase
- Matrix Vesicles
- Calcification
- Apoptosis

Vascular Ingrowth and Primary Bone Formation
Figure 3: (A) Long bone and the cartilaginous growth plate – [reproduced from open access source PLOS Biology distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited (Wolpert, 2010)]; (B) A histological depiction of the growth plate, illustrating chondrocyte development (Ballock & O’Keefe, 2003) Copyright Wolters Kluwer Health, Inc., used with permission.

For this normal process of growth plate mineralisation to occur, a sufficient supply of calcium, phosphate, and vitamin D must be available. While most attention is focused on calcium, phosphate also plays a vital role in rickets aetiology (Munns et al., 2016; Tiosano & Hochberg, 2009), and once serum phosphate concentrations decrease, defects in bone mineralisation occur, including rickets and osteomalacia. How hypophosphatemia leads to this is explained below.

Once serum calcium levels become insufficient [due to vitamin D deficiency in most cases worldwide, but in some very UVB rich (sunny) countries such as Bangladesh and Africa, may also occur in severe calcium intake deficiency (Fischer et al., 1999)], PTH levels rise in response. As discussed previously, PTH stimulates osteoclastic resorption of bone (via RANKL), liberating stored calcium and phosphate into the circulation. However, the effects of PTH on the kidney are key in rickets. PTH acts at the proximal tubule in order to maximise calcium resorption, but at the expense of phosphate loss. This causes lowered serum phosphate levels. Phosphate is a key promoter of chondrocyte apoptosis. This was initially shown in a series of experiments in embryonic chick tibial chondrocytes in which injection of phosphate caused dose and time dependant apoptosis (Mansfield, Rajpurohit, & Shapiro, 1999; Mansfield, Teixeira, Adams, & Shapiro, 2001). In states of lowered circulating phosphate, apoptosis of chondrocytes in the hyperplastic zone of the growth plate is therefore impaired, and further expansion and hyperplasia occurs in this zone (Munns et al., 2016; Tiosano & Hochberg, 2009). In addition, impaired cartilage mineralisation (including relative hypophosphataemia in the osteoblast) impairs vascular invasion at the level of the junction with the hypertrophic zone and metaphysis and hence new bone formation is impacted, with reduced removal and remodelling of cartilage in the hypertrophic zone. The resulting pathology at the tissue level is reflected in the classic radiological signs of rickets (Figure 4) including growth plate widening, cupping, and fraying (all due to under-mineralisation of the expanded hypertrophic zone) (Munns et al., 2016).
Figure 4: X-ray image of rachitic bone, and demonstration of classic changes including growth plate widening, cupping, and fraying (Tiosano & Hochberg, 2009). Copyright Japanese Society for Bone and Mineral Research.

The pathogenic mechanism as outlined above is common to all forms of rickets, both acquired and genetic, and is one method used to classify rickets: as PTH dependant (e.g. VDDR/nutritional rickets, VDR and post-receptor mutations); FGF23 dependant (e.g. X-linked or autosomally inherited forms of hypophosphatemic rickets); or renal (Fanconi syndrome) (Tiosano & Hochberg, 2009).
Figure 5: Child suffering from vitamin D deficiency rickets - image obtained from the Center for Disease Control via the Public Health Image Library (PHIL) at https://phil.cdc.gov/phil/details.asp (open access)
1.4.2 A brief history of rickets

Figure 6: Glissonius de Rachitide – “Glisson examines child with rickets as the mother looks on. Two more children with rickets play in the background and bones deformed by rickets hang on the wall.” The US National Library of Medicine digital collection http://resource.nlm.nih.gov/101434430 (open access)
The origins of the word “rickets” are somewhat unclear, but may originate from the German word “wricken” meaning “twisted” (Elder & Bishop, 2014). The first clear descriptions of rickets occurred in the 17th century, by English physicians Daniel Whistler in 1645, and Francis Glisson in 1650 (Glisson, Bate, Regemorter, & Pre-1801 Imprint Collection (Library of Congress), 1660). However, clearly the importance and impacts of vitamin D on human health were recognized well before this time. Not only was rickets described in early Greek and Roman medical writings of the 1st and 2nd century AD (Rajakumar, 2003), but the body’s thirst for vitamin D is considered to be the leading theory for the evolution of human skin colour (Yuen & Jablonski, 2010). This is supported by archeological evidence from the far north of Greenland, in which a colony of settlers appears to have become extinct secondary to “gross pelvic deformities of osteomalacia” (Murray, 1934).

In the 17th century, due to severe overcrowding and pollution in industrial cities, rickets was very common (the “first wave of rickets”), and despite excellent scientific descriptions of the disease, the aetiology remained poorly understood and the rationale of potential treatments poorly validated (Glisson apparently suggested splinting, cautery, and pendulous suspension of the infant to help straighten out crooked bones) (Rajakumar, 2003). It was not until the early 20th century, when Mellanby discovered the 4th vitamin (Mellanby, 1989), subsequently named “vitamin D” by McCollum (McCollum, Simmonds, Becker, & Shipley, 1922), that a proper scientific understanding of the basis of rickets could begin. Alfred Hess (and others), a paediatrician and nutrition researcher, pioneered the use of cod liver oil, rich in vitamin D, in an at-risk black community in 1917 (Hess & Unger, 1917). Interestingly, Hess also made observations of the increased risk of rickets with unsupplemented breastfeeding and seasonal variation (Hess & Unger, 1917; Hess & Weinstock, 1924a, 1924b). Also at this time, animal experiments by Shipley and Park (Shipley & Park, 1921) highlighted the healing of rickets with cod liver oil and UVB light exposure.

Despite these advances in knowledge and treatment, rickets continued to be common during the early 20th century. A local illustration of how common rickets was at this time comes from the Renwick hospital for sick infants in Sydney, Australia (Maddox, 1932). In this study, rickets was identified in 52% of 218 consecutive infants reviewed in the outpatient department. Maddox also offered some excellent advice on preventing rickets, much of which remains applicable today: “1) public education in the dangers to the child of an ill-balanced diet in the mothers as regards vitamin content during pregnancy and lactation…; 2) a stronger insistence
on daily exposure to the half-naked child to the direct rays of the morning sun; 3) The routine provision to all outpatients of a stronger preparation of cod-liver oil...”.

Use of cod liver oil and food fortification strategies (including increased use of infant formula) led to a decrease in rickets over the next few decades (Harrison, 1966). This was until increasing immigration to Europe and England of dark-skinned individuals from equatorial regions (e.g. India/South Asia, and the West Indies) led to the identification of a “second wave” of rickets (Allgrove, 2004). Again, successful public health campaigns targeting these new immigrants reduced rickets presentations (Dunnigan et al., 1985). Unfortunately, as will be discussed in the following epidemiology section, we are now in a “third wave” of rickets. In part, this is caused by lack of sun exposure due to modern lifestyles and sun avoidance measures, as well as lapses in awareness of existing public health policies for supplementation or targeted supplementation to at risk groups.

1.4.3 Epidemiology of vitamin D deficiency rickets
Clearly, as described in 1.3.2 a brief history of rickets (above), VDDR has always been with us, but worldwide concerns have more recently been raised that VDDR is again increasing in frequency (Allgrove, 2004; Elder & Bishop, 2014; Ladhani et al., 2004). Considerable data has been collected over the past 20 years and are collated in Table 3. When incidence has been calculated, this ranges from 2.9/100,000 – 60/100,000 depending on the population studied. Most of this data consists of retrospective case series, and are not national, but usually focused to specific areas or hospitals. In contrast, two recent prospective national paediatric surveillance unit studies have been conducted in Canada (Ward et al., 2007) and Australia (Munns et al., 2012), and these are likely able to give a more accurate idea of incidence for these countries. The first of these, conducted in Canada between 2002 and 2004, found 104 cases, giving an overall annual incidence of 2.9 cases/100,000 children aged < 18 years. The more recent Australian study was conducted between 2006 and 2007, and found a slightly higher overall incidence of 4.9 /100,000 children aged ≤ 15 years. To give these figures some clinical context, this is about the same as for childhood leukaemia at 4.4/100,000 (Kaatsch & Mergenthaler, 2008), and considerably less than for type 1 diabetes, one of the commonest chronic diseases in childhood, which has an estimated New Zealand incidence of 17.9 cases / 100,000 (Campbell-Stokes, Taylor, & New Zealand Children's Diabetes Working, 2005). Regarding incidence estimates from the retrospectively collected studies, the estimated incidence from Denmark was 2.9/100,000 (children aged 0-15 years), and 5.8/100,000 (aged 0-
3 years). In addition, for immigrant children in Denmark the incidence was 10-fold higher at 60/100,000 (Beck-Nielsen, Brock-Jacobsen, Gram, Brixen, & Jensen, 2009). It is also important to note that the above studies likely underestimate the total community burden of VDDR. This is because the data summarised in Table 3 are generally produced by hospital specialists and/or retrospective audit of hospital case records. As milder VDDR may be managed and/or incidentally diagnosed in the community by family doctors/general practitioners, these cases are missing from all published data.

Younger age appears to be an important risk factor, with incidence in the Canadian data 9/100,000 and 12/100,000 in those aged <1 and between 1 and 2 years, respectively (Ward et al., 2007). This is supported by retrospective regional incidence data from Japan in those <4 years, identical to Canada at 9/100,000 (Matsuo, Mukai, Suzuki, & Fujieda, 2009). In most of the retrospective case studies, we find mean/median (which ever was presented) ages usually less than 2 years (Blok, Grant, McNeil, & Reid, 2000; Kreiter et al., 2000; Nozza & Rodda, 2001). The prospective Australian study however is an outlier, with a median age at diagnosis of 6.24 years (Munns et al., 2012), although this is likely explained by the fact that most (75%) cases in this study were detected as part of screening of new refugee intakes into Australia.

Concerning gender, the risk is usually evenly distributed, particularly in the available prospective national data (Munns et al., 2012; Ward et al., 2007). There are discrepancies in this, with some retrospective data in younger children suggesting more males affected (Al-Atawi, Al-Alwan, Al-Mutair, Tamim, & Al-Jurayyan, 2009; Matsuo et al., 2009; Robinson et al., 2006), and one paper from the Denmark noting in children older than 4 years, veiled girls comprised 78% of the sample (Beck-Nielsen, Brock-Jacobsen, et al., 2009).

Supplementation with vitamin D is clearly also another vital factor. For example in the Canadian PSU study, there were no cases reported in children who had received vitamin D supplementation as recommended (400IU/day) (Ward et al., 2007). In fact, to the best of my knowledge, no cases of rickets have been reported in infants who have had the opportunity to receive adequate daily supplementation; this is also mentioned in the recent global consensus statement on nutritional rickets (Munns et al., 2016). Subsequently, un-supplemented breast-feeding is seen as a risk factor in most available studies (DeLucia, Mitnick, & Carpenter, 2003; Kreiter et al., 2000; Ladhani et al., 2004; Ward et al., 2007; Weisberg et al., 2004). This includes the prospective Canadian study, where 94% were breast-fed at the time of diagnosis, with only three exclusively fed formula (in these three cases, presentation with hypocalcaemia was seen.
shortly after birth, and maternal 25OHD levels were found to be “critically low” (Ward et al., 2007). The Australian PSU data also showed that duration of exclusive breastfeeding was inversely related to serum 25OHD levels in those aged < 3 years (Munns et al., 2012).

Other risk factors seen for VDDR are identical to vitamin D deficiency in general (discussed above in section 1.2.3), and these include: season (increased risk in winter/spring (Blok et al., 2000; Munns et al., 2012; Nozza & Rodda, 2001)); darker skin pigmentation (maternal and/or infant); non-European ethnicity (in the southern hemisphere usually African or south Asian (Blok et al., 2000; Munns et al., 2012)); and more northern/southern latitude.

VDDR is also more likely to occur in the presence of any chronic disease that may contribute to vitamin D deficiency, such as an underlying disorder of the liver, kidney, or fat malabsorption.
Table 3: Summary of main studies describing VDDR in children and adolescents (1998 – present)

<table>
<thead>
<tr>
<th>Author &amp; Year Published</th>
<th>Location &amp; Design (duration)</th>
<th>Rickets Definition</th>
<th>N</th>
<th>%male</th>
<th>Ethnicity</th>
<th>Age Mean / Median* (Range)</th>
<th>Rickets Incidence</th>
<th>Results of investigations Mean / median†</th>
<th>Presenting features</th>
<th>Other findings</th>
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<tr>
<td>Prospective study design</td>
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<tr>
<td>(Ward et al., 2007)</td>
<td>Canada - Prospective PSU* 2002 – 2004 (24m)</td>
<td>↑ALP And/or Xray ↓25OHD</td>
<td>104</td>
<td>52%</td>
<td>33% Black 23% Indigenous 13% ME 11% Caucasian</td>
<td>1.4 years (2weeks – 6.3 years)</td>
<td>0.03/1000 (0-18 years)</td>
<td>25OHD 15nmol/L ALP 1237 U/L Xray 93%</td>
<td>Limb Def 52% Seizures 19% Dev Delay 12% Fracture 11% Incidental 7% FTT 5%</td>
<td>Breast feeding 94% Dark skin 89% No children supplementation as per guidelines More cases winter/Spring</td>
</tr>
<tr>
<td>(Munns et al., 2012)</td>
<td>Australia - Prospective PSU* 2006-2007 (18m)</td>
<td>↑ALP And/or Xray ↓25OHD</td>
<td>398</td>
<td>55%</td>
<td>63% African 75% Refugees</td>
<td>Median 6.24yrs (0.2-15yrs)</td>
<td>0.05/1000 (0-15 years)</td>
<td>25OHD 18nmol/L ALP 407 (med) ↑ALP 100% Xray 71% ID 19% ↑PTH 49%</td>
<td>Screened 82%</td>
<td>Duration Breastfeeding ↑risk Dark skin 85% 31% mothers veiled Hypocalcaemia 12%</td>
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<tr>
<td>Retrospective study design</td>
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<tr>
<td>(Blok et al., 2000)</td>
<td>NZ - Retrospective Hospital case review 1998 (12m)</td>
<td>Xray ↓25OHD (&lt;25nmol/L)</td>
<td>18</td>
<td>55%</td>
<td>66% Indian 11% African 11% PI 5% Maori 5% Asian</td>
<td>1.2 years (3m–3yr)</td>
<td>----</td>
<td>25OHD &lt;14 ↑ALP 100% (&gt;350) Xray 100% ID 11%</td>
<td>Limb Def 39% Seizure 22% Incidental 22% FTT 11% Fracture 5%</td>
<td>Incomplete data on BF and Sun exposure 66% presented winter-Spring</td>
</tr>
<tr>
<td>(Kreiter et al., 2000)</td>
<td>USA (North Carolina) Retrospective Medical Centre Case review 1990 - 1999 (114m)</td>
<td>Xray</td>
<td>30</td>
<td>57%</td>
<td>100% Black</td>
<td>14.9m (5m–25m)</td>
<td>----</td>
<td>↑ALP 100% Xray 100% 60% ↓Ca 97% ↓PO 83% ↓25OHD</td>
<td>Limb Def 53% FTT 43% Seizure 7% D Delay 3%</td>
<td>100% BF Minimal Vit D supplements No info re Sunlight</td>
</tr>
<tr>
<td>Author &amp; Year Published</td>
<td>Location &amp; Design (duration)</td>
<td>Rickets Definition</td>
<td>Ethnicity</td>
<td>Age Mean / Median(^\d) (Range)</td>
<td>Rickets Incidence</td>
<td>Results of investigations Mean / median(^\d)</td>
<td>Presenting features</td>
<td>Other findings</td>
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<tr>
<td>(Nozza &amp; Rodda, 2001)</td>
<td>Australia (Melbourne) Retrospective Hospital case review 1994-1999 (57m)</td>
<td>Discharge coding</td>
<td>45% African 24% India/Pak 24% ME 5% Italy 2% Euro</td>
<td>16m (11d – 12years)</td>
<td>-----</td>
<td>94% ↑ALP 1165 89%(^\d) ↑PTH 29.54 55% ↓Ca 2.08 (1-2.6)</td>
<td>D walking 36% 25% Limb Def 22% Seizure 16% FTT 11% Incid 7% Bone pain 2% Fracture</td>
<td>42% presented in spring ↓Ca in 94% under 9m 56% of mothers had 25OHD – 81% &lt;25nmol/L, 90% &lt;40nmol/L.</td>
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<tr>
<td>(DeLucia et al., 2003)</td>
<td>USA (Conneticut) Retrospective Hospital case review 1986-2002 (132m)</td>
<td>Xray</td>
<td>79% Black 12% White 2% ME 5%LA 2% Asian</td>
<td>20m (4m–38m)</td>
<td>-----</td>
<td>↑ALP 100% Xray 100% 56% ↓Ca 56% ↓PO</td>
<td>Limb Def 76% FTT 43% Seizure 8%</td>
<td>94% BF 83% Low Ca diet Minimal Vit D supplements No info Sunlight</td>
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<tr>
<td>(Weisberg et al., 2004)</td>
<td>USA (17 States) Retrospective literature review of published cases 1986-2003 (204m)</td>
<td>Variable Xray and ALP</td>
<td>83% Black 4% Indian 2%LA 2%Indig &lt;1% Asian/ME</td>
<td>10.5-25m (4m–54m)</td>
<td>-----</td>
<td>↑ALP 99% Xray 98% 55% ↓Ca 64% ↓PO 68% ↓25OHD ↑PTH94%</td>
<td>Grouped % not given</td>
<td>96% BF Minimal Vit D supplements Low Diary overall Variable Sun exposure data Possibly some (minimal) Calcium deficient not Vitamin D</td>
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<tr>
<td>(Ladhani et al., 2004)</td>
<td>UK (London) Retrospective Hospital case review 1996-2001 (72m)</td>
<td>Variable Clin + Xray + Biochem</td>
<td>60% Asian (Indian in NZ) 40% Afro-Caribbean (Black)</td>
<td>Not clear</td>
<td>-----</td>
<td>Majority Not clearly stated Range ALP 266 – 8988 – median 989</td>
<td>Hypocalcaemia Peak in winter Majority of patients responded to treatment with Vitamin D Hypocalcaemia &lt;3 years or &gt;10 years</td>
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<tr>
<td>(Dawodu et al., 2005)</td>
<td>UAE (Al Ain Medical District) Retrospective Hospital case review 1999-2002</td>
<td></td>
<td>38</td>
<td>13.5m (2-30m)</td>
<td>-----</td>
<td>25OHD 8nmol/L ALP 834IU/L</td>
<td>Not stated</td>
<td>92 BF minimal sunlight exposure</td>
<td></td>
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<tr>
<td>Author &amp; Year Published</td>
<td>Location &amp; Design (duration)</td>
<td>Rickets Definition</td>
<td>N %male</td>
<td>Ethnicity</td>
<td>Age Mean / Median (Range)</td>
<td>Rickets Incidence</td>
<td>Results of investigations Mean / median</td>
<td>Presenting features</td>
<td>Other findings</td>
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<tr>
<td>Robinson et al., 2006</td>
<td>Australia (Sydney) Retrospective Hospital (3) case review</td>
<td>Biochemical and/or x-ray</td>
<td>126</td>
<td>37% South Asian 33% African 11% Middle East</td>
<td>15.1m -----</td>
<td>25OH D &lt;20nmol/L 73% 52% ↓Ca ↑PTH 80% ↑ALP 82%</td>
<td>Seizure 33% Bow legs 22% Screening 18% FTT 6%</td>
<td>Almost exclusively non-european (79% Australian born) BF 71% 68% winter/spring</td>
<td></td>
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<tr>
<td>Al-Atawi et al., 2009</td>
<td>Saudi Arabia (Riyadh) Retrospective hospital case review</td>
<td>Clinical, biochemical and Xray</td>
<td>283</td>
<td>Saudi</td>
<td>100% &lt;14m -----</td>
<td>25OH D &lt;12nmol/L 100% ↑PTH 94% ↓PO 70%</td>
<td>Seizure 34% Chest infection 33% Fracture 1%</td>
<td>70% BF</td>
<td></td>
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<tr>
<td>Matsuo et al., 2009</td>
<td>Japan (Hokkaido) Retrospective questionnaire based case review 1999-2004 (60m)</td>
<td>Xray and biochemical</td>
<td>31</td>
<td>Japanese</td>
<td>16m (0 – 41m) 0.09/1000 (aged &lt;4 years)</td>
<td>↑ALP 100% Xray 100% 25OH D 11nmol/L Ca / P - variable</td>
<td>Seizure 23% Bow legs 48% Incidental 29%</td>
<td>93% BF Peak winter/Spring (peak April)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beck-Nielsen, Brock-Jacobsen et al., 2009</td>
<td>Denmark National retrospective cohort of medical records</td>
<td>Biochemical and/or xray</td>
<td>112</td>
<td>Danish 26% Immigrant 74%</td>
<td>0.03/1000 (aged 0-15 years) 0.6/1000 immigrant children (0-15 years)</td>
<td>Xray evidence 75% 25OH D &lt;12.5nmol/L 78% ↑ALP 97% ↓Ca 68% ↑PTH 92%</td>
<td>Poor weight bearing Seizure 23%</td>
<td>75% cases January - June No prior vitamin D supplementation in 88% 78% of girls &gt;4 years veiled</td>
<td></td>
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</tbody>
</table>

#Not all had test taken; *PSU – Paediatric Surveillance unit; †Mean or median stated (variably reported from each individual reference); BF – Breastfed; PTH – parathyroid hormone; 25OH D – 25-hydroxyvitamin D; Ca - Calcium; P – phosphate; ALP – alkaline phosphatase; FTT – failure to thrive; def – deformity; ID – iron deficiency
1.4.4 Clinical presentation/features of rickets

The classic clinical presentations of children presenting with rickets are demonstrated in Figure 5 and Table 4. Presentation is often dependant on the age of the child. Hypocalcaemia is almost exclusively seen in those < 3 years of age (Ladhani et al., 2004; Nozza & Rodda, 2001; Ward et al., 2007). Fracture as a presentation remains quite controversial, particularly after a range of publications from Paterson (a controversial advocate for “temporary brittle bone disease” and vitamin D deficiency as an alternative explanation for fracture and trauma attributed to non-accidental injury (Paterson, 2009a, 2009b)). In the latest global consensus statement, fracture is acknowledged to occur with rickets, but only when clear radiological evidence of rickets is present (as opposed to simple vitamin D deficiency, or solely biochemical evidence of rickets) (Munns et al., 2016).

Table 4: Clinical presentation of rickets

<table>
<thead>
<tr>
<th>Skeletal abnormality/deformity‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor growth / Failure to thrive</td>
</tr>
<tr>
<td>Delayed motor milestones / weakness</td>
</tr>
<tr>
<td>Symptomatic hypocalcaemia – Seizure /Tetany (generally confined to those &lt;3 years)</td>
</tr>
<tr>
<td>Bone pain / Limp</td>
</tr>
<tr>
<td>Irritability</td>
</tr>
<tr>
<td>Respiratory disease</td>
</tr>
<tr>
<td>Eczema</td>
</tr>
<tr>
<td>Incidental finding / Found on screening e.g. sibling with rickets / refugee screening</td>
</tr>
<tr>
<td>Fracture</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
</tr>
</tbody>
</table>

‡ lower limb deformity (bowed legs); swollen wrists or ankles; large anterior fontanelle/splayed sutures; frontal bossing; delayed tooth eruption; rachitic rosary (enlarged costochodral joints of the ribs); craniotabes (a softening of the skull found in infants)
1.4.5 The diagnosis of vitamin D deficiency rickets

The diagnosis of VDDR is made by clinical (as discussed above 1.3.4) and biochemical evaluation and confirmed by xray (Munns et al., 2016). Figure 4 demonstrates a photograph of a child with classic vitamin D deficiency rickets.

The classic radiographic signs were discussed in section 1.3.1 above, and demonstrated in Figure 4. However, it should be noted that while the recent global consensus statement requires x-ray confirmation, a subset of young infants have been described who present with symptomatic hypocalcaemia and biochemical evidence of severe vitamin D deficiency / VDDR, but who do not have x-ray changes (Ladhani et al., 2004). In these infants it has been proposed due to their very young age, rapid growth, high calcium need, and inability to adequately mobilise calcium from the skeleton, symptomatic hypocalcaemia can occur before radiological changes have had time to develop (Ladhani et al., 2004).

The key biochemical markers of VDDR are:

1) Low vitamin D status as measured by serum 25OHD. This is essential to define VDDR and in most cases this should reveal deficiency (<50nmol/L) and in most cases severe deficiency, usually less than 30-34 nmol/L (Munns et al., 2016).

2) Elevated total Alkaline phosphatase (ALP). In addition to the liver, ALP is found in the cell membrane of osteoblasts, and therefore increased levels of total serum ALP may indicate increased bone formation and/or turnover (Corathers, 2006; Munns et al., 2016). Rickets represents a situation of markedly increased bone turnover. When measured, ALP is elevated in 94-100% of VDDR cases reported in the literature (even if ALP was not used in the initial definition of rickets) (Blok et al., 2000; DeLucia et al., 2003; Kreiter et al., 2000; Munns et al., 2012; Ward et al., 2007). This has led to ALP being used as part of the diagnostic criteria in the only two published prospective reviews of rickets incidence and characteristics (Munns et al., 2012; Ward et al., 2007).

3) Serum PTH, released in response to decreased ionised calcium, is raised in most cases of VDD (Elder & Bishop, 2014; Munns et al., 2016)

4) Classically in severe VDDR, serum calcium and phosphate are both low (particularly in younger children). However, in early or milder cases of VDDR, as both serum calcium and phosphate are tightly regulated and essential for life, bone can be mobilised (hence the rickets) to maintain normal serum status.
5) Serum measures of 1,25(OH)\textsubscript{2}D are not helpful in the acute situation for VDDR, as levels may be elevated, normal or low (Robinson et al., 2006).

Therefore, review of the above factors, as with the two prospective rickets trials to date, indicates that the best definition of simple VDDR consists of evidence of vitamin D deficiency (<50nmol/L), an elevated ALP and ideally accompanied by confirmatory x-ray findings at the wrist or knee (large and rapidly growing bones). Other findings such as an elevated PTH, or hypophosphataemia and hypocalcaemia, are supportive but not essential.

Finally, for a diagnosis of simple vitamin D deficiency rickets to be made, rickets secondary to any heritable disorder of vitamin D deficiency, including but not limited to: 1-alpha hydroxylase deficiency, vitamin D receptor defects, hypophosphataemic rickets, and secondary hyperparathyroidism of any other cause other than vitamin D deficiency, must also be excluded.

1.4.6 NZ vitamin D deficiency rickets data

Prior to the work presented in this thesis there has been relatively little NZ specific study of rickets, and nothing before 2000 (Blok et al., 2000). Blok et al (Blok et al., 2000) were the first to explore VDDR in New Zealand children, identifying 18 children, all <5 years of age, presenting with rickets to Auckland Starship Children’s Hospital in 1998. The key characteristics were: a median age of 12 months; 12/18 Indian origin, with no European children described; 12/18 presented in Winter-Spring (July-December) with a peak in August; and 10/18 had a history of breastfeeding, with a mean duration of 16 months. The most common presentation was with bony deformity/delayed walking in 7/18, while 3/18 presented with hypocalcaemic seizures all aged 12 months or younger. Biochemically, 11/18 had hypocalcaemia at presentation, 15/18 had 25OHD levels <12.5nmol/L, and all had raised ALPs. However, it is possible cases were overlooked, particularly milder cases, or infants with hypocalcaemia, as xray confirmation was essential for inclusion.

Only two other New Zealand papers mention VDDR. One, published in 2006 and focused on maternal status during pregnancy (Judkins & Eagleton, 2006), has a passing reference to rickets anecdotally found in a single Wellington GP practice database review which identified 10 cases in children < 5 years of age, over a three year period prior to publication. It was beyond the scope of this paper to describe the characteristics or features of rickets seen or even mention what diagnostic criteria were used. Finally, a single case report from 2008 (Wallis, 2008)
describes a classic case of rickets presenting as hypocalcaemia in a black African 5 month old male in Dunedin.

1.4.7 Summary
Vitamin D is essential for calcium and bone metabolism. Vitamin D deficiency is relatively common, and the most severe consequence of deficiency is rickets. VDDR is a disease unique to growing children, as it is a mineralisation defect affecting the open growth plate. Manifestations include bony deformity, poor growth, delayed dentition, slowed motor development, and symptomatic hypocalcaemia (seizures and tetany). The risk factors for rickets are relatively well defined, but most current epidemiological data relies on retrospective hospital case series. Prior to the present work (Chapter two), only two international prospective studies on rickets have been conducted, and few studies exist in the New Zealand context.

1.5 A focus on vitamin D during pregnancy and lactation

1.5.1 Vitamin D and Pregnancy
Vitamin D status and pregnancy epidemiology have been covered in previous aspects of this thesis, and highlight the point that deficiency during pregnancy is common in New Zealand and worldwide. As seen in the previous section, rickets is the most severe consequence of childhood vitamin D deficiency. Pregnancy plays an important role in the aetiology of rickets in the child, and this section will therefore focus on the impacts of maternal deficiency on the foetus and neonate, intervention trials in pregnancy to improve neonatal vitamin D status, as well as a spotlight on previous longitudinal studies looking at vitamin D status spanning pregnancy (and beyond).

1.5.1.1 Impacts of maternal deficiency on the foetus and neonate
Pregnancy is clearly one of the most important stages of the human lifecycle, and therefore nutrition during pregnancy is of critical importance. Regarding vitamin D nutrition, pregnancy is a vital period for not only maternal health, but also infant status, particularly with regard to skeletal development and mineralisation. The third trimester is the most important aspect and is where the majority of foetal skeletal mineralisation occurs (Givens & Macy, 1933; Trotter & Hixon, 1974). This is particularly important in the modern world, as once born, many infants
will have minimal sun exposure for the first 6 months of life (AAP, 1999; Ministry of Health, 2013), and a favourable milieu during in utero life may stand the child in better stead.

As early as 1924, Hess (one of the early vitamin D pioneers) stated “It would be interesting to ascertain how often women suffering from osteomalacia give birth to infants with congenital rickets” (Hess & Weinstock, 1924b). It has been subsequently confirmed that 25OHD readily crosses the placenta, and infant status at birth is closely correlated to maternal vitamin D status (Bowyer et al., 2009; Grant et al., 2014; Rodda et al., 2015; S. D. Thomas et al., 2011). Thus, Hess’s speculation was very precisely on the mark, and indeed it is from his inspired insight that much of the work in the present document flows. Multiple subsequent studies including those identifying a relationship between poor maternal D status and neonatal hypocalcaemic convulsions (Roberts, Cohen, & Forfar, 1973), and newer local data by Nozza et al. who showed that in young Australian children with rickets, 90% had mothers with severe deficiency themselves (81% <25nmol/L) (Nozza & Rodda, 2001) have since been conducted.

In addition, maternal status in pregnancy may impact on foetal growth (Bowyer et al., 2009), foetal bone accrual and subsequent bone size (Viljakainen et al., 2011; Viljakainen et al., 2010). Impacts on foetal bone have been seen as early as 19 weeks gestation using high resolution 3D ultrasound (Mahon et al., 2010), and out to 20 years post-partum (Zhu et al., 2014). RCT studies from regions with high rates of infant growth problems, have also suggested improvements in early postnatal linear growth (Roth et al., 2013).

Dental health also appears important. This was first seen in an early study from 1973, showing the presence of enamel hypoplasia in those with early neonatal tetany associated with maternal vitamin-D deficiency (Purvis et al., 1973). More recently, increased prevalence of early onset dental caries has been associated with poorer prenatal maternal vitamin D status (Schroth et al., 2014).

1.5.1.2 Supplementation trials in pregnancy and neonatal vitamin D status

Given the above, it was clearly apparent that pregnancy is an ideal time to ensure adequate vitamin D status. There are many reasons for this, including the high rates of deficiency described in previous sections, and noting the attractive potential of treating two patients in one go. The ideal situation would be safely increasing both maternal and foetal status enough during pregnancy, so that no infant supplementation would be required during lactation.
While there is limited data with follow up out to 4-6 months of exclusive breast-feeding, some evidence exists (Grant et al., 2014; Hollis, Johnson, Hulsey, Ebeling, & Wagner, 2011; March et al., 2015; Roth et al., 2013), with the most recent study by March et al (March et al., 2015). These studies have demonstrated that supplemental vitamin D doses ranging between 400 to 2000 IU/d during pregnancy result in substantially higher cord blood 25(OH)D (ranging from 73 to 95 nmol/L). March et al, supplemented mothers with 2000 IU/d from mid-pregnancy, and this appeared to protect 98% of unsupplemented breastfed infants against vitamin D deficiency (serum 25(OH)D < 30 nmol/L) out to at least until 8 weeks postpartum, the end point of the study (March et al., 2015).

In the only New Zealand study examining this to date, maternal pregnancy doses of 1000-2000 IU daily were given from 27 weeks gestation until delivery (Grant et al., 2014). This treatment considerably increased maternal status at 36 weeks compared to placebo, by 35nmol/L (95% CI 27.5-42.5), as well as infant cord blood status (by 27.5nmol/L). When combined with subsequent infant supplementation at 400IU or 800IU / day from birth to 6 months of age, 82-92% of infants had 25OHD status >50nmol/L at aged 6 months. In addition, no safety concerns were raised (at the above vitamin D supplementation doses) by this or any of the above pregnancy intervention studies. It must be noted that in order to achieve these rates of sufficiency at 6 months of age, postnatal infant supplementation was also required.

These studies highlight some important issues. Firstly, cord blood 25OHD levels reflect maternal status at delivery (but are overall lower, on average 35nmol/L lower than maternal 36 week gestation levels in the grant et al study) (Grant et al., 2014). This means that maternal pregnancy 25OHD status must be clearly > 50nmol/L in order to avoid deficiency in all infants at birth, highlighting the importance of maternal supplementation during pregnancy. Secondly, it is likely some form of infant supplementation is required regardless of maternal supplementation in pregnancy, to ensure sufficiency in exclusively breastfed infants to 4-6 months postnatally. At this stage, the currently available data only supports infant protection from 2000IU daily maternal pregnancy supplementation out to 8 weeks post-partum (March et al., 2015), at which point, in order to maintain sufficiency, additional infant supplementation is likely required.
1.5.1.3 Longitudinal studies in pregnancy

There are a number of published longitudinal studies investigating pregnancy vitamin D status and beyond to varying degrees, of which only three had been published when the longitudinal study detailed in Chapter 4 was commenced. In addition, to date all available studies are from the northern hemisphere, latitudes ranging from 39°N - 63.8°N (Cross, Hillman, Allen, Krause, & Vieira, 1995; Haliloglu et al., 2011; Holmes, Barnes, Alexander, McFaul, & Wallace, 2009; Lundqvist, Sandstrom, Stenlund, Johansson, & Hultdin, 2016; Milman, Hvas, & Bergholt, 2011; Ritchie et al., 1998; Zhang, Lucey, Horgan, Kenny, & Kiely, 2014).

The first, published in 1995, followed 10 women (39°N Kansas, US) who were part of a larger calcium supplementation trial, and therefore 6 out of 10 participants were in a calcium intervention arm during the study (Cross et al., 1995). Key findings were an increase in 25OHD over pregnancy, while on a pregnancy supplement containing vitamin D, and PTH concentrations that were higher post-weaning than during pregnancy (p<0.01). 1,25(OH)2D levels also increased across pregnancy and then declined during lactation and post-weaning. An important factor in these participants was the sufficient 25OHD in all subjects throughout the study (likely due to dietary fortification and pregnancy supplement use). This makes the findings less translatable to the NZ setting where deficiency is common, and season is a major determinant of status.

The second, published in 1998 (Latitude 40°N Berkley, US) is similar, again largely focusing on calcium, and with only 14 participants. Like Cross et al. above, as the focus was on calcium, season is not mentioned nor taken into account which is an important confounder of 25OHD status, and again all participants were taking vitamin D and calcium supplements (Cross et al., 1995; Ritchie et al., 1998). Similarly, PTH also appeared lower in pregnancy, but this did not achieve statistical significance. If this were a valid observation, a potential contributory mechanism could be secondary to 1,25(OH)2D increases in pregnancy (Ritchie et al., 1998).

The third study, published in 2009, was confined to pregnancy, with no postnatal assessment, and was in 99 women, at latitude 54-55°N Belfast, Ireland. Similar to the study by Cross et al., assessments were taken around 12, 20, and 35 weeks (Holmes et al., 2009). Compared to Cross and Ritchie, only 22% were on a vitamin D containing multivitamin preparation. Spanning pregnancy, between 76-96% of all participants had a 25OHD level <50nmol/L. Season and low supplementation were risk factors. Non-pregnant controls were included, and 25OHD status in pregnancy was significantly lower than non-pregnancy, with 25OHD levels increasing at the end
of pregnancy and postpartum (although season clearly played a role in this increase as 1st trimester was winter for all, with all deliveries in summer).

A trend of decreasing maternal 25OHD as pregnancy progressed was also seen in a recent study from Zhang et al, who reported this pattern irrespective of season (Zhang et al., 2014). This has also been seen compared to non-pregnancy controls in one study (Holmes et al., 2009). However, Milman et al (Milman et al., 2011) found an increase in 25OHD until 32 weeks followed by a decrease to delivery. From Scandinavia, Lundqvist et al (Lundqvist et al., 2016) similarly found a steady increase over pregnancy, as has Park et al. based on a single measurement at 26-29 weeks (Park et al., 2016). The reason for these contrasting results remains uncertain, but differing season, dietary intake/supplement use/other life style factors, and ethnicity potentially all may play a role. Physiological explanations may also be possible, including variations in the VDR, or vitamin D binding protein (VDBP). Analysis issues may also contribute, for instance the detection or not of the C3-epimer of vitamin D (Yazdanpanah, Bailey, Walsh, Wan, & Adeli, 2013), which has also been shown to increase in pregnancy (Park et al., 2016).

Fluctuation in other aspects of vitamin D metabolism have been revealed in pregnancy by these longitudinal studies, and may have a role in these 25OHD changes. This includes increasing VDBP levels during pregnancy (Park et al., 2016; Zhang et al., 2014), and the possible PTH suppression mentioned above and in Haliloglu et al. (Haliloglu et al., 2011)). Certainly, compared to non-pregnancy, the relationship between PTH and 25OHD appears weaker. 1,25OHD levels have also been reported to increase as pregnancy progresses (Hollis et al., 2011), and this is likely interconnected to these possible VDBP and PTH changes (Cross et al., 1995; Ritchie et al., 1998; Wilson, Retallack, Kent, Worth, & Gutteridge, 1990). Increases in 1,25(OH)₂D are likely due to increased maternal renal and placental 1α-hydroxylase activity (Turner, Barre, Benjamin, Goltzman, & Gascon-Barre, 1988).

There are limitations in this available northern hemispheric longitudinal data. These include: 1) variable and often high rates of maternal supplement use, varying from 22-100% of participants; 2) high rates of dietary fortification (when documented), estimated to equate to approximately 200IU/day; 2) infant data is not included; 3) variable reporting of PTH; and 4) variable reporting and distribution of participants by season (Cross et al., 1995; Haliloglu et al., 2011; Holmes et al., 2009; Lundqvist et al., 2016; Milman et al., 2011; Ritchie et al., 1998; Zhang et al., 2014). The mixed findings of these studies, and their potential limitations, leave many questions
unanswered regarding vitamin D status and metabolism longitudinally during pregnancy and lactation.

Finally, an important message coming from these studies is that overall, vitamin D deficiency (25OHD <50nmol/L) is not uncommon, being seen in 19-96% of participants at one or more time points (Holmes et al., 2009; Milman et al., 2011). Based on this, all these studies conclude with a statement on the importance of supplementation with vitamin D during pregnancy in the Northern hemisphere context. Public health policy in New Zealand currently uses a targeted, albeit vague strategy, stating that women at “higher risk” may benefit from supplementation (Ministry of Health, 2013). Further, other than in large doses (50,000IU capsules), there is currently no funded daily vitamin D supplement in New Zealand. However, the policy also specifically states these guidelines will be updated when new research comes to light, and acknowledges that New Zealand studies are ongoing.

Chapter 4 of this thesis focuses on a longitudinal descriptive study in pregnancy and contributes new knowledge, including describing status in largely unsupplemented infants and mothers out to month five of lactation. An aim of the project is to provide further information to inform public policy on vitamin D during pregnancy and lactation, and in particular, to add detailed longitudinal data from southern NZ.

1.5.2 Vitamin D and Lactation

Breast milk is the ideal food for infants, with current global recommendations aiming for 6 months of exclusive breastfeeding (Kramer & Kakuma, 2002; Ministry of Health, 2013). However, breast milk is not normally a significant source of vitamin D for the neonate unless maternal supplement intakes are >2000IU/day (A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011). This is compounded by the fact that vitamin D deficiency is common in pregnancy and in women of childbearing age (Saadi et al., 2007). In addition, as mentioned previously, unsupplemented breastfeeding (particularly when prolonged) is an independent risk factor for VDDR.

Given this, a number of countries and guidelines recommend universal supplementation of breastfed infants with daily vitamin D (usually 400 IU/day) (Finberg, 1981; Munns et al., 2016; Wagner et al., 2008). However, uptake of infant supplementation can be variable (Crocker et al., 2011; Gallo et al., 2010; Perrine et al., 2010; Taylor et al., 2010), with up to 80% of breastfed
infants failing to reach RDIs of vitamin D (Ahrens et al., 2016). This has led to a number of studies investigating strategies of increasing breast milk vitamin D content via maternal supplementation. This section will therefore address the literature on breastmilk vitamin D, and supplementation trials to increase breastmilk vitamin D concentrations.

1.5.2.1 How much vitamin D is there in breast milk?

It is well established that breast milk is not naturally a significant source of vitamin D. While Hess and early researchers in the 1920s noticed that there were high rates of rickets even in breastfed babies (Hess & Weinstock, 1924b), it was not until the 1980s that Hollis et al. in a number of studies using high-performance liquid chromatography (HPLC), confirmed the naturally low vitamin D concentrations in milk (Hollis, 1983; Hollis, Roos, Draper, & Lambert, 1981). These and other more recent studies have confirmed in natural, non-fortified (e.g. exposed to high dose maternal supplements/UVB) human milk the vitamin D mean activity appears to range from 35-52nmol/L (Hollis & Wagner, 2004; Wall et al., 2016).

This vitamin D activity of human milk is predominantly composed of dietary cholecalciferol or ergocalciferol, with only a small proportion coming from 25OHD (Greer, Hollis, & Napoli, 1984; Oberhelman et al., 2013). As a result, milk vitamin D activity is largely dependent on how much D$_2$ or D$_3$ is consumed, or generated by UVB exposure (D$_3$ only), and this D$_3$ is only detectable in milk for a very short period (out to 3-7 days) post-ingestion (Oberhelman et al., 2013).

It should also be noted that measuring vitamin D activity in milk is a complex and expensive process, particularly relating to obtaining an appropriate milk sample (as most vitamin D activity is in the fat of hind milk (Hollis, 1983; Hollis & Wagner, 2004)); and extraction of lipid and other sample preparation prior to analysis (Hollis, 1983). These factors have limited the analysis (and for those analysing - the accurate analysis) of breastmilk vitamin D activity, with only a handful of studies actually achieving this (Hollis & Wagner, 2004; Saadi et al., 2009; Wagner, Hulsey, Fanning, Ebeling, & Hollis, 2006; Wall et al., 2016). Once specimen preparation has occurred the final analysis includes the measurement and addition of relevant compounds including vitamin D$_2$, D$_3$, 25OHD$_2$ and 25OHD$_3$ (Wall et al., 2016).

These findings have led to major guidelines, including the global consensus recommendations on the prevention and management of nutritional rickets recommending traditional RDIs of
vitamin D for lactating mothers to support their own needs, but not as a means of supplementing their infants (Munns et al., 2016).

1.5.2.2 Can we increase the vitamin D content of breast milk?

The naturally low human milk levels leads to an important question of whether maternal supplementation could provide adequate vitamin D intake for both mothers and their babies during lactation.

Hess was the first to ask this question, and he conducted a number of experiments using cod liver oil in rats. Unfortunately, all failed to show a benefit (Hess & Weinstock, 1924b). This led to little interest in breastmilk as a vehicle for preventing or treating rickets until the late 20th century. In the 1980s, the first studies by Greer and Hollis confirmed that breastmilk vitamin D activity could be substantially increased, and in one publication up to a staggering equivalent of 7660 IU/L (compared to the 400 IU/L of most infant formula milks) (Greer et al., 1984). In another of these early works, they showed that using UVB exposure to the mother, milk concentrations of up to 148IU/L were achievable. An important conclusion of this work was that, even with this UVB intervention, the human milk concentration was still well below half of the RDI for infants (400 IU/L).

The first randomised study to investigate the impact of maternal supplementation on both maternal and infant status was conducted by Hollis and Wagner (Hollis & Wagner, 2004). They supplemented 18 lactating women beginning at 1 month postpartum with either 1600 or 3600 IU/day of ergocalciferol (vitamin D2) for 3 months, together with a supplement of 400 IU/D vitamin D3. At 4 months of age, infants had attained a mean of 25OHD concentration of 69 and 77 nmol/L, for the two doses respectively. The authors concluded that maternal vitamin D intakes ≥ 4000 IU/d appear to ensure adequate infant vitamin D status. There are some important limitations with this study, in particular the lack of a control group, lack of blinding, and accounting for the concurrent daily supplementation with daily vitamin D (400IU/day) that all infants received. Moreover, a number of observational studies examining vitamin D status over time in unsupplemented infants have observed similar increases in infant 25OHD (Challa et al., 2005; Grant et al., 2014; Kim et al., 2010; Narchi, Kochiyil, Zayed, Abdulrazzak, & Agarwal, 2011; Perumal, Al Mahmud, Baqui, & Roth, 2017; Ziegler, Hollis, Nelson, & Jeter, 2006). Similar issues regarding lack of controls and often inadequate blinding have plagued all available maternal supplementation trials to date (Hollis & Wagner, 2004; Hollis et al., 2015; March et
al., 2015; Oberhelman et al., 2013; Saadi et al., 2009). Only the well-designed New Zealand study by Wall et al. avoids all of these limitations (Wall et al., 2016). However, the maternal intervention was limited to pregnancy, with no postnatal supplementation. In addition, at 2 weeks postpartum the mean milk vitamin D activity was only 74IU/L in the women taking the largest pregnancy supplementation dose (2000IU/day), compared to 51IU/L in the placebo group. These levels are, again, well below RDIs for breastfed infants, and consistent with what is known about the duration of D₃ in human milk (Oberhelman et al., 2013).

The results from the available maternal breastmilk supplementation studies are highlighted in Figure 7.

![Figure 7: Maternal supplementation trials and milk outcomes (Munns et al., 2016). Used with permission Oxford University Press and The Endocrine Society.](image)

At the time the present study detailed in Chapter 3 was commenced, little data was available on intermittent moderate-to-high dose maternal supplementation. Intermittent dosing is an attractive option for maternal adherence, as even in the trial setting 10-20% of participants in the original Hollis/Wagner study reported poor compliance e.g., missed doses (Hollis & Wagner, 2004). Subsequently, the Oberhelman study, detailed above in Figure 7 was published (Oberhelman et al., 2013). This was a two-arm study comparing a single maternal vitamin D₃
dose of 150,000 IU to 5000 IU/day over 28 days. Maternal and infant outcomes with either regimen were similar, with significant increases in both maternal and infant serum 25OHD concentrations from baseline (40.8 nmol/L) to Day 28 (96.8 nmol/L) (Oberhelman et al., 2013). However, this study only administered a single 150,000 IU dose. The safety of this dosage with regular use (e.g. every 4 weeks spanning 4-6 months of lactation) is unknown, and currently exceeds the tolerable upper intake level of daily intake suggested by the U.S. IOM by over 30% (A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011).

1.5.2.3 Summary

Pregnancy and lactation are of great importance for both maternal and infant vitamin D status. Studies spanning this vital period of the human life cycle highlight that maternal vitamin D status in pregnancy determines infant status at birth, with the two closely correlated. There are few studies longitudinally assessing pregnancy and lactation, especially providing dual maternal and infant data. The available studies confirm that vitamin D deficiency during pregnancy and lactation is common, and that a number of aspects of vitamin D and calcium metabolism appear altered during these stages.

While breastmilk is the ideal food source for infants, it is not naturally a rich source of vitamin D. However, there is growing evidence that breast milk concentrations can be increased, and at times quite impressively, with moderate-to-high dose daily, and intermittent maternal supplementation. The results of the studies investigating this to date must also be interpreted with some caution, due to a number of methodological limitations. In addition, to date, only high maternal doses, exceeding those recommended by the IOM, have been shown to achieve milk vitamin D activities similar to or exceeding infant RDIs. For this reason, pending new information, global recommendations for preventing rickets continue to recommend daily infant supplementation only (Munns et al., 2016; A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011).
1.6 Thesis Objectives

This thesis aims to investigate aspects of the role of vitamin D during pregnancy, lactation, and childhood. This encompasses the broad goal of increasing understanding and awareness of vitamin D deficiency rickets in children, and vitamin D status in pregnancy, as well as a better understanding of maternal supplementation to prevent vitamin D deficiency in exclusively breast-fed infants and their mothers.

Specific Aims

Chapter 2:

1) To determine the annual incidence of vitamin D deficiency rickets in New Zealand children

2) To describe the associated clinical and demographic characteristics of New Zealand children with rickets.

Chapter 3:

3) To evaluate, by way of a randomised placebo controlled trial, the effect of two different intermittent monthly doses of maternal Cholecalciferol supplementation, over 5 months of exclusive breastfeeding, on serum 25OHD status of both the infant and mother.

Chapter 4:

4) To describe longitudinal vitamin D and PTH status throughout pregnancy, and for the first 5 months of exclusive lactation in a cohort of Dunedin women and infants living at 45°S.
2 Paper 1 - Incidence and characteristics of vitamin D deficiency rickets in New Zealand children

2.1 Preface


As established in chapter one, worldwide vitamin D deficiency is common during pregnancy, and childhood. However, for rickets, most data in children comes from retrospective case series studies, often taken from hospital records. To date, and at the time this study was conducted and published, only two prospective national data sets were available, from Canada (Ward et al., 2007) and Australia (Munns et al., 2012). In addition, the only New Zealand data available was published in 1998, a retrospective audit of rickets presentations to the Starship Children’s Hospital.

Therefore, the aims of this chapter/project were to prospectively determine the annual incidence of vitamin D deficiency rickets in New Zealand children as recognised by paediatricians, and to describe the associated clinical and demographic characteristics.
2.2 Incidence and characteristics of vitamin D deficiency rickets in New Zealand children: a New Zealand Paediatric Surveillance Unit study

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Statement of Contribution:

BJW and BJT conceived this study and along with NPD and LMW designed it. BJW further refined and finalized the research protocol and data collection tools (adapted from a previous study by LMW, and using the NZPSU systems put in place by NPD and BJT). BJW conducted the research including data collection and contact with all participants. BJW conducted all analysis and produced the first draft of the research manuscript. All authors subsequently contributed to the review and revision of the manuscript, and read and approved the final manuscript. BJW gave all subsequent presentations of the study at scientific meetings and university seminars.
Abstract

Objective

To investigate the incidence and characteristics of vitamin D deficiency rickets in New Zealand.

Methods

Prospective surveillance of Vitamin D Deficiency Rickets among specialist paediatricians was conducted by the New Zealand Paediatric Surveillance Unit (NZPSU), for 36 months, from July 2010 – June 2013 inclusive. Inclusion criteria were: children and adolescents <15 years of age with vitamin D deficiency rickets (defined by low serum 25-hydroxyvitamin D and elevated alkaline phosphatase levels, and/or radiological rickets).

Results

Fifty-eight children with confirmed vitamin D deficiency rickets were identified. Median age was 1.4 (range 0.3 – 11) years, 47% were male, and 95% of children were born in New Zealand, however the majority of the mothers (68%) were born outside New Zealand. Overall annual incidence of rickets in children aged <15 years was 2.2/100,000 (95% CI 1.4-3.5); with incidence in those < 3 years, 10.5/100,000 (95% CI 6.7-16.6). Skeletal abnormalities, poor growth, and developmental motor delay were the most common presenting features, with hypocalcaemic convulsion in 16% of children. Key risk factors identified were: darker skin pigment, Indian and African ethnicity, age <3 years, exclusive breast feeding, and southern latitude, particularly when combined with season (winter/spring). Of the patients reported, none had received appropriate vitamin D supplementation.

Conclusions

Vitamin D deficiency rickets remains a health problem for New Zealand children. Key risk factors remain similar to those identified in the international literature. Preventative targeted vitamin D supplementation, as per existing national guidelines, was lacking in all cases reported.

Implications

Vitamin D deficiency rickets is the most significant manifestation of vitamin D deficiency in growing children. To reduce the incidence of this disease among those at high risk, increasing awareness and implementation of current public health policies for targeted maternal, infant, and child supplementation are required.
2.2.1 Introduction

Vitamin D is critical for calcium homeostasis and mineralization of the skeleton, especially during periods of growth. A severe deficiency in vitamin D can result in rickets (a mineralization defect at the epiphyseal growth plates, specific to growing children), and osteomalacia (a mineralization defect of bone tissue). Clinically, rickets may be associated with pain, fractures, skeletal deformity, growth retardation, defects in dental enamel, delayed developmental milestones, and hypocalcaemic tetany and seizure (Ladhani et al., 2004; Ward et al., 2007; Weisberg et al., 2004).

The main determinants of vitamin D status in humans are: any factor which limits the exposure of the epidermal layers of the skin to ultraviolet B radiation (UVB) from sunlight including darker skin, clothing/veiling, sunscreen, indoor lifestyle, and seasonal changes in countries at higher latitudes, such as New Zealand (35 to 47 degrees south) (Elder & Bishop, 2014; M. F. Holick, 2007); and dietary intake (including human milk), which in the absence of fortification is not normally an important source. Worldwide, there has been increasing concern that the number of children suffering from vitamin D deficiency and vitamin D deficiency rickets may be increasing (Allgrove, 2004; Elder & Bishop, 2014).

Currently, there are no national statistics on vitamin D deficiency rickets in New Zealand. However, there is increasing evidence that vitamin D deficiency in pregnant women, infants and young children is relatively common (Blokh et al., 2000; Camargo et al., 2010; Grant et al., 2009; Houghton et al.; Judkins & Eagleton, 2006). The aim of this study was to determine the current, annual incidence of vitamin D deficiency rickets in New Zealand children as recognized by paediatricians, and to describe the associated clinical and demographic characteristics.

2.2.2 Methods

The New Zealand Paediatric Surveillance Unit (NZPSU) (Dow et al., 1999) was used to prospectively collect cases of vitamin D deficiency rickets for 36 months, between July 2010 and June 2013 inclusive. The NZPSU undertakes surveillance of a number of uncommon childhood conditions through a reporting card or email sent to New Zealand paediatricians (approximately n=220, representing approximately 92% of New Zealand paediatricians registered with the Medical Council) on a monthly basis, asking if they have seen any cases of conditions under surveillance. We included vitamin D deficiency rickets in children and adolescents under 15
years of age on the monthly card/email over this period. During the study period, the mean NZPSU response rate was 92%.

In response to a notification of vitamin D deficiency rickets, a questionnaire was then sent to obtain relevant de-identified clinical, biochemical, management, and demographic data. Demographics collected included date of birth, gender, parental/child ethnicity and place of birth, pregnancy/ birth details, feeding and breastfeeding history, previous use of supplements and/or formula, clinician observed child/parental skin colour, and history of veiling.

Vitamin D deficiency rickets was defined as: 25-hydroxyvitamin D (25OHD) <50 nmol/L, and elevated alkaline phosphatase (ALP) levels (as per local pathology service reference values), and/or radiological rickets. Exclusion criteria were any case of vitamin D deficiency rickets associated with an underlying chronic disease such as fat malabsorption, liver disease and renal insufficiency, all genetic forms of rickets, and being on total parenteral nutrition. Forms were returned directly to the investigators and reviewed, to determine inclusion.

Descriptive results are expressed as median (range). Incidence rates were annualised based on the number of cases over the 36-month study period. Estimates of population by age group were provided by Statistics NZ 2013 national census data (Statistics New Zealand, 2014) and 95% confidence intervals were calculated using the Poisson distribution. Ethical approval for this study was granted by Lower South Regional Ethics Committee, Ministry of Health, New Zealand.

2.2.3 Results
Over the 36-month study period, 73 cases of vitamin D deficiency rickets were notified from 38 different specialists. Of these, 15 cases were excluded due to either not meeting case definition e.g. having isolated vitamin D deficiency, and/or not having a raised ALP and/or radiological evidence of rickets (n=13), or being duplicate cases (n=2), hence 58 cases were confirmed.

Incidence of reported vitamin D-deficiency rickets

The overall annual incidence of reported rickets was 2.2/100,000 (95% CI 1.4-3.5) for children and adolescents under 15 years of age. For children less than 3 years, the incidence was higher at 10.5/100,000 (95% CI 6.7-16.6). The incidence rates for all confirmed cases of vitamin D deficiency rickets by region are presented in Table 5, with highest rates among case children residing at the highest latitude in the South Island.
Table 5: Incidence of vitamin D deficiency rickets in New Zealand by province (ranked north to south) and age group

<table>
<thead>
<tr>
<th>Age group, years</th>
<th>&lt;3</th>
<th>3 - &lt;15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined Provincial Regions</td>
<td>Total no. (%) of confirmed cases</td>
<td>No. of confirmed cases</td>
</tr>
<tr>
<td>Auckland/Northland</td>
<td>19 (33)</td>
<td>19</td>
</tr>
<tr>
<td>Waikato/Bay of Plenty/Taranaki/ Gisborne</td>
<td>9 (15)</td>
<td>8</td>
</tr>
<tr>
<td>Wellington/ Hawke’s Bay/ Wanganui</td>
<td>14 (24)</td>
<td>13</td>
</tr>
<tr>
<td>Canterbury/Nelson-Marlborough/ Westland</td>
<td>5 (9)</td>
<td>5</td>
</tr>
<tr>
<td>Otago/Southland</td>
<td>11 (19)</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>58 (100)</td>
<td>55</td>
</tr>
</tbody>
</table>

‡Population estimates provided by statistics New Zealand, from 2013 census data.
Description of the children with rickets

Median age of children presenting with vitamin D deficiency rickets was 1.4 (range 0.3 – 11) years and 17 (47.0%) were male. Median birth weight was 3185 (1970-4300) grams. Of those with known birth gestation (52), most (45 [86.5%]) were born full term (gestation ≥37 weeks), with 7 (13.5%) born between 32-36 weeks, and none under 32 weeks gestation.

The vast majority of the children (55 [95%]) were born in New Zealand, as opposed to only 13 (22.4%) of their mothers. Table 6 shows the country of birth of the child and of the mother; most of the latter were born in Africa (14 [24.1%]) and India (13 [22%]). Similarly, maternal ethnicity, which differs in some cases from place of birth, in descending order of frequency was as follows: 24 (41.4%) Asian (Indian 21, Pakistani 1, Nepalese 1, Malaysian 1); 16 (27.6%) Middle Eastern/Latin American/African (African 14, Middle Eastern 2); 10 (17.2%) European; 5 (8.6%) Pacific; and 3 (5.2%) Maori. Paternal ethnicity as reported was very similar: Asian 23 (40.0%); Middle Eastern/Latin American/African 16 (27.6%); European 10 (17.2%); Maori 5 (8.6%); Pacific 3 (5.2%). Most of the children had dark (29 [50%]), or intermediate (18 [31%]) skin colour, with the remaining cases identified as being fair-skinned.

Table 6: Region of birth of children with vitamin D deficiency rickets and their mothers

<table>
<thead>
<tr>
<th>Child country of birth</th>
<th>n (%)</th>
<th>Mother</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Zealand</td>
<td>55 (94.8%)</td>
<td>Africa</td>
<td>14 (24.1%)</td>
</tr>
<tr>
<td>Pacific</td>
<td>1 (1.7%)</td>
<td>India</td>
<td>13 (22.4%)</td>
</tr>
<tr>
<td>Middle East</td>
<td>1 (1.7%)</td>
<td>New Zealand</td>
<td>13 (22.4%)</td>
</tr>
<tr>
<td>Africa</td>
<td>1 (1.7%)</td>
<td>Pacific</td>
<td>8 (13.8%)</td>
</tr>
<tr>
<td>Other Asian</td>
<td></td>
<td></td>
<td>4 (6.9%)</td>
</tr>
<tr>
<td>Middle East</td>
<td></td>
<td></td>
<td>2 (3.4%)</td>
</tr>
<tr>
<td>North America</td>
<td></td>
<td></td>
<td>1 (1.7%)</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
<td>3 (5.2%)</td>
</tr>
</tbody>
</table>
A history of exclusive breastfeeding (past and/or current) was reported in most cases (54/58 [93.1%]), with a median duration of 11 (range 2 – 26) months. Any exposure to commercial infant formula was present in 18 (31.0%) children. Only 5 [(8.6%) had received any vitamin D supplementation prior to diagnosis. Of these, four had been prescribed 400 IU vitamin D per day as infants (< 1 year of age), however, no data were available on compliance during this supplementation period, and all four children were subsequently diagnosed after 2 years of age. The remaining case had received 50,000 IU as a single dose just prior to formal diagnosis. Likewise, only 2 (3.4%) of all case mothers had received vitamin D supplementation during their pregnancy.

Skeletal abnormality, poor growth, and motor delay were the most common presenting features (Table 7). Median age of those presenting with symptomatic hypocalcaemia was 0.9 (0.5-2.5) years. Of the five children presenting with fracture, four were ambulant, and one aged 3 months experienced multiple fractures (with the possibly of additional non-accidental injury subsequently raised), and had biochemical evidence of rickets (25-hydroxyvitamin D 5 nmol/L [RR >50]; ALP 746 IU/L [RR 80-360]; PTH 12.5 pmol/L [RR 1-7]; phosphate 1.0 mmol/L [RR 1.15-2.15]) without x-ray changes at ulna/femur. All biochemistry returned to within normal ranges with vitamin D treatment, and no further fractures occurred during/after treatment with alternative care in place.

Biochemical and x-ray data are outlined in Table 8. Median age of those without x-ray confirmation was 0.8 (0.3 – 2.8) years. Maternal 25-hydroxyvitamin D was available in nearly one-third of the mothers (18 of 58) with median concentration of 30 (5 – 68) nmol/L. Veiling was reported in 6 (10%) of case mothers, but none of the children. Figure 8 depicts the cases of vitamin D deficiency rickets by month of diagnosis, with the highest number of cases diagnosed during the spring season.
<table>
<thead>
<tr>
<th>Reason for presentation</th>
<th>No. of cases (%)</th>
<th>Total n=58†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal abnormality/deformity‡</td>
<td>17 (29.3%)</td>
<td></td>
</tr>
<tr>
<td>Poor growth</td>
<td>15 (25.9%)</td>
<td></td>
</tr>
<tr>
<td>Motor Delay</td>
<td>12 (20.7%)</td>
<td></td>
</tr>
<tr>
<td>Symptomatic hypocalcaemia – Seizure (8) /Tetany (1)</td>
<td>9 (15.5%)</td>
<td></td>
</tr>
<tr>
<td>Bone pain</td>
<td>7 (12.1%)</td>
<td></td>
</tr>
<tr>
<td>Respiratory disease</td>
<td>5 (8.6%)</td>
<td></td>
</tr>
<tr>
<td>Fracture</td>
<td>5 (8.6%)</td>
<td></td>
</tr>
<tr>
<td>Incidental finding</td>
<td>4 (7%)</td>
<td></td>
</tr>
<tr>
<td>Eczema</td>
<td>3 (5.2%)</td>
<td></td>
</tr>
<tr>
<td>Screening (as sibling with rickets)</td>
<td>2 (3.4%)</td>
<td></td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>1 (1.7%)</td>
<td></td>
</tr>
</tbody>
</table>

†Percentages add up to >100% as more than one presenting feature was present in some instances
‡14 with limb deformity; 1 with “swollen wrists”; 2 with large anterior fontanelle/splayed sutures
Table 8: Biochemical analysis and x-ray status for cases of vitamin D deficiency rickets, reported between July 2010, and June 2013

<table>
<thead>
<tr>
<th></th>
<th>Number of Children (n)</th>
<th>Reference range</th>
<th>Median (Range)</th>
<th>Number abnormal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-hydroxyvitamin D (nmol/L)</td>
<td>58</td>
<td>&lt;50</td>
<td>12</td>
<td>5 - 48</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>58</td>
<td>80 - 360†</td>
<td>768</td>
<td>361 - 3100</td>
</tr>
<tr>
<td>Parathyroid hormone (pmol/L)</td>
<td>43</td>
<td>1.0 – 7.0</td>
<td>24.7</td>
<td>1.1 – 96.5</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>55</td>
<td>2.1 – 2.65*</td>
<td>2.3</td>
<td>1.1 – 2.7</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>55</td>
<td>0.9 – 2.2*</td>
<td>1.3</td>
<td>0.5 – 2.2†</td>
</tr>
<tr>
<td>Ferritin* (mcg/L)</td>
<td>28</td>
<td>15 - 150</td>
<td>25.5</td>
<td>2.9 - 288</td>
</tr>
<tr>
<td>Mean cell volume (f/L)</td>
<td>52</td>
<td>*</td>
<td>75.6</td>
<td>53 - 98</td>
</tr>
<tr>
<td>X-ray changes present (distal ulna/femur)</td>
<td>41</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

†Quoted reference range for Canterbury Health Laboratories. Laboratory ranges varied by age and location, leading to 100% incidence of elevated levels.

*Reference ranges vary based on age. Cut-point determined by age appropriate ranges.

‡No phosphate was elevated based on age appropriate cut points.

*Ferritin used as a marker of iron status.
Figure 8: Cases of vitamin D deficiency rickets by month of diagnosis

Number of cases (n)

- Summer
- Autumn
- Winter
- Spring
2.2.4 Discussion

In this prospective analysis of vitamin D deficient rickets, we found an incidence rate for children under three years of 10.6/100,000. This is the first national New Zealand study, and one of the few studies worldwide, to prospectively assess the incidence of vitamin D deficiency rickets. Our findings raise concerns about the implementation and awareness of current targeted guidelines for prevention of vitamin D deficiency and rickets. For the majority of cases, given they had defined risk factors and were born in New Zealand, prevention guidelines were not followed, as seen by the lack of vitamin D supplementation, in both the cases and their mothers.

Rickets is the most profound manifestation of vitamin D deficiency. In the New Zealand context, a number of prevention guidelines are available (Ministry of Health, 2006, 2013; Paxton et al., 2013). Over the time period of the study, the New Zealand Ministry of Health recommended that health practitioners identify breastfeeding women and infants at risk of vitamin D deficiency, and encourage the consumption of oily fish, eggs, and vitamin D fortified margarine. In addition, it has been recommended that targeted supplementation of 400 IU to the mother and infant should be considered (Ministry of Health, 2006). In April 2013 (two months before the study completed), a formal targeted supplementation strategy for breastfed infants at risk was adopted (Ministry of Health, 2013). It remains important to emphasise that while breast milk remains the ideal fluid source for infants, it is well known that it is not a rich source of vitamin D. Because of this, and the potential difficulties in implementation of a targeted strategy, many higher latitude countries have recommended universal supplementation of predominantly breastfed infants (Gartner, Greer, Section on, & Committee on Nutrition. American Academy of, 2003; Wagner et al., 2008). Additionally, as maternal vitamin D deficiency in pregnancy is a significant risk factor for rickets (Nozza & Rodda, 2001; Thomson, Morley, Grover, & Zacharin, 2004; Wharton & Bishop, 2003), targeted pregnancy screening and supplementation has also been recommended in a recent Australian and New Zealand consensus statement (Paxton et al., 2013). With minimal supplementation seen for reported cases in high-risk mothers during pregnancy and their offspring, our data highlight the importance of implementing current maternal and child vitamin D guidelines for targeted supplementation.

The overall incidence rate in all children < 15 years of 2.2/100,000, is similar to Canada (2.9/100,000) (Ward et al., 2007), but less than recently published Australian data (4.9/100,000) (Munns et al., 2012). However, when excluding those screened in Australia as refugees, the rate would be considerably higher than the Australian data, where refugee screening
contributed 75% of cases. It is no surprise that the non-refugee New Zealand incidence, excluding those screened at immigration is higher given the latitude of New Zealand. This is most markedly shown in the data from the southern (44-46 degrees south) Otago/Southland region, where the incidence is >30/100,000. Additionally, 10.6/100,000 incidence in the under 3 age group appears higher than Australian data (<4 years, 5.9/100000). Our data supports past reports (Ladhani et al., 2004; Ward et al., 2007), highlighting age < 3 years as a peak time for development and diagnosis of rickets, as well as the serious associated complications of seizures and symptomatic hypocalcaemia.

Our data support existing literature, highlighting risk factors such as breastfeeding, darker skin, and migrant status of the mother (Munns et al., 2012; Nozza & Rodda, 2001; Ward et al., 2007; Weisberg et al., 2004). In addition, the observed influence of season and latitude were contributing factors, as seen in similar prospective Australian data (Munns et al., 2012). Indian, and African ethnicities account for the majority of vitamin D deficiency rickets cases reported. This finding builds on a previous case series from New Zealand (Blok et al., 2000), as well as data from around the world, emphasising skin colour and specific ethnicities, that are frequently associated, as key risk factors (Kreiter et al., 2000; Ladhani et al., 2004; Robinson et al., 2006; Weisberg et al., 2004).

Skeletal deformity, poor growth, and motor delay were the most common presenting features. Symptomatic hypocalcaemia was also present in 16% of children, exclusively in those ≤ 2 years, as seen in Ward et al (Ward et al., 2007), but less than in retrospective hospital case series (Ladhani et al., 2004; Nozza & Rodda, 2001; Robinson et al., 2006). Skeletal deformity (excluding older screened refugees, which made up >80% of Australian data) was also the most common presenting feature in the only other prospective data for comparison (Munns et al., 2012; Ward et al., 2007). Again, similar to all previous prospective studies, x-ray changes were present in approximately 80% (Munns et al., 2012; Ward et al., 2007).

A particular strength of this study is the prospective data collection, using an established surveillance network that encompasses New Zealand’s currently practising paediatricians. The detailed data collection provides robust information on characteristics of those diagnosed with vitamin D deficiency rickets. Study weaknesses include the issue of under-reporting/under diagnosis. The study was limited to paediatricians and will therefore underestimate the overall incidence. Under reporting of cases is a possibility; in particular, the Canterbury region of the South Island experienced two catastrophic earthquakes in the first year of reporting, perhaps
accounting for the lower number of cases reported from this region. In addition there is possible under diagnosis, which is likely as few cases were reported as incidental findings as part of screening programs. These factors would mean that overall observed incidence rate calculated is at least 2.2/100,000 for children and adolescents under 15 years of age and 10.5/100,000 for children less than 3 years.

In conclusion, vitamin D deficiency rickets is a health concern for New Zealand children, with a higher incidence seen, in those with mothers from India and Africa, children under three years of age who are currently or were previously breastfed, and those living further south at a higher latitude. The question remains as to whether rickets has re-emerged in recent years, or whether it has simply remained a persistent problem. To reduce the incidence of this disease among those at risk, efforts should be focused on increasing awareness, and implementation, of existing policies for targeted maternal, infant, and child supplementation.
3 Paper 2 – The Vitamin D and Breastfeeding study

3.1 Preface


In chapter one it was highlighted that vitamin D is unique among vitamins as its main source is not dietary, but direct synthesis in the skin following exposure to UVB radiation, from sunlight. However, UVB radiation exposure varies based on latitude, skin colour, sunscreen use and clothing (McKenzie et al., 2009). Unfortunately, changes in human lifestyle including sun avoidance practices, indoor occupations and recreation, added to NZ’s geographical location at 35ºS to 47ºS, mean that children and adults cannot depend on adequate skin exposure to sunlight for vitamin D synthesis, especially during winter months (McKenzie et al., 2009; Rockell et al., 2008). At latitude 45 ºS the Otago region is particularly at risk.

Dietary intake is a secondary source, but without additional supplementation there is little in foods most humans normally ingest. As opposed to many countries NZ currently has no mandatory, and little voluntary food supplementation (Ministry of Health, 2013). Compounding this, human milk, which is advocated as the ideal fluid source for infants, is generally low in vitamin D and this has led to recommendations for universal supplementation of breast feeding infants in some countries (Wagner et al., 2008).

One of the main reasons breast milk may not be a rich source of vitamin D is that many breast feeding mothers are themselves vitamin D deficient (Judkins & Eagleton, 2006; Nozza & Rodda, 2001). In addition, some studies suggest that daily or intermittent supplementation of the breast feeding mother with high dose vitamin D can safely lead to not only improved vitamin D levels for her, but also in her milk and subsequently her nursing infant (Hollis & Wagner, 2004; Oberhelman et al., 2013). However, the results of these studies must be interpreted with caution due to the lack of control group, unblinded treatment, and/or concurrent infant vitamin D supplementation.

If effective, supplementation of mothers (and hence their breast milk) would be an attractive alternative to universal supplementation of breast-fed infants. This would support current
WHO recommendations on the importance of breast-feeding and also avoid the issue of direct supplementation of exclusively breast-fed infants which in New Zealand can be controversial and poorly complied with by families (Taylor et al., 2010).

The primary aim of this chapter/project was to determine the effect of 2 different intermittent monthly doses of maternal cholecalciferol supplementation (weeks 4–20 postpartum) on serum 25(OH)D status of non–vitamin D supplemented breastfed infants.
3.2 High dose monthly maternal cholecalciferol supplementation during breastfeeding increases affects maternal and infant vitamin D status at 5 months post-partum: A randomized controlled trial.

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Statement of contribution:

BJW, BJT, and LAH conceived and designed the study. BJW obtained funding and ethics and locality approvals. BJW, BJT, PH, SJ, and LAH further refined and finalized the research protocol; BJW and SJ conducted the research including coordinating data collection and recruitment (AM contributed to recruitment also); BJW, SJ, MJH, and LAH oversaw the laboratory analyses; PH, JJH, and BJW all analyses. BJW drafted the research manuscript. All authors, in particular BJT, LAH, and JJH contributed to the review and revision of the manuscript, and all read and approved the final manuscript. BJW gave all subsequent presentations of the study at scientific meetings and university seminars.
Abstract

**Background:** Many countries recommend daily infant vitamin D supplementation during breast feeding, but compliance is often poor. A monthly, high dose maternal regimen may offer an alternative strategy for dual maternal and infant supplementation, but its efficacy is unknown.

**Objectives:** The objective of this study was to determine the effect of two different monthly maternal doses of cholecalciferol (D₃) on both maternal and infant 25-hydroxyvitamin D (25[OH]D) status during the first 5 months of breastfeeding.

**Methods:** Using a randomized, double blind, placebo-controlled design, breastfeeding women (n=90, mean age 32.1 years) were assigned to receive either placebo/mo, 50,000 IU/mo, or 100,000 IU/mo of D₃ from Week 4 to Week 20 postpartum. Comparison between intervention and placebo groups for change in maternal and infant mean serum 25OHD from baseline to week 20 was made using linear regression.

**Results:** Compared to the control group, the effect size for mean change in maternal serum 25OHD from baseline to Week 20 was 12.8 nmol/L (95%CI 0.4 to 25.2; P=0.043) higher for the 50,000 IU/mo and 21.5 nmol/L (95%CI 9.2 to 33.8; P=0.001) higher for 100,000 IU/mo groups. For infants, the mean changes in serum 25OHD were higher in the intervention groups compared to the control, but not statistically significant: 50,000 IU /mo (4.5 nmol/L 95%CI -16.2 to 25.0; P=0.67) and 100,000 IU /mo (15.8nmol/L 95%CI -4.7 to 36.4; P=0.13) groups. However, after adjustment for season of birth, vitamin D-fortified formula intake, and infant skin color, the effect size for the 100,000 IU group was 19.1 nmol/L (95% CI 2.5 to 35.6; P=0.025) higher compared to placebo.

**Conclusions:** For infants, maternal D₃ supplementation at a dose of 100,000 IU /mo during the first 5 months of breastfeeding potentially benefits vitamin D status. While attractive from a compliance perspective, further studies are required to determine optimum dose and dosing frequency.

**Clinical Trial Registration:** [www.anzctr.org.au](http://www.anzctr.org.au) ACTRN12611000108910
3.2.1 Introduction

Vitamin D is essential for calcium and bone metabolism. It is unique among vitamins, in that its main source is derived from synthesis in the skin following exposure to ultraviolet-B radiation. In the absence of fortification, few foods are rich in vitamin D, including low levels present in human milk (Hollis et al., 1981). This suboptimal intake leads to an increased risk of deficiency in higher latitude countries like New Zealand, particularly during winter months (Grant et al., 2014; Houghton et al., 2010; McKenzie et al., 2009; Rockell et al., 2005; Wall, Grant, & Jones, 2013). For infants, this risk is compounded as direct sunlight exposure is not recommended in the first 6 months of life (AAP, 1999).

The most profound manifestation of vitamin D deficiency in growing children is rickets, a mineralization defect of the growth plate, characterized by bone deformities, impaired growth, biochemical abnormalities, and, depending on the stage of deficiency, seizures (Munns et al., 2012; Pettifor & Prentice, 2011; Ward et al., 2007; B. J. Wheeler et al., 2015). Recent prospective surveillance studies in New Zealand, Canada, and Australia have drawn attention to this continuing problem, especially among young children (Munns et al., 2012; Ward et al., 2007; B. J. Wheeler et al., 2015). Common risk factors identified include darker pigmented skin, maternal vitamin D deficiency during pregnancy, season of birth, age <3 years, and exclusive breastfeeding (Munns et al., 2012; Ward et al., 2007; B. J. Wheeler et al., 2015).

In light of this, several countries have recommended universal vitamin D supplementation for all breastfed infants (Wagner et al., 2008) for the prevention of rickets. However, compliance is variable (Crocker et al., 2011; Gallo et al., 2010; Perrine et al., 2010; Taylor et al., 2010), with recent data published from US National Health and Nutrition Examination Survey (NHANES) suggesting that 80% of US breast-fed infants failed to achieve the recommended daily intake of 400 IU (Ahrens et al., 2016). In addition, cases of rickets continue to be reported in countries using this preventive approach (Ward et al., 2007; Weisberg et al., 2004).

A potential alternative strategy to improve the vitamin D status in breastfed infants is high dose cholecalciferol (D₃) supplementation to pregnant and lactating women. This has been shown to increase both maternal and infant serum 25-hydroxyvitamin D (25OHD) concentrations (Basile, Taylor, Wagner, Horst, & Hollis, 2006; Hollis & Wagner, 2004; March et al., 2015; Oberhelman et al., 2013; Saadi et al., 2009); however, interpretation of these studies
is often complicated by lack of a control group, un-blinded treatment, and concurrent vitamin D supplementation to infant study participants.

New Zealand is a unique environment in that it has both negligible food fortification, and at the time of this study, a public health policy to only “consider” supplementation to infants “at risk”. Thus, the primary aim of this randomized placebo-controlled study was to determine the effect of two different intermittent monthly doses of maternal D₃ supplementation (Weeks 4 to 20 postpartum) on vitamin D status of non-supplemented, breastfed infants. A monthly dose was chosen to improve compliance.

3.2.2 Subjects and Methods
Study population and design

Healthy pregnant women planning to exclusively breastfeed for at least five months following delivery were recruited from July 2012 to June 2013 through the Queen Mary Maternity Centre (QMMC), Dunedin Hospital, Dunedin, New Zealand (45ºS latitude). QMMC provides publically funded, tertiary level maternity and newborn care to the Otago/Southland region of New Zealand (population ~310,000), and is the only birthing unit in Dunedin, with approximately 97% of all city births (the remaining 3% being home births). Exclusion criteria included: 1) preterm delivery prior to 37 weeks gestation; 2) intent to use vitamin D supplements (either mother or infant) in the postnatal period; 3) a history of disorders known to affect calcium and/or vitamin D metabolism, including abnormal calcium levels/urine Ca/Cr ratio at study baseline; and 4) planned travel outside of New Zealand over the study period; 5) living outside of Dunedin. The study was approved by the New Zealand Lower South Regional Ethics Committee (LRS/11/02/007), and written informed consent was obtained from each mother. The study was registered prior to study commencement with the Australian New Zealand Clinical Trials Registry at www.anzctr.org.au as ACTRN12611000108910.

The study was a 16 week randomized, double-blind, placebo-controlled trial of 90 mother and infant pairs, beginning at 4 weeks postpartum. Women were enrolled from 20 weeks gestation until delivery. Following delivery, lactation support was provided, along with breast pumps as needed. At 4 weeks postpartum, mothers were randomized in blocks of 15 to one of three treatment arms: placebo, 50,000 IU, or 100,000 IU to be administered every month until 16 weeks postpartum. Randomization was not stratified by season. These intervention
doses were selected as they conformed with commonly available D₃ preparations, and
administration of 50,000 IU D₃ /mo is a commonly prescribed and funded regimen for the
treatment of vitamin D insufficiency in New Zealand adults (with no daily dosing options
publically funded); and as previously discussed, daily maternal and infant dosing have been
subject to issues of noncompliance. All tablets were supplied to the participants, and self-
administered with written and verbal prompting (e.g. reminder phone calls) to take one tablet
at baseline (4 weeks postpartum) and every month thereafter with the last dose administered
at 16 weeks postpartum.

Socio-demographic data were obtained from interviews at enrolment (maternal only), and at
4 and 20 weeks postpartum (both mother and baby). Information was obtained on maternal
age, education, ethnicity, smoking status, vitamin and mineral supplement use, breastfeeding
and use of infant formula (frequency and duration), sun exposure, and sunscreen use.
Maternal height and weight measurements were taken at enrolment and at 4 weeks post-
partum using standardized techniques. Infant length and weight measurements were taken at
birth, 4, and 20 weeks of age. Body Mass Index (BMI; in kg/m²) was calculated, and infant z-
scores utilized the WHO Child Growth Standards (Group, 2006). Blood was drawn at birth
(cord), 4 weeks (maternal), and 20 weeks postpartum (infant and maternal). Lastly, maternal
and infant skin reflectance was measured at each visit by spectrophotometer (CM2006d,
Minolta Co. Ltd, Osaka, Japan) and converted to a final color assessment of “very light”,
“light”, “intermediate”, “dark” and “very dark”. Measurement sites included the medial
aspect of the upper arm (natural skin color) and dorsal aspect of the forearm (sun exposed
surface).

Supplements

The study supplements were manufactured by OPTIMUS Healthcare Limited (Auckland, New
Zealand) as identical hard tablets each containing lactose powder (placebo), and a target of
either 50,000 IU D₃, or 100,000 IU D₃. Vitamin D content was measured at an independent
laboratory, Eurofins NZ Laboratory Services Ltd. The placebo tablet contained no detectable
D₃, and the mean D₃ content of tablets used for the 50,000 IU D₃ and 100,000 IU D₃ were
51,139 and 93,630 IU D₃/tablet, respectively. Supplements were coded by a third party
(Dunedin Public Hospital Pharmacy) and participants, investigators and biostatisticians were
blinded to treatment. The randomization list was kept strictly confidential in a sealed
envelope until all aspects of the study had been performed. Participants were instructed to
take one tablet every month and to return all unused pills. They were also advised to avoid any additional supplemental vitamin D ingestion and overseas travel, and to use sunscreen for the duration of the study. Mothers were contacted every month during the study to remind them to take their monthly dose, and compliance was assessed by counting returned pills at the end of the study.

**Dietary vitamin D intake**

From birth to week 20, mothers recorded in a diary the brand of infant formula used and daily volume consumed (if any). The quantity of vitamin D consumed was estimated from the vitamin D content of infant formula (IU/100 g) by mean daily intake over this 20 week study period.

**Blood/urine sampling and laboratory analysis**

Fasting, second void urine of the morning for maternal calcium/creatinine (Ca/Cr) ratio was also measured at 4 weeks and 20 weeks postpartum. Non-fasting venous maternal, cord, and infant blood samples were collected. Serum calcium, phosphate, albumin, alkaline phosphatase (ALP) were measured immediately, and remaining serum was aliquoted and stored at −80°C until study completion. Creatinine was measured using photometric analysis via fully automated cobas® 8000 c502 (Roche Diagnostics, Mannheim, Germany), with the remaining assays analyzed by cobas® 8000 c702 analyzer (Roche Diagnostics, Mannheim, Germany). The clinical cut-offs used to define maternal serum hypercalcemia and elevated urine Ca/Cr ratios at any time point were > 2.6 mmol/L (Chan et al., 2009) and > 0.6 mole ratio (Metz, 2006), respectively. For infants at 20 weeks, serum hypercalcaemia was defined as >2.8 mmol/L (Chan et al., 2009).

Serum samples for 25OHD and parathyroid hormone (PTH) from each participant pair were analyzed in the same run to avoid inter-assay variation. Serum levels of 25OHD were measured by isotope-dilution liquid chromatography tandem mass spectrometry (Maunsell et al., 2005), using an API 3200 instrument (Applied Biosystems) connected to a Dionex Ultimate 3000 HPLC system. The limit of quantification for the assay was < 5 nmol/L for both metabolites. To assess accuracy and inter-assay variability, external quality control serum material (UTAK Laboratories) containing low and medium levels of both metabolites were analyzed with every run. Measurements fell within the expected range with mean ± SD values of 29.0 ± 1.2 nmol/L and 67.3 ± 2.1 nmol/L for 25OHD₃ (UTAK verified values 25.0, 69.9
nmol/L, and 26.2 ± 1.4 nmol/L and 67.0 ± 2.9 nmol/L for 25OH D$_2$ (UTAK verified values 29.1, 67.9 nmol/L). Internal quality control pooled serum samples were also analyzed, the inter-assay CV for 25OH D$_3$ being 3.7% at 56.9 nmol/L, 25OH D$_2$ was below the limit of quantification.

PTH was measured using an automated electrochemiluminescence immunoassay (Elecsys®2010, Roche Diagnostics, Mannheim, Germany). The PTH assay showed a detection sensitivity of 1.2 pg/mL. The control samples provided by the manufacturer were within the recommended target value and the inter-assay CV based on a pooled serum was 4.4% (n=22).

**Statistical analyses**

Sample size was calculated on the basis of detecting a minimum 15 nmol/L difference in infant serum 25OHD between placebo and intervention at study end. To have 80% power to detect such an effect with a standard deviation of 20 nmol/L, it was estimated that 28 infants would be required in each treatment arm. Allowing for an estimated 10% loss to follow-up, we planned to recruit a total of 90 mother-infant pairs.

Baseline characteristics were summarized as means ± SD for continuous variables and as the percentage distribution for categorical variables. The primary study outcomes were 1) change in infant 25OHD from baseline to week 20, and 2) proportion defined with deficiency at week 20 (as per Australian and New Zealand guidelines (Paxton et al., 2013)). For the change in infant 25OHD, the data were analyzed using linear regression with 20 week 25OHD as the outcome, randomized group as a fixed effect with the control group as reference, and baseline 25OHD as a covariate. As season of birth, infant underarm skin color, and infant formula intake are likely prognostic variables for breast-fed infant 25OHD levels, a secondary analysis for infants was carried out adjusting for these variables. A Fisher’s exact test was used to determine if the proportion of infants that were classified as deficient (serum 25OHD <50 nmol/L) or moderately/severely deficient (serum 25OHD <30 nmol/L) at 20 weeks was different between intervention groups (28). These proportions were plotted on a bar graph as percentages.

Secondary outcome measures included change in maternal 25OHD from baseline to Week 20 and proportion of maternal deficiency at 20 weeks, which were assessed using the same methods as for infants, with the exception of the secondary adjusted analysis that included season of birth and maternal underarm skin color as covariates.
To examine whether supplementation affected other biochemical/safety indices such as PTH, calcium, phosphate, and urine calcium creatinine ratio, change in these variables for both mother and infant (as appropriate) was assessed using linear regression, with randomized group as a fixed effect with control group as reference, and baseline level as a covariate. Clinically meaningful elevations were defined as above, and proportions compared between groups.

To illustrate how 25OHD changed in each group over the time of the trial, mean serum 25OHD and standard error bars were plotted with a line graph for both mothers and infants. Pearson’s correlation coefficient and p-value were calculated between baseline maternal 25OHD and infant cord 25OHD. Change in forearm skin color over the trial period was also investigated, as a proxy to sun exposure. Forearm skin color was categorized into “very light”, “light”, “intermediate”, “dark” and “very dark” (as per spectrophotometry described above) at baseline and at Week 20. Mothers and infants were then further categorized into three categories: “became lighter” (if their skin color changed to a lighter category); “unchanged”; or “became darker” (if their skin color changed to a darker category). A chi-squared test was used to determine if skin color change categories were different between treatment groups.

All statistical analyses were performed using Stata (version 13.1; StataCorp, College Station, TX). Residuals were calculated and plotted to visually assess whether the assumptions of the statistical models had been met (no transformations were made). A two-sided P value <0.05 was considered statistically significant.

### 3.2.3 Results

Of the 90 women and infant pairs randomized, 87 completed the trial (Figure 9), giving a 97% retention rate. Across the treatment groups the loss to follow-up was similar: 3% (n=1) for the placebo, 6% (n=2) in the 50,000 IU D₃ group, and 0% (n=0) in the 100,000 IU D₃ group.

Participant characteristics of mothers and infants are shown in Table 9. Median weight, length and BMI at 1 month were 3.5 kg (weight-for-age z score -1.2), 51 cm (length-for-age z score -1.2), and 13.0 kg/m² (BMI-for-age z score -1.0) respectively for girls and 3.7 kg (weight-for-age z score -1.3), 53 cm (length-for-age z score -0.8), and 13.5 kg/m² (BMI-for-age z score -1.1) for boys.
Figure 9: CONSORT diagram depicting participant flow and follow up.
Table 9: Baseline participant demographics for maternal vitamin D₃ treated and control groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>50,000 IU /mo</th>
<th>100,000 IU /mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>31.2 ± 5.5</td>
<td>32.3 ± 4.0</td>
<td>32.8 ± 5.6</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZ European</td>
<td>18 (60%)</td>
<td>23 (77%)</td>
<td>24 (80%)</td>
</tr>
<tr>
<td>Maori</td>
<td>3 (10%)</td>
<td>3 (10%)</td>
<td>0</td>
</tr>
<tr>
<td>Pacific</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>4 (13%)</td>
<td>6 (20%)</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>Skin color:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very light</td>
<td>15 (50%)</td>
<td>18 (60%)</td>
<td>16 (53%)</td>
</tr>
<tr>
<td>Light</td>
<td>11 (37%)</td>
<td>11 (37%)</td>
<td>11 (37%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>1 (3%)</td>
<td>1 (3%)</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Dark</td>
<td>3 (10%)</td>
<td>0</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Primiparous</td>
<td>14 (47%)</td>
<td>13 (43%)</td>
<td>13 (43%)</td>
</tr>
<tr>
<td>Tertiary education²</td>
<td>23 (77%)</td>
<td>18 (60%)</td>
<td>21 (70%)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.5 ± 4.4</td>
<td>29.7 ± 4.2</td>
<td>29.6 ± 6.3</td>
</tr>
<tr>
<td>Smoker</td>
<td>4 (13%)</td>
<td>3 (10%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>25OHD, nmol/L</td>
<td>51.7 ± 23.4</td>
<td>51.6 ± 24.9</td>
<td>50.6 ± 23.1</td>
</tr>
<tr>
<td>PTH, pg/mL</td>
<td>34 ± 14</td>
<td>30 ± 12</td>
<td>38 ± 21</td>
</tr>
<tr>
<td>Corrected calcium ,mmol/L</td>
<td>2.3 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>Phosphate, mmol/L</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Urine Ca/Cr</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.2</td>
</tr>
<tr>
<td>Infant:</td>
<td>Control</td>
<td>50,000 IU /mo</td>
<td>100,000 IU /mo</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Gestation, wk</td>
<td>39.6 ± 1.0</td>
<td>39.7 ± 1.0</td>
<td>39.7 ± 1.1</td>
</tr>
<tr>
<td>Gender, male</td>
<td>16 (53%)</td>
<td>12 (40%)</td>
<td>15 (50%)</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>3613 (559)</td>
<td>3546 (495)</td>
<td>3376 (435)</td>
</tr>
<tr>
<td>Birth Season:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>9 (30%)</td>
<td>9 (30%)</td>
<td>9 (30%)</td>
</tr>
<tr>
<td>Summer</td>
<td>4 (13%)</td>
<td>3 (10%)</td>
<td>5 (17%)</td>
</tr>
<tr>
<td>Autumn</td>
<td>5 (17%)</td>
<td>6 (20%)</td>
<td>4 (13%)</td>
</tr>
<tr>
<td>Winter</td>
<td>12 (40%)</td>
<td>12 (40%)</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Skin color:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very light</td>
<td>3 (10%)</td>
<td>3 (10%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Light</td>
<td>17 (59%)</td>
<td>15 (52%)</td>
<td>15 (50%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>9 (31%)</td>
<td>9 (31%)</td>
<td>11 (37%)</td>
</tr>
<tr>
<td>Dark</td>
<td>0</td>
<td>2 (7%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Exclusive breast feeding</td>
<td>22 (73%)</td>
<td>22 (73%)</td>
<td>20 (67%)</td>
</tr>
<tr>
<td>Infant vitamin D exposure</td>
<td>14 ± 47</td>
<td>16 ± 42</td>
<td>23 ± 70</td>
</tr>
<tr>
<td>25OHD total, nmol/L</td>
<td>37 ± 21</td>
<td>36 ± 18</td>
<td>40 ± 20</td>
</tr>
<tr>
<td>PTH, pg/mL</td>
<td>6.1 ± 7.9</td>
<td>4.6 ± 1.7</td>
<td>4.8 ± 3.7</td>
</tr>
<tr>
<td>Corrected calcium, mmol/L</td>
<td>2.6 ± 0.2</td>
<td>2.6 ± 0.1</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>Phosphate, mmol/L</td>
<td>2.0 ± 0.4</td>
<td>1.9 ± 0.4</td>
<td>2.0 ± 0.4</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs or n (%), and all laboratory analyses in serum, unless otherwise indicated, n = 30 for each intervention group, no statistical tests for between group differences at baseline were undertaken as per the CONSORT statement (Moher et al., 2010);
2 Completed university or other higher education qualification
3 No exposure to infant formula over 20 weeks
4 Birth – week 20
Overall, compliance with respect to supplement intake was high (96%) and similar for the 3 groups: 94% for placebo, 94% for 50,000 IU D₃ group, and 100% for 100,000 IU D₃ group. As a surrogate for sun exposure, 70% of infants, and 8% of mothers had a lighter forearm skin color at Week 20 compared to baseline, 27% of infants and 55% of mothers were unchanged, and 3% of infants and 30% of mothers were darker. There were no statistically significant differences in skin color change between groups of infants and mothers from baseline to Week 20.

In terms of baseline vitamin D status, overall mean maternal serum 25OHD concentration at 4 weeks postpartum was 51.3 ± 23.6 nmol/L, with 55% (48 of 87) and 14% (12 of 87) of women having a serum 25OHD concentration <50 and <30 nmol/L, respectively (Table 9 depicts data by treatment group). Mean infant cord 25OHD concentrations of 37.3 ± 19.5 nmol/L were substantially lower than maternal concentrations, and over three quarters (77%; 66 of 86) of the infant participants had a cord 25OHD below 50 nmol/L. A statistically significant correlation was observed between cord 25OHD and maternal 25OHD (r = 0.81, P < 0.001).

Over the 20 week study period, mean maternal serum 25OHD increased in all 3 groups (Figure 10), although the changes in mean serum 25OHD among mothers randomly assigned to monthly vitamin D supplementation after 16 weeks were significantly greater than those of the placebo group (Table 10). Adjustment for season of delivery, and maternal skin color improved the precision of the estimates for the difference in changes in maternal serum 25OHD levels. Effect size (95%CI) = 10.8 (0.5, 21.1) nmol/L, P=0.041 for the 50,000 IU group compared to placebo, and 20.7 (10.6, 30.9) nmol/L, P<0.001 for the 100,000 IU group compared to placebo.
Table 10: Mean change in maternal and infant serum 25OHD and other serum biochemical indices, from baseline to week 20, for control and vitamin D₃ treated groups

<table>
<thead>
<tr>
<th></th>
<th>Control (n=28)</th>
<th>50,000 IU/mo (n=28)</th>
<th>100,000 IU/mo (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal (week 4 to week 20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25OHD, nmol/L</td>
<td>17.3 ± 32.7</td>
<td>30.2 ± 25.9</td>
<td>40.0 ± 29.3</td>
</tr>
<tr>
<td>Change in 25OHD compared to</td>
<td>Reference</td>
<td>12.8 (0.4, 25.2)</td>
<td>21.5 (9.2, 33.8)</td>
</tr>
<tr>
<td>control, nmol/L/16wk</td>
<td></td>
<td>0.043</td>
<td>0.001</td>
</tr>
<tr>
<td>PTH, pg/mL</td>
<td>-1.1 ± 12.8</td>
<td>-0.8 ± 14.2</td>
<td>-5.5 ± 19.6</td>
</tr>
<tr>
<td>Change in PTH compared to</td>
<td>Reference</td>
<td>-1.6 (-8.7, 5.4)</td>
<td>-1.9 (-8.8, 5.1)</td>
</tr>
<tr>
<td>control, pg/mL/16wk</td>
<td></td>
<td>0.65</td>
<td>0.59</td>
</tr>
<tr>
<td>Corrected calcium, mmol/L</td>
<td>0.07 ± 0.11</td>
<td>0.07 ± 0.13</td>
<td>0.11 ± 0.12</td>
</tr>
<tr>
<td>Change in corrected calcium</td>
<td>Reference</td>
<td>-0.01 (-0.07, 0.04)</td>
<td>0.02 (-0.03, 0.07)</td>
</tr>
<tr>
<td>compared to control, mmol/L/16wk</td>
<td></td>
<td>0.63</td>
<td>0.46</td>
</tr>
<tr>
<td>Phosphate, mmol/L</td>
<td>0.01 ± 0.20</td>
<td>-0.01 ± 0.18</td>
<td>0.05 ± 0.28</td>
</tr>
<tr>
<td>Change in phosphate compared to</td>
<td>Reference</td>
<td>-0.05 (-0.14, 0.04)</td>
<td>0.01 (-0.07, 0.10)</td>
</tr>
<tr>
<td>control, mmol/L/16wk</td>
<td></td>
<td>0.27</td>
<td>0.75</td>
</tr>
<tr>
<td>Infant (birth to 20 weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>25OHD, nmol/L</strong></td>
<td>37.4 ± 51.9</td>
<td>44.7 ± 44.7</td>
<td>52.8 ± 46.4</td>
</tr>
<tr>
<td>Change in 25OHD compared to control, nmol/L/20wk</td>
<td>Reference</td>
<td>4.5 (-16.1, 25.0)</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>PTH, pg/mL</strong></td>
<td>31.9 ± 61.4</td>
<td>17.6 ± 10.9</td>
<td>13.6 ± 7.8)</td>
</tr>
<tr>
<td>Change in PTH compared to control, pg/mL/20wk</td>
<td>Reference</td>
<td>-17.0 (-38.2, 4.2)</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Corrected calcium, mmol/L</strong></td>
<td>0.06 ± 0.22</td>
<td>0.04 ± 0.19</td>
<td>0.04 ± 0.21</td>
</tr>
<tr>
<td>Change in corrected calcium compared to control, mmol/L/20wk</td>
<td>Reference</td>
<td>0.02 (-0.02, 0.07)</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>Phosphate, mmol/L</strong></td>
<td>-0.22 ± 0.43</td>
<td>-0.06 ± 0.43</td>
<td>-0.13 ± 0.48</td>
</tr>
<tr>
<td>Change in phosphate compared to control, mmol/L/20wk</td>
<td>Reference</td>
<td>0.06 (-0.02, 0.15)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

1 Values are ± SDs or (95% CI), unless otherwise indicated. 25OHD, 25-hydroxyvitamin D; PTH, parathyroid hormone
2 Placebo compared with cholecalciferol interventions with the use of linear regression.
Figure 10: Mean ± SE maternal (A) and infant (B) serum 25-hydroxyvitamin D at baseline and week 20, for vitamin D₃ treated and control groups. Descriptive data only, see table 2 for statistical analysis of primary outcomes. 25OHD, 25-hydroxyvitamin D; SE, standard error bars.
Among infants, the mean change in serum 25OHD concentrations among the treatment and placebo groups from cord blood to 20 weeks postnatally are shown in Table 10. In contrast to change in maternal serum 25OHD concentrations, the magnitude of the change in infant serum 25OHD over time did not significantly differ among the 3 groups. Adjustment for the confounding effects of infant skin color, season of birth, and infant formula intake was undertaken as these were important prognostic variables identified in the literature. As a result the effect estimates for the difference in changes in infant 25OHD levels were strengthened and more precise. The effect size (95% CI) was 5.0 (-11.3, 21.3) nmol/L, \( P=0.54 \), for the 50,000 IU group compared to control, and 19.1 (2.5, 35.6) nmol/L, \( P=0.025 \), for the 100,000 IU group compared to control.

The prevalence of having a maternal serum of 25OHD < 50 nmol/L was 26% (7 of 27) in the placebo group at the end of the 20 weeks postpartum, which was significantly higher than the 50,000 IU group (4%; 1 of 27) and the 100,000 IU group (0%) (\( P=0.002 \)) (Figure 11). At study conclusion, none of the maternal participants taking vitamin D supplements had a serum 25OHD <30 nmol/L whereas 7% (2 of 27) on placebo remained below 30 nmol/L. Among the infant participants, there was no difference in the prevalence of having a serum 25OHD level <50 nmol/L between groups at study completion (prevalence = 27%, 29%, and 19% for the placebo, 50,000IU, and 100,000IU groups respectively, \( P=0.65 \)) (Figure 11). While the number of infants with a 25OHD levels < 30 nmol/L was greater in the placebo group (23%, 6 of 26) compared to the 50,000 IU group (11%; 3 of 28) and 100,000 IU (4%; 1 of 27) at the end of trial, these proportions were not significantly different (\( P=0.09 \)).
Figure 11: Percentage of maternal and infant vitamin D₃ treated and control groups that were deficient, serum 25OHD <50nmol/L (A), or moderately/severely deficient, serum 25OHD <30nmol/L (B), at 20 weeks postnatal age. Vitamin D₃ supplement intake (IU/mo) is represented in 3 categories, for maternal groups: placebo (n=27), 50,000 IU/mo (n=27), and 100,000 IU/mo (n=28); for infant groups: placebo (n=26), 50,000 IU/mo (n=28), and 100,000 IU/mo (n=27). Fisher’s exact test was used to test for difference between: maternal groups <30nmol/L, not testable; maternal groups <50 nmol/L, P=0.002; infant groups <30 nmol/L, P=0.09; infant groups <50nmol/L, P=0.65. 25OHD, 25-hydroxyvitamin D; vitamin D₃, cholecalciferol.
Additional biochemistry and safety data

In both mothers and infants, there were no statistically significant differences in the mean changes of serum PTH, corrected calcium, and phosphate among groups over the trial period (Table 2). However, two infants in the placebo group did have clinically meaningful elevations in PTH (above the upper normal limit of 65) of 120 and 281 pg/mL at the end of the intervention. Although normal values for PTH concentration in infants and young children have not been clearly defined, in these two infants, PTH values were also both associated with severe vitamin D deficiency, (25OHD levels of 6.4 and 8.6 nmol/L) and elevated ALP values (352 and 507 U/L) – all biochemically suggestive of vitamin D deficiency rickets.

No cases of hyper- or hypocalcaemia were found in any maternal or infant participant. Mean change in maternal urinary Ca/Cr ratios over the study intervention were comparable between control and intervention groups (50,000 IU D₃/mo P=0.96; 100,000 IU D₃/mo P=0.22). Nevertheless, six mothers had a higher than normal Ca/Cr ratio at 20 weeks postpartum (normal range <0.6; n=2 placebo, n=1 50,000 IU and n=3 100,000 IU groups). All of these were considered non-fasting specimens and a repeat fasting specimen as per study protocol was not undertaken.

3.2.4 Discussion

The results of the present study reveal new insights into the potential relevance of a maternal vitamin D dosing strategy for breastfed infants, that have been lacking from previous studies due to lack of controls, blinding and/or concomitant daily infant vitamin D administration (Hollis & Wagner, 2004; Hollis et al., 2015; March et al., 2015; Oberhelman et al., 2013; Saadi et al., 2009). As expected, once monthly moderate (50,000 IU) or high (100,000 IU) dose maternal D₃ elevated serum 25OHD concentrations in mothers by Week 20, and were well tolerated. However, the results in infants are more complex. For the primary analysis, no significant differential effect of maternal supplementation on infant vitamin D status, or prevalence of having a serum 25OHD below 50 nmol/L was seen at the end of the study period. However, once adjusted for potentially important confounders including season of birth, infant formula exposure, and infant skin color, high dose maternal monthly vitamin D₃ (100,000 IU) supplementation resulted in a statistically significant and clinically meaningful increase in infant serum 25OHD of 19 nmol/L compared to the placebo group. Moreover, this dose (100,000 IU) may also offer some protection against moderate to severe infant deficiency with only one infant in this group (as compared to six infants in the placebo) presenting with a 25OHD level of less than 30 nmol/L.
To our knowledge, this is the first randomized, double-blinded placebo-controlled trial to document the effect of maternal vitamin D supplementation on the vitamin D status of breastfed infants. Importantly, in the present study, infants did not consume any other oral vitamin D supplements. Thus, the observed changes can therefore be attributed to the supplementation of the mothers. Our secondary findings of effect for infants in the 100,000 IU intervention, align with previous maternal supplementation studies in lactating women (Hollis & Wagner, 2004; Hollis et al., 2015; March et al., 2015; Oberhelman et al., 2013; Saadi et al., 2009). These studies have trialed both daily and monthly supplementation, however all lack controls, in addition to often inadequate blinding. One of the first maternal vitamin D supplementation trials conducted (Hollis & Wagner, 2004), supplemented 18 lactating women beginning at 1 month postpartum with either 1600 IU/d or 3600 IU/d ergocalciferol (vitamin D2) for a period of 3 months together with a supplement of 400 IU/D vitamin D3. At 4 months of age, infants had attained a mean of 25OHD concentration of 69 and 77 nmol/L, respectively. The authors concluded that maternal vitamin D intakes ≥ 4000 IU/d appear to ensure adequate infant vitamin D status. Of importance, we report a similar mean serum infant 25OHD concentration of 76 nmol/L at Week 20 in study infants whose mothers were receiving placebo. This highlights one of the most notable findings of our study, which was the observed increase in 25OHD concentrations of placebo assigned infants over the 20 week postnatal period. Similar increases in 25OHD have been shown in a number of longitudinal studies among unsupplemented breastfed infants (Challa et al., 2005; Grant et al., 2014; Kim et al., 2010; Narchi et al., 2011; Perumal et al., 2017; Ziegler et al., 2006). The reasons for this rise in 25OHD are complex but may be the result of a seasonal imbalance in recruitment and/or a physiological increase in vitamin D binding proteins (DBP) with age – a key determinant of 25OHD levels (Carpenter et al., 2013). Information on DBP in early infancy is limited, but it is known that adult levels of albumin concentration and binding affinity are not reached until 10 to 12 months of age. Regardless, these results emphasize the importance of a control group. In light of our findings, the reported effect size of previous uncontrolled studies may have been potentially confounded by a natural temporal trend towards increased vitamin D status in the first year of life.

It is unknown whether a higher monthly maternal dose would have achieved greater improvements in infant vitamin D status, but the lower 50,000 IU vitamin D3 dose every 4 weeks appeared ineffective. A recent uncontrolled, blinded study administering a single maternal vitamin D3 dose of 150,000 IU demonstrated a significant increase in infant serum 25OHD concentrations from baseline (40.8 nmol/L) to Day 28 (96.8 nmol/L) (Oberhelman et al., 2013). However, safety of this dosage with regular use (every 4 weeks) is unknown and currently exceeds the tolerable upper intake level.
of daily intake suggested by the Institute of Medicine by over 30% (A. C. Ross et al., 2011). In our current investigation, we found that doses of up to 100,000 IU every 4 weeks were well tolerated, and the incidence of maternal hypercalcemia and hypercalciuria did not differ between groups, nor was vitamin D treatment associated with any laboratory, clinical, or serious adverse effect. In addition, we did not observe hypervitaminosis D (defined as 25OHD concentration > 225 nmol/L) in any of our participants (peak maternal and infant 136 nmol/L and 140 nmol/L, respectively).

An alternative strategy for improving the vitamin D status of breastfed infants is to increase fetal vitamin D status via maternal supplementation during pregnancy, as cord blood levels of 25OHD in the present study highly correlated with maternal levels. A number of studies have supported this finding (Grant et al., 2014; Hollis et al., 2011; March et al., 2015; Roth et al., 2013), with the most recent study by March et al (March et al., 2015), demonstrating that doses between 400 to 2000 IU/d during pregnancy resulted in substantially higher cord blood 25OHD, ranging from 73 to 95 nmol/L. In the present study, the mean cord 25OHD of unsupplemented mothers was 37.3 nmol/L. Supplementation with 2000 IU/d antenatally also appeared to protect 98% of unsupplemented breastfed infants against vitamin D deficiency (serum 25OHD < 30 nmol/L) at least until 8 weeks postpartum (March et al., 2015).

A particular strength of this study is that this is the first known randomized, double blind, placebo controlled design conducted in a setting with little interference from food fortification. In addition, there was no policy for universal vitamin D supplementation for breastfed infants at the time the study was conducted. The longer duration of our study over the recommended period of exclusive breastfeeding with high rates reported in each group (67-73%), and close to 100% rate of current breastfeeding at study completion further strengthened the design in comparison to previous studies (Grant et al., 2014; Hollis & Wagner, 2004; Hollis et al., 2015; March et al., 2015; Oberhelman et al., 2013; Saadi et al., 2009). Study limitations include the convenience sample of healthy women in the south island of New Zealand who were mainly of European descent (72%). As a result, it is unknown whether these results can be generalized to other ethnic groups or other regions of varying latitude in New Zealand. Another limitation is breast milk analysis for vitamin D content was not available, which would have been ideal as a secondary outcome measure, albeit the half-life of vitamin D₃ in milk is approximately 24-72 hours (Oberhelman et al., 2013). Lastly, the lack of effect in the primary analysis may be due to type 2 error, and is a limitation reflecting both a potentially small sample size and an imbalance of recruitment by season.
In conclusion, our study shows that maternal vitamin D supplementation of lactating women during the first 5 months of breastfeeding improved the vitamin D status of the mother, and suggests that doses of 100,000 IU D₃ / mo are not only safe, but may improve the vitamin D status of the breastfeeding infant, and subsequently prevent deficiency. In addition, these data demonstrate a general rise in serum 25OHD concentrations over the first 20 weeks of life, which questions the effect size and beneficial effects of previous uncontrolled maternal supplementation studies. Further work is warranted to support our findings, and to more fully investigate the efficacy of varying maternal dosage regimens (e.g. daily or weekly) on both maternal and infant vitamin D status.
4 Paper 3 – A longitudinal study of maternal and infant vitamin D and parathyroid hormone status throughout pregnancy and lactation in New Zealand mothers and their infants at 45° South

4.1 Preface

The following chapter contains a published original manuscript titled “A longitudinal study of 25-hydroxy vitamin D and parathyroid hormone status throughout pregnancy and exclusive lactation in New Zealand mothers and their infants at 45° South”. This manuscript was published in Nutrients 2018. *Nutrients* 2018, 10(1), 86; doi:10.3390/nu10010086 (B. Wheeler et al., 2018).

The importance of pregnancy and the early postnatal period have been highlighted in Chapter 1. Pregnancy is a unique time in terms of vitamin D and calcium metabolism, with not only the mineral needs of the mother, but also the fetus needing to be maintained and developed. This is particularly the case in the third trimester when peak fetal bone mineral accrual occurs (Givens & Macy, 1933; Trotter & Hixon, 1974). In addition, as previously stated, maternal vitamin D status during pregnancy is also essential in establishing final infant status, with infant 25OHD levels at birth closely correlated to maternal pregnancy vitamin D status (Bowyer et al., 2009; Grant et al., 2014; S. D. Thomas et al., 2011).

Given how important pregnancy is, there are but a few studies assessing pregnancy and lactation longitudinally. The data available confirms that vitamin D deficiency during pregnancy and lactation appears common, and that a number of aspects of vitamin D and calcium metabolism appear altered during these stages. Limitations in these papers include a lack of infant data, and questions around generalisability to the southern hemisphere, with all available studies originating from the northern hemisphere. This suggests a need to better understand vitamin D status and metabolism during pregnancy and lactation. This is particularly so for regions of the world where mothers are at risk of suboptimal vitamin D status. New Zealand is one such country due to its southern latitude (spanning 35 - 47° South), negligible vitamin D food fortification, and public health policy to only consider vitamin D supplementation to women during pregnancy and to breastfed infants who are considered “at risk” (Ministry of Health, 2013).

Therefore, the primary aim of this chapter/project was to describe longitudinal 25OHD and parathyroid hormone (PTH) status during pregnancy, and postnatally to 5 months, in a sample of New Zealand women and their infants living at a latitude of 45° south.
4.2 A longitudinal study of 25-hydroxy vitamin D and parathyroid hormone status throughout pregnancy and exclusive lactation in New Zealand mothers and their infants at 45° South.

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Statement of contribution:

B.J.W., L.A.H. and B.J.T. conceived and designed the study. B.J.W. obtained funding and wrote the first draft of the manuscript. B.J.W. with the help of S.J. conducted all aspects of the study, including recruitment and data collection. M.J.H. conducted the laboratory analysis. A.M. provided clinical pregnancy care and recruitment for the study. B.J.W. and M.L. performed all data analysis. All authors contributed to review and revision, and read and approved the final manuscript. BJW gave all subsequent presentations of the study meetings and university seminars.
Abstract

Vitamin D status and associated metabolism during pregnancy and lactation have been assessed in only a limited number of longitudinal studies, all from the northern hemisphere, with no infant data concurrently reported. Therefore, we aimed to describe longitudinal maternal and infant 25-hydroxy vitamin D (25OHD) and parathyroid hormone (PTH) status during pregnancy and out to 5 months postnatal age, in New Zealand women and their infants living at 45ºS latitude. Between September 2011 and June 2013, 126 pregnant women intending to exclusively breastfeed for at least 20 weeks were recruited. Longitudinal data were collected at three time points spanning pregnancy, and following birth and at 20 weeks postpartum. Vitamin D deficiency (25OHD <50nmol/L) was common, found at one or more time points in 65% and 76% of mothers and their infants, respectively. Mean cord 25OHD was 41nmol/L, and three infants exhibited secondary hyperparathyroidism by postnatal week 20. Maternal late pregnancy 25OHD (gestation 32-38 weeks) was closely correlated with infant cord 25OHD, r² = 0.87 (95%CI 0.8-0.91), while no correlation was seen between early pregnancy (<20 weeks gestation) maternal and cord 25OHD, r²=0.06 (95%CI -0.16-0.28). Among other variables, pregnancy 25OHD status and therefore infant status at birth, were influenced by season of conception. In conclusion, vitamin D deficiency in women and their infants is very common during pregnancy and lactation in New Zealand at 45ºS. These data raise questions regarding the applicability of current pregnancy and lactation policy at this latitude, particularly recommendations relating to first trimester maternal vitamin D screening and targeted supplementation for those “at risk”.
4.2.1 Introduction

Pregnancy is a unique and demanding life stage in terms of vitamin D and calcium metabolism due to increased need for fetal development of mineralised structures while maintaining optimal maternal status. This is particularly the case in the third trimester of pregnancy when peak fetal bone mineral accrual occurs (Givens & Macy, 1933; Trotter & Hixon, 1974). 25-Hydroxyvitamin D (25OHD) readily crosses the placenta. As a result, 25OHD levels of mother-infant cord blood are positively correlated. Maternal vitamin D status during pregnancy plays a key role in establishing the size of their neonate reserves at birth (Bowyer et al., 2009; Grant et al., 2014; S. D. Thomas et al., 2011), with supplementation studies during pregnancy demonstrating significantly improved infant status at birth and beyond (Grant et al., 2014; March et al., 2015; Roth et al., 2013).

The potential adverse consequences to the neonate of maternal vitamin D deficiency have been demonstrated with both maternal vitamin D deficiency and lack of prenatal supplementation associated with an increased risk of infancy and childhood rickets (Moncrieff & Fadhahnsi, 1974; Nozza & Rodda, 2001; Roberts et al., 1973; Ward et al., 2007). In addition, other aspects of foetal and child bone health may also be impacted by maternal vitamin D status, including foetal growth (Bowyer et al., 2009), foetal bone accrual and subsequent bone size (Viljakainen et al., 2011; Viljakainen et al., 2010), and dental health including enamel hypoplasia (Purvis et al., 1973) and dental caries (Schroth et al., 2014). These impacts of low maternal vitamin D status on foetal bone have been reported as early as 19 weeks gestation using high resolution 3D ultrasound showing a poorer fetal femoral development (Mahon et al., 2010), as well as longer-term follow up studies demonstrating lower peak offspring bone mass at 20 years of age (Zhu et al., 2014).

Maternal vitamin D status during pregnancy is influenced by a range of factors, including season, skin colour, supplementation, latitude, and potential pregnancy specific variations in metabolism (Bowyer et al., 2009; Ekeroma et al., 2015; Lundqvist et al., 2016). To better understand the magnitude of these factors, maternal vitamin D status and associated metabolism have been assessed in a number of longitudinal studies (Cross et al., 1995; Haliloglou et al., 2011; Holmes et al., 2009; Lundqvist et al., 2016; Milman et al., 2011; Ritchie et al., 1998; Zhang et al., 2014). Nonetheless, all of the studies to date have been conducted in the northern hemisphere (spanning 39°N - 63.8°N) where supplement use is common. Many of these studies were also limited by unbalanced seasonal sampling or lack of seasonal data. In addition, post-pregnancy maternal follow-up during lactation was variable and the respective infant data was not reported.
A better understanding of vitamin D status and metabolism during pregnancy and lactation is needed, particularly in regions of the world where mothers are at risk of suboptimal vitamin D status. New Zealand is one such country due to its southern latitude (spanning 35 - 47° South), and negligible vitamin D food fortification. Pregnancy and infant vitamin D deficiency (25 OHD <50nmol/L) in New Zealand also appear common with high rates reported across diverse ethnicities and latitudes (Camargo et al., 2010; Ekeroma et al., 2015; Judkins & Eagleton, 2006; B. J. Wheeler et al., 2016). In addition, current public health policy is to only consider vitamin D supplementation to women during pregnancy and to breastfed infants who are “at risk” i.e. having 1 or more of the following: naturally dark skin; complete sun avoidance; a sibling with rickets; have liver or kidney disease, or on certain medications that affect vitamin D levels; and infants who are breastfed over winter (Ministry of Health, 2013).

Therefore, we aimed to describe longitudinal 25OHD and parathyroid hormone (PTH) status throughout pregnancy as well as 20 weeks postnatally in a sample of New Zealand women and their infants living at a latitude of 45° south.

### 4.2.2 Subjects and Methods

#### Study population and design

This longitudinal descriptive study includes 126 pregnant women intending to exclusively breastfeed for at least 20 weeks, and their infants. Blood samples obtained antenatally during routine care were collapsed into the following three categories: 1) < 20 weeks gestation “first trimester” screening bloods (with specific sample timing determined by when pregnancy was confirmed and antenatal care commenced); 2) 28 week maternal gestational diabetes screening (range 20 – 31 weeks); and 3) 36 week routine maternal iron status determination (range 32-38 weeks). There were two additional postnatal time points measured in both mothers and infants, at 4 and 20 weeks for mothers, and cord blood (at delivery) and 20 weeks for infants. Overall, 40 women provided all five maternal observations, 40 provided four observations, 24 provided two observations, and 22 women only one observation – providing 431 maternal observations for analysis in total.

This sample population of women and their infants was drawn from those recruited and screened as part of a previously published randomized controlled trial (RCT). Detailed methods for this study have been published elsewhere (B. J. Wheeler et al., 2016). In brief, healthy pregnant women planning to exclusively breastfeed for at least five months following delivery were recruited from September 2011
to June 2013 through the Queen Mary Maternity Centre (QMMC), Dunedin Hospital, Dunedin, New Zealand (45°S latitude). QMMC provides publically funded, tertiary level maternity and newborn care to the Otago/Southland region of New Zealand (population ~320,000), and is the only birthing unit in Dunedin, with approximately 97% of all city births (the remaining 3% being home births). Exclusion criteria included: 1) delivery prior to 37 weeks gestation; 2) intent to use postnatal vitamin D supplements (mother or infant); 3) a history of disorders known to affect calcium and/or vitamin D metabolism, including abnormal calcium levels/urine Ca/Cr ratio at study baseline; and 4) planned travel outside of New Zealand over the study period.

The study consisted of a 16 week randomized, double-blind, placebo-controlled trial of 90 mother and infant pairs, beginning at 4 weeks postpartum. Following delivery, lactation support was provided, along with breast pumps as needed. At 4 weeks postpartum, mothers were randomized in blocks of 15 to one of three treatment arms: placebo, 50,000 IU, or 100,000 IU cholecalciferol to be administered every month until 16 weeks postpartum (inclusive). Women were enrolled from 20 weeks gestation until delivery, and antenatal data were regularly collected for subsequent analysis for this longitudinal study. Postnatal week 20 data for both mothers and infants for those in the two intervention arms of this study have been excluded for the purposes of this longitudinal study due to the potential impact of this intervention on their “free-living” vitamin D status.

In addition, in the present study, an additional 36 women who were recruited prior to RCT commencement (due to initial delays with the availability of independently verified placebo and intervention vitamin supplement) from September 2011 – June 2012, were also included. These women were enrolled from the same population, using identical inclusion/exclusion criteria (Figure 12).
Figure 12: Diagram depicting longitudinal study participant flow
Socio-demographic data were obtained from interviews at antenatal study enrolment (maternal only), and at 4 and 20 weeks postpartum (both mother and baby). Information was obtained on maternal age, education, ethnicity (in 7 instances dual ethnicities were given, in these cases for the purposes of description in this study, these were allocated to a single ethnic group based order of priority: Māori, Pacific, Asian and European/Other), smoking status (yes/no – pre-pregnancy and pregnancy), vitamin and mineral supplement use (recall of number of days/week taken during each month of pregnancy), breastfeeding and use of infant formula (frequency and duration), sun exposure (including degree of veiling/covering behaviour, and hours of exposure previous month), and sunscreen use (frequency).

Maternal height and weight measurements were taken at enrolment and at 4 weeks post-partum using standardized techniques, and body mass index (BMI) was subsequently calculated. Infant length and weight measurements were taken at birth, 4, and 20 weeks of age. Lastly, maternal and infant skin reflectance was measured at each visit by spectrophotometer (CM2600d, Minolta Co. Ltd, Osaka, Japan) and converted to a final skin colour assessment by calculating individual typology angles (ITA) using the following formula: ITA = (ArcTangent ((L - 50)/b)) - 180/π. Skin colour was then classified using the ITA into the following groups: very light > 55 > light > 41 intermediate > 28 > dark > -10 > very dark. Measurement sites included the medial aspect of the upper arm (natural skin colour) and dorsal aspect of the forearm (sun exposed surface).

Dietary vitamin D intake

From antenatal enrolment to week 20 postpartum, mothers recorded use of dietary supplements (if any), as well as the brand of infant formula used and daily volume consumed (if any) for the infant. These data were reviewed by study personnel at each study visit. Maternal supplemental vitamin D intake was then determined by estimating mean supplement intake (IU/d) over the duration of pregnancy (there was no postnatal use once the RCT commenced). No infant vitamin supplements were used. However, the amount of dietary vitamin D intake consumed by the infants was estimated from the mean daily intake of infant formula (mL/d) multiplied by the vitamin D content (IU/100 g) over the 20 week study period.

Blood sampling and laboratory analysis

Non-fasting venous maternal, cord, and infant blood samples were collected, and stored at –80°C until study completion. Serum samples for 25(OH)D and PTH from each participant were analysed in the same run to avoid inter-assay variation. Serum levels of 25(OH)D were measured by isotope-dilution liquid chromatography tandem mass spectrometry (Maunsell et al., 2005), using an API 3200 instrument (Applied Biosystems) connected to a Dionex Ultimate 3000 HPLC system. The limit of
quantification for the assay was < 5 nmol/L for both metabolites. To assess accuracy and inter-assay variability, external quality control serum material (UTAK Laboratories) containing low and medium levels of both metabolites were analyzed with every run. Measurements fell within the expected range with mean ± SD values of 29.0 ± 1.2 nmol/L and 67.3 ± 2.1 nmol/L for 25(OH)D₃ (UTAK verified values 25.0, 69.9 nmol/L), and 26.2 ± 1.4 nmol/L and 67.0 ± 2.9 nmol/L for 25(OH)D₂ (UTAK verified values 29.1, 67.9 nmol/L). Internal quality control pooled serum samples were also analyzed, the inter-assay CV for 25(OH)D₃ being 3.7% at 56.9 nmol/L, 25(OH)D₂ was below the limit of quantification.

PTH was measured using an automated electrochemiluminescence immunoassay (Elecsys®2010, Roche Diagnostics, Mannheim, Germany). The PTH assay showed a detection sensitivity of 0.1 pmol/L. The control samples provided by the manufacturer were within the recommended target value and the inter-assay CV based on a pooled serum was 4.4% (n=22).

Statistical analyses

Baseline descriptive characteristics have been presented as means ± SDs for continuous variables and as counts and percentages for categorical variables. Student’s t-tests, Fisher’s Exact tests, and Chi-squared tests were used to examine differences between groups. Pearson’s correlation coefficients were used to assess associations between continuous variables. For all regression analyses, 25OHD values were log transformed (natural logs), these were back transformed to geometric means for the purposes of Figure 2. Linear models were used to analyse the relationships between cord 25OHD and season; maternal 25OHD and PTH; and maternal 25OHD and BMI, maternal skin colour, parity, season of 25OHD measurement, pregnancy (or not), and vitamin D supplement intake. Next, for maternal 25OHD, factors that were significantly associated or considered biologically plausible were then included in a multivariable mixed model analysis. Mixed models included a random intercept for each mother, using BMI (>30kg/m²), vitamin D supplement intake, parity, season of 25OHD measurement, pregnancy, and maternal skin colour as predictors. To examine the predictive potential of very early maternal pregnancy 25OHD status on final maternal 25OHD status at delivery, further modelling was undertaken to graphically illustrate longitudinal variation in maternal 25OHD status based on season of conception. Modelled means of maternal 25OHD status grouped by season of conception, spanning pregnancy (earliest sampling points were at 6 weeks gestation) out to 20 weeks post-partum, centred by date of delivery (gestational ages adjusted relative to date of delivery) were plotted adjusting for all factors included in the mixed model analysis above, excluding only month of measurement, and pregnancy (both variables changing along the x-axis). For simplicity, whenever described, and for all analyses undertaken, season has been defined by calendar month: summer: December, January,
February; autumn: March, April, May; winter: June, July, August; and spring: September, October, November.

A 2-sided $P$ value of $<0.05$ was considered significant. All statistical analyses were performed using R version 3.4 (R Core Team, 2017), using the lme4, and lmerTest libraries (Bates, Machler, Bolker, & Walker, 2015).

This study was approved by the New Zealand Lower South Regional Ethics Committee (LRS/11/02/007), and the study registered prior to commencement with the Australian New Zealand Clinical Trials Registry at www.anzctr.org.au as ACTRN12611000108910.

4.2.3 Results

Baseline characteristics of the 126 women and their infants are presented in Table 1. There were no differences in basic demographics between those who provided samples at all time points vs. those with missing data points, other than there were fewer births in spring/summer in the “all time points” group 34% vs 53% respectively, $p=0.02$. The majority of mothers and their infants had no vitamin D supplement exposure during the study (Table 1).
<table>
<thead>
<tr>
<th>Characteristics (total n=126)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal</strong></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>32.8 ± 5.0</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>104 (83%)</td>
</tr>
<tr>
<td>Maori</td>
<td>8 (6%)</td>
</tr>
<tr>
<td>Pacific</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Other</td>
<td>13 (10%)</td>
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<tr>
<td>Skin color</td>
<td></td>
</tr>
<tr>
<td>Very light</td>
<td>67 (53%)</td>
</tr>
<tr>
<td>Light</td>
<td>51 (41%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>5 (4%)</td>
</tr>
<tr>
<td>Dark</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Primiparous</td>
<td>37 (29%)</td>
</tr>
<tr>
<td>Tertiary education</td>
<td></td>
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<tr>
<td></td>
<td>90 (71%)</td>
</tr>
<tr>
<td>Pre-pregnancy BMI, kg/m²</td>
<td>25.0 ± 6.2</td>
</tr>
<tr>
<td>Smoker</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 (7%)</td>
</tr>
<tr>
<td>Antenatal vitamin D supplement use</td>
<td>35 (28%)</td>
</tr>
<tr>
<td>Supplement intake IU/day</td>
<td></td>
</tr>
<tr>
<td>Consumers only</td>
<td>339 ± 269</td>
</tr>
<tr>
<td><strong>Infant</strong></td>
<td></td>
</tr>
<tr>
<td>Gestation, wk</td>
<td>39.3 ± 1.1</td>
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<tr>
<td>Gender, male</td>
<td>61 (48%)</td>
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</tbody>
</table>
The longitudinal serum 25OHD and PTH values spanning the three time points in pregnancy, and two time points postpartum (out to 20 weeks) in both mothers and infants are shown in Table 12. Table 12 also shows the proportion of mothers at each time point with vitamin D deficiency (25OHD<50nmol/L) by season. Maternal vitamin D deficiency was common. In those where both pregnancy and postnatal values were available, deficiency was found at one or more time points.
during the full longitudinal study (antenatal plus postnatal) in 65% (52/80), and during pregnancy in 48% (38/80). Infant vitamin D deficiency was also very common, seen in 76% at one or more time points, with 68% deficient at birth, and a mean cord blood of 41nmol/L. Mean cord 25OHD was 25
nmol/L greater when season of delivery was in summer (mean ± SD, 57 ± 23 nmol/l), compared to winter (32 nmol ± 16) (p < 0.001). At 20 weeks postpartum, the majority remained exclusively breastfed (71% no dietary vitamin D exposure), but increased their 25OHD status to a mean of 57 ± 42 nmol/L. However, 51% remained deficient, and in three infants, there was potentially clinically important secondary hyperparathyroidism, serum PTH values between 12.6 and 29.5 pmol/L (upper limit normal 7 pmol/L). Although normal values for serum PTH in infants have not been consistently defined, these PTH values were accompanied by severe vitamin D deficiency (25OHD concentrations of 4.7, 6.4, and 8.6nmol/l) and in two infants, elevated alkaline phosphatase levels (352 and 507U/L), all factors that suggest possible vitamin D deficiency rickets. These three infants (only one with dark skin) were all born between late summer and mid-autumn, meaning this 20-week sample was taken in mid-winter/early spring – at the anticipated nadir of 25OHD status.

Positive correlations were observed between infant cord blood 25OHD and two of three maternal 25OHD antenatal sampling time points, as well as at 4 weeks postpartum. The maternal AN3 antenatal time point (32-38 Weeks) was most closely correlated with infant cord blood status, r² = 0.87 (95%CI 0.8-0.91), followed by week 4 postpartum, r² = 0.81 (95%CI 0.74-0.86), and AN2 (week 20-31), r²=0.4 (95%CI 0.19-0.57). There was no correlation seen between with the maternal AN1 (<20 week gestation) 25OHD and infant cord 25OHD, r²=0.06 (95%CI -0.16-0.28). This is demonstrated in Figure 13. Additionally, there was a weak, negative correlation between maternal 25OHD and PTH (r² ranging between -0.12 - -0.31), with little difference in the correlations between the five available maternal time points (both throughout pregnancy and postpartum), with similar overlapping confidence intervals, (spanning -0.52 – 0.11). The strongest negative correlation between 25OHD and PTH was seen in the infant at 5 months of age, r²=-0.35 (95%CI -0.50--0.16, p=0.0003). No correlation was seen between cord 25OHD and cord PTH, r²=-0.11 (95%CI -0.29-0.08, p=0.2).

Variables affecting maternal serum 25OHD fully adjusted for season, pregnancy, supplement intake, skin colour, parity, and BMI are shown in Table 13. Due to the significant impact of time of year on 25OHD status, maternal 25OHD status measured over time (gestation and postnatal) by season of conception is shown in Figure 14 demonstrating the variability in serum 25OHD at this latitude over time, and gestation (model adjusted for all variables in Table 13, excluding month and pregnancy stage).
Table 12: Serum total 25-hydroxyvitamin D and parathyroid hormone concentrations in mothers and their infants during pregnancy and lactation (first 20 post-natal weeks)¹

<table>
<thead>
<tr>
<th>Sampling occasion</th>
<th>Maternal AN1 (n=80)</th>
<th>Maternal AN2 (n=80)</th>
<th>Maternal AN3 (n=80)</th>
<th>Maternal PN1 (n=123)</th>
<th>Maternal PN2 (n=66)</th>
<th>Infant Cord (n=122)</th>
<th>Infant PN2 (n=66)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation/postnatal week</td>
<td>Week 6-19</td>
<td>Week 20-31</td>
<td>Week 32-38</td>
<td>Week 4</td>
<td>Week 20</td>
<td>Date of birth</td>
<td>Week 20</td>
</tr>
<tr>
<td>Serum 25OHD, nmol/L</td>
<td>70 ± 25</td>
<td>78 ± 32</td>
<td>76 ± 34</td>
<td>55 ± 24</td>
<td>59 ± 25</td>
<td>41 ± 21</td>
<td>57 ± 42</td>
</tr>
<tr>
<td>Serum PTH, pmol/L</td>
<td>1.7 ± 0.7</td>
<td>1.6 ± 0.88</td>
<td>1.5 ± 0.7</td>
<td>3.5 ± 1.7</td>
<td>3.8 ± 1.7</td>
<td>0.5 ± 0.5</td>
<td>2.8 ± 3.9</td>
</tr>
<tr>
<td>Proportion with 25OHD deficiency (&lt;50nmol/L) by season, %</td>
<td>Spring</td>
<td>26% (8/31)</td>
<td>56% (9/16)</td>
<td>50% (6/12)</td>
<td>59% (19/32)</td>
<td>50% (9/18)</td>
<td>93% (27/29)</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>5% (1/19)</td>
<td>6% (1/17)</td>
<td>6% (1/16)</td>
<td>20% (4/20)</td>
<td>9% (1/11)</td>
<td>37% (10/27)</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>21% (3/14)</td>
<td>7% (2/30)</td>
<td>18% (5/28)</td>
<td>41% (12/29)</td>
<td>36% (5/14)</td>
<td>50% (14/28)</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>50% (8/16)</td>
<td>47% (8/17)</td>
<td>33% (8/24)</td>
<td>57% (24/42)</td>
<td>70% (16/23)</td>
<td>84% (32/38)</td>
</tr>
</tbody>
</table>

¹ Values are means ± SDs or % (n); AN, Antenatal; PN, Postnatal; AN1, <20 week gestation; AN2, week 20-31 gestation; AN3, week 32-38 gestation; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone
Figure 13: (A) Correlation between infant cord 25-hydroxy vitamin D (25OHD) and maternal late pregnancy serum 25OHD (week 32-38), $r^2 = 0.87$ (95%CI 0.8-0.91); (B) Correlation between infant cord 25OHD and maternal early pregnancy (<20 week gestation) serum 25OHD, $r^2=0.06$ (95%CI 0.16-0.28).
Table 13: Predictors of maternal serum 25-hydroxyvitamin D status during pregnancy and lactation (first 20 post-natal weeks)\(^1\)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Estimate</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Season</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>Ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>0.72</td>
<td>0.67 - 0.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Winter</td>
<td>0.58</td>
<td>0.54 - 0.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spring</td>
<td>0.70</td>
<td>0.65 - 0.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pregnancy(^2)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Supplement Use</td>
<td>1.20</td>
<td>1.09 - 1.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Skin Colour(^3)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very light</td>
<td>Ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>1.07</td>
<td>0.95 - 1.21</td>
<td>0.29</td>
</tr>
<tr>
<td>Intermediate</td>
<td>1.01</td>
<td>0.74 - 1.36</td>
<td>0.97</td>
</tr>
<tr>
<td>Dark</td>
<td>0.55</td>
<td>0.38 - 0.80</td>
<td>0.003</td>
</tr>
<tr>
<td>First Pregnancy</td>
<td>0.88</td>
<td>0.78 - 1.01</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>BMI(^4)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;30kg/m(^2)</td>
<td>0.95</td>
<td>0.85 - 1.05</td>
<td>0.32</td>
</tr>
</tbody>
</table>

\(^1\)Multivariable mixed model analysis; \(^2\)Pregnancy life stage compared to postnatal (birth to 20 weeks); \(^3\)Skin colour as determined by spectrophotometry; \(^4\)BMI, Body mass index (13%>30kg/m\(^2\))
Figure 14: Variation in estimated mean maternal 25-hydroxyvitamin D, by season of conception

Geometric Means ± 95% CI presented. Time 0 on the x-axis represents time of birth, with negative values the antenatal months pre-delivery (no data was available earlier than 6 weeks gestation), and positive values postnatal months. Season of conception deduced from gestational age. Model adjusting for all variables in Table 13, excluding pregnancy status and season (both variables changing along the x-axis). 25OHD, 25-hydroxyvitamin D.
4.2.4 Discussion

The results of the present study contribute to the growing literature on vitamin D and PTH status during pregnancy and lactation in both mother and offspring, and to the best of our knowledge, are the first longitudinal data from the southern hemisphere. The main findings showed that during pregnancy and out to 5 months postnatally, at one or more time points, both infant and maternal vitamin D deficiency were very common, especially during winter and spring. Infant cord 25OHD status was most strongly correlated with maternal status towards the end of the third trimester, and importantly had no correlation with maternal status prior to 20 weeks gestation. In addition, pregnancy itself was an independent predictor of maternal vitamin D status with levels being higher at all measured time points throughout pregnancy compared with post-pregnancy values, a finding that remained after adjustment for all other measured variables.

Maternal and infant vitamin D deficiency found in the present study was greater than other comparative longitudinal pregnancy cohorts (rates of 19-33% (25OHD<50nmol/L)), albeit these studies were conducted in Scandinavia where dietary fortification and supplementation is more prevalent (Lundqvist et al., 2016; Milman et al., 2011). Our reported rates of deficiency are also greater than the 42% (maternal), and 57% (infant) deficiency rates (25OHD<50nmol/L) found in more northern New Zealand cohorts at 36-43°S (Camargo et al., 2010; Ekeroma et al., 2015). In fact, deficiency rates in the present study (of generally “low” risk women) are more comparable to the rate reported in a “high risk” multi-ethnic population from Wellington, at 41°S (Judkins & Eagleton, 2006). The significance of the levels of deficiency seen is emphasised by the three otherwise healthy breastfed infants (only one dark skinned) at 5 months of age (sampled during mid-winter/spring) who had biochemical values consistent with vitamin D deficiency rickets, a clinical consequence not seen in any participants of the aforementioned studies.

Our findings have important implications for those living in the south of the southern hemisphere. Firstly, the lack of correlation between cord 25OHD and early pregnancy maternal serum 25OHD (Figure 12), raises questions about the utility of screening for maternal vitamin D deficiency in early pregnancy as recommend by regional guidelines (Paxton et al., 2013). This, combined with the high rates of deficiency found (irrespective of season) in largely “low risk” light skinned European women and infants, means that consideration should be given to strengthening current guidelines, including further thought given to recommending a universal supplementation strategy during pregnancy and exclusive lactation. Currently, New Zealand’s supplementation policy recommends that supplementation be only considered for those who are: dark skinned; completely avoid sun exposure; have liver or kidney disease; or live in southern regions during winter (Ministry of Health, 2013). These
data show that without supplementation, these individuals are clearly not achieving optimal vitamin D status throughout pregnancy (Ministry of Health, 2013; A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium.). Furthermore, as pregnancy and lactation span multiple seasons, targeting only pregnant women in the “winter” is also not efficacious. Figure 14, which examines 25OHD status by season of conception, clearly demonstrates this, with first trimester summer participants having the lowest 25OHD status by the time of delivery (Figure 14).

Our longitudinal data highlights that 25OHD status during pregnancy and lactation is influenced by many variables. These include a dominant influence of season, which is well described in the literature in both pregnant and non-pregnant life stages (Ekeroma et al., 2015; Houghton et al., 2010; Rockell et al., 2005; A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium.). In addition, maternal 25OHD status appears higher in pregnancy than after delivery, an association that appears to exist beyond that explained by the other variables in our model. It is possible that this association is partially explained by other variables not in the model, such as sun exposure behaviour following pregnancy, but also suggest some of this longitudinal variability may be directly related to the physiological state of pregnancy itself. This would be consistent with other longitudinal pregnancy studies, which have shown a decrease in 25OHD status by the time of delivery (Holmes et al., 2009; Zhang et al., 2014). Milman et al found an increase in 25OHD status up to 32 weeks followed by a decrease until delivery (Milman et al., 2011). Likewise, Lundqvist et al. found 25OHD status increased until at least 35 weeks gestation, followed by a fall found at 12 weeks postnatal (Lundqvist et al., 2016). Physiological changes in pregnancy may account for this including variation in the vitamin D binding protein (VDBP) which has been shown to increase in pregnancy (Park et al., 2016; Zhang et al., 2014). The possible decline at the end of the third trimester could also reflect increasing 1,25-dihydroxyvitamin D production which is seen at this time (Hollis et al., 2011; Turner et al., 1988). Analytical measurement issues may have also contributed to inconsistent results, for instance the detection or not of the C3-epimer of vitamin D (Yazdanpanah et al., 2013), which has also been shown to increase in pregnancy (Park et al., 2016). Finally, PTH in pregnancy (and at birth and throughout lactation) in our data was shown to have a relatively weak inverse correlation with 25OHD status. This has also been reported previously (Haddow et al., 2011), and therefore does not appear to be a reliable predictor of adequate 25OHD status during pregnancy.

Sampling of both 25OHD and PTH in a setting of minimal dietary fortification and very little vitamin D supplementation to both mothers and infants throughout pregnancy and postpartum are particular strengths of the present study. Additionally, the longitudinal nature of the data collection, spanning
multiple stages of pregnancy to 5 months of lactation is another strength, as is the traditionally “low risk” largely ethnically homogeneous population. The latter point, however, is also a limitation, as it remains uncertain how generalizable the findings of this convenience population sample are to other latitudes (particularly those closer to the equator, where fluctuation in 25OHD status with season will be less) and to other ethnic groups. A final limitation is the relatively small sample size, which may have influenced some of our findings.

In conclusion, vitamin D deficiency in women and their infants is very common during pregnancy and lactation in New Zealand at 45°S. The main predictors of maternal vitamin D status were season, supplement use, skin colour, and pregnancy itself. However, the frequency, and at times severity of the vitamin D deficiency seen in this longitudinal cohort raise significant questions regarding the relevance and efficacy of current public health policies for those living in southern New Zealand, particularly the validity of first trimester screening and targeted supplementation for those currently defined “at risk”.
5 Discussion

This thesis has aimed to investigate the incidence and characteristics of vitamin D deficiency rickets in New Zealand children; to longitudinally examine vitamin D and parathyroid hormone status in New Zealand women and infants throughout pregnancy and exclusive lactation; and to investigate a maternal vitamin D supplementation strategy during exclusive lactation to dual-supplement both mothers and their infants. These research objectives were developed in order to complement and build on the existing literature in this field, and to utilise multiple research methodologies. Investigation or prevention of vitamin D deficiency and its consequences in childhood tie together the three investigation chapters of this thesis.

First described in the 1600s (Glisson et al., 1660), understood in the 1900s (McCollum et al., 1922; Mellanby, 1989), and “conquered” in the 1960s (Harrison, 1966), vitamin D deficiency rickets is the most important and severe consequence of childhood vitamin D deficiency. As previous recent studies on rickets incidence have confirmed, with a global incidence of between 2.9 - 60/100,000 children (Beck-Nielsen, Brock-Jacobsen, et al., 2009; Munns et al., 2012; Ward et al., 2007), rickets is occurring worldwide, and has definitely not been “conquered”. The findings from the prospective Paediatric Surveillance Unit study presented in chapter 2 highlight this, with an overall NZ childhood VDDR incidence of 2.2/100,000, increasing to 10.5/100,000 in those under three years of age. This work is the first national study from New Zealand, and only the third worldwide, which has prospectively collected national data on VDDR.

Of direct relevance to the study detailed in chapter 3 of this thesis, exclusive breastfeeding was a risk factor in our national rickets data. This was also found in the Canadian and Australian prospective studies (Munns et al., 2012; Ward et al., 2007). The project detailed in chapter 3 specifically targets this period of exclusive lactation, and provides early evidence that high-dose monthly maternal cholecalciferol (100,000IU/month) may offer an alternative to daily infant supplementation in order to improve infant vitamin D status, thereby potentially preventing rickets in exclusively breastfeeding Dunedin infants. The key results from this study were that, after adjustment for important variables (season, skin colour, and supplementation exposure), maternal cholecalciferol at 100,000IU/month resulted in a significant and clinically meaningful increase in infant 25OHD by 19.1 nmol/L, compared with that of the placebo group (p=0.025). This, combined with promising results from other studies using high-dose daily or once only dosing of cholecalciferol (Basile et al., 2006; Hollis & Wagner, 2004; Hollis et al., 2015; Oberhelman et al., 2013), suggests further consideration of maternal supplementation strategies is warranted.
Southern latitude was a further risk factor for childhood VDDR, as outlined in chapter 2. This finding is fully supported by findings from chapter 4, which provide (to the best of my knowledge) the first longitudinal vitamin D data during pregnancy and lactation that includes both maternal and infant measures, as well as the first longitudinal southern hemisphere pregnancy data. One of the key findings from this study was that at 45°S in largely unsupplemented women and their infants, rates of vitamin D deficiency are very high, found in 65% and 76% of all participants (women and their infants respectively) at one or more time points. These high rates of deficiency in traditionally “low” risk healthy light-skinned southern women/infants is much higher than comparable northern NZ (Camargo et al., 2010; Ekeroma et al., 2015; Grant et al., 2014), and northern hemisphere datasets (Lundqvist et al., 2016; Milman et al., 2011). The direct relevance of this finding to childhood rickets (chapter two) was further emphasised by the three cases of secondary hyperparathyroidism (and possible biochemical VDDR) found by week 20 of exclusive breastfeeding in this chapter four cohort. To the best of my knowledge, deficiency of this magnitude has not been reported before in any prospective/longitudinal descriptive studies in otherwise healthy women and their infants.

These findings from chapters 2 and 4 highlight the importance of infant supplementation during exclusive breastfeeding. While breastfeeding is the ideal nutrition source for infants (Kramer & Kakuma, 2002; Ministry of Health, 2013), breastmilk does not normally contain sufficient vitamin D to meet recommended daily requirements. This led to one of the conclusions from our rickets paper (published prior to completed analysis of chapters 3 and 4) that most of the cases we described would have been prevented, had the current NZ targeted vitamin D supplementation policy been adhered to (no cases with risk factors had received appropriate public health preventative measures). Clearly raising awareness of the need for supplementation, at minimum in those with readily identifiable risk factors, is required. However, based on the data from chapter 4 with very high rates of southern deficiency found in both mothers and their infants, considerably higher than comparable northern hemisphere, and northern NZ data, an argument could be made for universal supplementation, at least at southern NZ latitudes, in line with Northern hemisphere policy (Wagner et al., 2008). This makes sense, as the risk factors seen in our rickets data are essentially identical to more northern datasets, and the rates of maternal and infant deficiency found in our longitudinal study are in fact greater than current northern data. The important variation longitudinally during pregnancy, and therefore at birth, of vitamin D status when examined by season of conception, adds further weight to arguments for universal supplementation in pregnancy and lactation.

The alternative option to universal infant supplementation is high dose maternal supplementation, as shown in chapter 3. This offers some potential advantages over daily infant supplementation. These
include: the benefit of dual supplementation of both mother and baby; no interference with exclusive breastfeeding; and potential improvements in adherence (as opposed to the poor rates of adherence reported using daily infant supplementation seen in some studies (Ahrens et al., 2016). In addition, 50,000IU capsules are the only maternal supplement option currently funded in NZ, with no funded daily maternal option available. Funded infant options are also limited, the only infant option being vitadol C™, which also contains vitamin A and C (Ministry of Health, 2013). Daily doses using vitadol C™ above 400 IU cholecalciferol (e.g. as required for management of more severe deficiency or rickets) are therefore not advisable, due to the risk of vitamin A toxicity with this preparation. These New Zealand specific funding issues, for both infants and mothers, make a higher dose intermittent supplementation option attractive in the New Zealand setting.

Finally, as seen in chapter 3 and 4, a number of natural physiological phenomena are likely occurring during pregnancy and infancy that impact on vitamin D status and metabolism. These remain incompletely understood. The first of these phenomena is highlighted in both chapters 3 and 4, with the observed increase in infant 25OHD concentrations from birth to week 20, in the placebo and unsupplemented observation study groups. This potential natural temporal trend towards increased vitamin D status in the first year of life has also been found in a number of comparable studies (Challa et al., 2005; Grant et al., 2014; Kim et al., 2010; Narchi et al., 2011). Of course, this may be influenced by season, and an imbalance in season of recruitment was seen in chapter 3. However, a physiologic increase in the circulation of vitamin D binding proteins, a key determinant of 25OHD concentrations is also possible (Carpenter et al., 2013). Either way, these findings reveal the importance of a placebo group for nutritional studies of this nature, something lacking in many similar trials. An association between vitamin D and gestational/post-gestational age also appears to exist beyond that explained by the other variables in our model. This suggests that there is some longitudinal variation in 25OHD metabolism directly related to stage of pregnancy. While it remains possible that this association is partially explained by other variables not in our model, this finding is consistent with our current limited understanding of vitamin D metabolism during pregnancy. Other longitudinal pregnancy studies have shown a similar decrease in 25OHD status by the end of pregnancy (Holmes et al., 2009; Zhang et al., 2014), and our data is almost identical to Lundqvist et al. where this was found (Lundqvist et al., 2016). Similarly, Milman et al found an increase till 32 weeks, followed by a decrease through to delivery (Milman et al., 2011). A number of physiological changes in pregnancy may account for this, including genetic variations in the VDR (depending on the population studied), or variations in vitamin D binding proteins (VDBP), which, as in infancy (above), have been shown to also increase in pregnancy (Park et al., 2016; Zhang et al., 2014). The dip some have found in the third trimester could also reflect
increasing 1,25-dihydroxyvitamin D production, which is seen at this time of peak foetal calcium need (Hollis et al., 2011; Turner et al., 1988). Analysis issues may also contribute, for instance the detection or not of the C3-epimer of vitamin D (Yazdanpanah et al., 2013), which has also been shown to increase in pregnancy (Park et al., 2016).

5.1 Strengths and limitations

Study design is a key strength of the three studies of differing methodology that make up this thesis. This is highlighted firstly in chapter 2, with the national, prospective nature of the rickets surveillance study, using the established infrastructure of the NZPSU. The NZPSU represents 92% of the paediatricians registered with the NZ Medical council, and has a >90% response rate (B. J. Wheeler et al., 2015). For chapter 3, the randomised, double-blind, placebo-controlled trial design, as well as environment with little other concurrent infant supplementation/dietary fortification are major strengths, and separates this study from the majority of the existing trials on high-dose maternal vitamin D supplementation (Basile et al., 2006; Hollis & Wagner, 2004; Oberhelman et al., 2013; Saadi et al., 2009). Thirdly, the longitudinal data presented in chapter 4, which includes both infants and mothers from three time points in pregnancy to week 20 postnatal is a strength and point of difference from most descriptive studies of vitamin D status during pregnancy or lactation. Again, the New Zealand context of negligible dietary fortification, and minimal supplementation are additional advantages of descriptive studies of vitamin D status in New Zealand.

The high rates of exclusive and concurrent breastfeeding at completion of both the RCT and longitudinal descriptive study are also important strengths, given that the first 20 weeks of lactation were particular focuses of both these projects. This is a point of difference from other studies conducted during lactation (Grant et al., 2014; Hollis & Wagner, 2004; Hollis et al., 2015; March et al., 2015; Oberhelman et al., 2013; Saadi et al., 2009). In addition, the robust and detailed data collection from the three studies is a strength, this includes the detailed reporting of rickets by New Zealand paediatricians, as well as the detailed collection of data for chapters three and four on skin colour (by objective spectrophotometry), supplement use, season, and other sociodemographic factors. Vital factors such as season have been overlooked or restricted in some previous longitudinal studies (Cross et al., 1995; Ritchie et al., 1998; Zhang et al., 2014).

As with all research, the work described in this thesis has limitations. For the rickets surveillance study the biggest weakness lies in possible under-reporting or under-diagnosis of cases of VDDR. This is possible as reporting was restricted to Paediatricians. In addition, the Canterbury region also
experienced two catastrophic earthquakes in the first year of reporting, and there did appear to be smaller numbers of cases reported from this region. Under diagnosis may be reflected in the low numbers of incidentally diagnosed rickets. In the Australian data 80% of cases were found on “screening” (Munns et al., 2012), although frequency of incidental cases in the Canadian data, was identical to my NZPSU study (Ward et al., 2007).

For the projects detailed in chapters 3 and 4, it is unknown how generalizable the results are to other ethnicities and latitudes. Given that the majority of participants were of “low” risk light-skinned European ethnicity, the findings of these studies are likely to be similar or more profound in those with darker skins or non-European ethnicity. A further limitation, is ideally additional analysis of breast milk and of blood for additional vitamin D metabolites and binding proteins would have been conducted to give a more complete picture of vitamin D status and metabolism. Unfortunately funding did not allow for this. However, 25OHD was the primary outcome of interest for both our RCT and longitudinal study. In addition, measurement of PTH, and also 25OHD status in infants are important strengths and points of difference in the longitudinal study (chapter 4).

Finally, for chapters 3 and 4, type 2 error remains a possibility due to limitations in sample size, and for the RCT, distribution by season. This is one explanation for the clinically (15.8nmol/L) but not statistically significant (p=0.13) change in 25OHD status compared to placebo in the 100,00 IU group of the RCT in the primary analysis, and why a statistically significant change was only found after adjustment for season, skin colour, and supplement intake.

5.2 Future directions

The RCT detailed in chapter 3 found that maternal vitamin D supplementation in lactating women appears safe and may improve infant status. This suggests that further studies examining maternal supplementation strategies for dual supplementation of lactating mothers and their infants is required before this could be recommended as a public health strategy. In particular, given the data we now have, future larger studies could look at varying dosage regimens (daily vs intermittent e.g. weekly, fortnightly, monthly) as alternative infant supplementation options to the currently recommended daily infant strategy, as well as confirm, or not, our findings and effect size. Combining these with a third trimester maternal supplementation strategy similar to Grant et al. (Grant et al., 2014) or March et al. (March et al., 2015) is also likely to provide additional efficacy. This combined approach is likely to be of most benefit as a future public health intervention.
While that is occurring, efforts to increase awareness of New Zealand’s current targeted maternal and infant vitamin D supplementation policy are required, as highlighted in the rickets cases diagnosed in chapter two. At the same time advocacy for consideration of universal supplementation, at least at southern latitudes, is also required. Once these initiatives to increase awareness and supplementation have occurred, then a repeat of our prospective rickets study via the NZPSU would be of value – to measure success or not of public health policy implementation in this area.

In addition, I have now obtained a grant for a study investigating subsequent bone outcomes (in our RCT cohort) that may be more important than the surrogate 25OHD status that we have measured in these studies. When vitamin D status during pregnancy or infancy is linked to concrete health outcomes, this will provide stronger evidence/support for promoting maternal and infant supplementation strategies. To this end, in collaboration with the colleagues from the University of Otago Dental School, we plan a histological, molecular composition, and clinical follow up study to examine exfoliated teeth and dental caries/enamel defects in the children from the longitudinal cohort study detailed in Chapter 4. These various dental outcomes will then be compared to infant cord vitamin D status and maternal pregnancy status, measures taken over a time course that reflects the period of peak fetal bone calcium accrual.
5.3 Conclusion

In conclusion, this thesis has examined several aspects of vitamin D status and pathology spanning pregnancy, lactation, and childhood. It has demonstrated that in New Zealand, vitamin D deficiency during pregnancy, lactation and childhood remains an important health concern. In Dunedin, at 45°S, both infant and maternal deficiency during pregnancy and lactation is very common, and sometimes severe. Severe deficiency can lead to childhood rickets, and this thesis has demonstrated that rickets, an important bone pathology that can be easily prevented with vitamin D supplementation, is still occurring in New Zealand, with a higher incidence in children with mothers from India and Africa, and in children younger than three years, who are currently or previously breastfed. While infancy and breastfeeding are risk factors for rickets, high-dose monthly maternal supplementation may also hold promise as an alternative dual maternal and infant supplementation strategy. Further research is now required to expand on the findings of the projects at the heart of this thesis.
References


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Rockell, J. E., Skeaff, C. M., Venn, B. J., Williams, S. M., & Green, T. J. (2008). Vitamin D insufficiency in New Zealanders during the winter is associated with higher parathyroid hormone concentrations: implications for bone health? *N Z Med J, 121*(1286), 75-84.


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Appendices

Appendix 1. NZPSU - Protocol - Vitamin D deficiency rickets study

VITAMIN D DEFICIENCY RICKETS

Background to Study:
Vitamin D is critical for calcium homeostasis and for mineralization of the skeleton, especially during periods of growth. The most severe consequence of vitamin D deficiency during childhood is rickets (a mineralization defect at the epiphyseal growth plates). Rickets is associated with pain, fractures, skeletal deformity, growth restriction, dental defects, delayed developmental milestones and, in severe cases, hypocalcaemic seizures. Deficiency of vitamin D in children, without overt rickets, is also associated with low bone mineral density which has potential long-term implications particularly in adulthood. Of added concern, it has been recognised that Vitamin D has a major role to play in the developing and developed immune system. Low levels during foetal life, early infancy and into adulthood potentially have a major effect on the development of infection, autoimmune and cardiovascular disease, and possibly cancer.

Vitamin D is unique among vitamins as its main source is not dietary, but direct synthesis in the skin following exposure to UVB radiation from sunshine. UVB radiation exposure varies based on latitude, skin colour, sunscreen use and clothing. Changes in human lifestyle including sun avoidance practices, indoor occupations and recreation, added to New Zealand’s geographical location at 35ºS to 47ºS mean that children and adults cannot depend on adequate skin exposure to sunlight for vitamin D synthesis, especially during winter months. Dietary intake is a secondary source, but for a few exceptions, without additional supplementation there is little in the foods humans normally ingest. Compounding this issue, human milk, is often not rich in vitamin D.

There is evidence from across NZ and around the globe that vitamin D deficiency and rickets are increasingly common. Particularly at risk are infants who are breast-fed by mothers with poor vitamin D intake or inadequate sun exposure (due to veiling/southern latitudes/dark skin).

Despite this knowledge, there are no national recommendations for supplementation (targeted or universal) of exclusively breast-fed infants, and as opposed to many countries, NZ currently has no mandatory and little voluntary food supplementation. The current NZ Ministry of Health position is that supplementation is unnecessary.

The precise incidence of vitamin D deficiency rickets in New Zealand is unknown. This lack of available incidence and prevalence data and the concern that this preventable disease is on the rise underline the importance of a prospective surveillance study.
Objectives:
1. To ascertain the incidence of simple vitamin D deficiency rickets (also known as nutritional rickets) diagnosed by specialist paediatricians over a two-year period
2. To obtain demographic and medical information which will assist in the:
   a. Identification of risk factors for development of the disease in NZ
   b. Evaluation of current preventive strategies.
3. To supply data that will help develop public health policies to prevent vitamin D deficiency rickets among children living in New Zealand.

CASE DEFINITION
Children up to and including 15 years of age with rickets secondary to simple Vitamin D deficiency (also known as nutritional rickets) confirmed biochemically and/or radiographically.

Inclusion criteria (Biochemical)
1. Low serum 25-hydroxyvitamin D (25OHD) (<50nmol/L)
2. Elevated serum alkaline phosphatase (ALP) (age specific)

Supplemental data ideally to be obtained prior to treatment (and expected results†):
1. Serum calcium (normal or low) and albumin
2. Serum phosphate (normal or low)
3. Serum PTH (elevated)
4. Serum 1,25-dihydroxyvitamin D (1,25(OH)2D; low, normal or high)
5. X-ray confirmation of rickets at the distal ulnar or femoral epiphysis‡

* These results are not essential for reporting.
† Ionized calcium is also acceptable.
‡ In rare instances, the x-ray features of rickets may not be present at diagnosis e.g. if linear growth is arrested (and growth plate activity is blunted) or in the very early phase of the disease when x-ray changes at the growth plate are not yet visible. For this reason, although x-ray confirmation of rickets is not a strict inclusion criterion it should be obtained during the initial patient evaluation.

Follow-up of positive returns:
A questionnaire requesting further details will be sent to notifying paediatricians.

If you have any questions please contact:
Dr Ben Wheeler (Principal Investigator)
Senior Lecturer / Paediatrician
Dunedin School of Medicine
University of Otago
Email: ben.wheeler@otago.ac.nz
Phone: 027 4701980
Fax: 03 4747817
Appendix 2. NZPSU - Data collection form - Vitamin D deficiency rickets study

VITAMIN D DEFICIENCY RICKETS (VDDR)
NZPSU Reporting form
Principal Investigator: Dr Ben Wheeler
Please contact Dr Ben Wheeler at 027 4701980 or ben.wheeler@botago.ac.nz if you have any questions about this form

CASE DEFINITION FOR VITAMIN D DEFICIENCY RICKETS
Children ≤15 years of age with rickets secondary to simple Vitamin D deficiency (also known as nutritional rickets) confirmed biochemically and/or radiologically.

Inclusion criteria (Biochemical)
1. Low serum 25-hydroxyvitamin D (25OHD)
2. Elevated serum alkaline phosphatase

Supplemental data ideally to be obtained prior to treatment (and expected results):
1. Serum calcium and albumin (normal or low)
2. Serum phosphate (normal or low)
3. Serum PTH (elevated)
4. X-ray confirmation of rickets at the distal ulnar or femoral epiphysis
5. Haemoglobin, MCV and serum ferritin

These results are not essential for reporting.

Ionized Calcium is also acceptable

In rare instances, the x-ray features of rickets may not be obtained at diagnosis e.g. if linear growth is arrested (and growth plate activity is blunted) or in the very early phase of the disease when x-ray changes at the growth plate are not yet visible. For this reason, x-ray confirmation of rickets is not a strict inclusion criterion but should be obtained during the initial patient evaluation.

Exclusion criteria
1. Vitamin D deficiency rickets associated with underlying disease, such as fat malabsorption, liver disease and renal insufficiency. Patients receiving total parenteral nutrition are also excluded.
2. Vitamin D deficiency secondary to heritable disorders of vitamin D metabolism, including:
   • 1a-hydroxylase deficiency (pseudo-vitamin D deficiency rickets)
   • Vitamin D receptor defects (hypocalcemic vitamin D resistant rickets)
3. Phosphopara rickets of any aetiology (where hypophosphatemia is the primary cause of the rickets, and not due to calcipenic rickets with secondary hyperparathyroidism)

REPORTING CLINICIAN
1. Name ________________________________ 2. Month/Year of Report ______/____
3. Date questionnaire completed ______/____/____

PATIENT DETAILS
4. First 2 letters of first name _________ 5. First 2 letters of surname: _________
6. Date of Birth ______/____/____
7. Sex: ☐ M ☐ F
8. Date of diagnosis: ______/____/____
9. Location (City/Town) __________________________

11. Has the child’s mother immigrated to New Zealand? Yes ☐ No ☐ Unknown ☐
    If Yes, from what country? __________________________
    When (month/year)? ______/____
If this patient is primarily cared for by another physician whom you believe will report the case, please write the other physician’s name and complete questionnaire details above this line and return. If no other report is received for this child we will contact you for further information. Please keep the patient’s name and other details on your NZPSU file.

The primary clinician caring for this child is: **Name:** ___________________________ **Hospital:** ___________________________

**Instructions:** Please answer each question by ticking the appropriate box or writing your response in the space provided.

*DK = Don’t Know  NA = Not Applicable*

**FAMILY DETAILS**

12. **Ethnicity of Mother:** *(tick as many as apply)*

- NZ European [ ]
- Maori [ ]
- Samoan [ ]
- Cook Island Maori [ ]
- Tongan [ ]
- Chinese [ ]
- Indian [ ]
- Niuean [ ]
- Other [ ] Please state: ___________________________

13. **Mother’s Country of birth:** ___________________________

14. **Ethnicity of Father:** *(tick as many as apply)*

- NZ European [ ]
- Maori [ ]
- Samoan [ ]
- Cook Island Maori [ ]
- Tongan [ ]
- Chinese [ ]
- Indian [ ]
- Niuean [ ]
- Other [ ] Please state: ___________________________

15. **Father’s Country of birth:** ___________________________

16. **Where did the child spend most of his/her life?**

- City/Town: ___________________________
- Province: ___________________________
- DK [ ]

17. a) **Number of children in the family:**

- DK [ ]

b) **Number diagnosed with vitamin D deficiency rickets:**

- DK [ ]

**MEDICAL HISTORY:**

18. **Does the child have other medical conditions (including allergies)?**

- Yes [ ]
- No [ ]
- DK [ ]

*If yes, please specify: ___________________________

19. **Was the child on any medications at diagnosis (other than Vitamin D)?**

- Yes [ ]
- No [ ]
- DK [ ]

*If yes, please specify: ___________________________

20. **Age of mother at delivery:**

- DK [ ]

21. **Gestational age:**

- DK [ ]

22. **Birth Weight:**

- DK [ ]
NUTRITION HISTORY:
A) CHILD
23. Was/is the child breastfed? Yes ☐ No ☐ DK ☐
If yes, specify duration: ________ months Exclusively? Yes ☐ No ☐ DK ☐
24. Did the child receive commercial infant formula? Yes ☐ No ☐ DK ☐
If yes, at what age? ________
25. Does/did the child drink unmodified cow’s milk? Yes ☐ No ☐ DK ☐
26. Did the child receive multi-vitamin/vitamin D supplements prior to diagnosis of rickets? Yes ☐ No ☐ DK ☐
If yes, specify which vitamin preparation was used? ____________________________ DK ☐
If yes, prescribed dose:_______ DK ☐ Age started:_______ DK ☐ Duration given: _______ DK ☐

B) MOTHER
27. Did the mother receive vitamin D supplementation during her pregnancy? Yes ☐ No ☐ DK ☐
If yes, specify which vitamin preparation was used? ____________________________ DK ☐
If yes, prescribed dose:_______ DK ☐ Duration taken (months): _______ DK ☐
28. Did the mother receive vitamin D supplementation after delivery? Yes ☐ No ☐ DK ☐
If yes, specify which vitamin preparation was used? ____________________________ DK ☐
If yes, prescribed dose:_______ DK ☐ Duration taken (months): _______ DK ☐
OTHER RISK FACTORS FOR VITAMIN D DEFICIENCY
29. What is the child’s skin colour? Dark ☐ Intermediate ☐ Fair ☐
30. What is the mother’s skin colour? Dark ☐ Intermediate ☐ Fair ☐
31. Did/Does the child use sunscreen? Always ☐ Usually ☐ Never ☐ DK ☐
32. Was the mother veiled during the pregnancy? Yes ☐ No ☐ DK ☐
If yes, please tick the appropriate category below (tick one only): DK ☐
☐ consistently covered – covered up, including arms, hair and neck, when outdoors
☐ inconsistently covered – did not usually cover fully in her own backyard/garden
☐ uncovered – did not generally cover up arms, hair and neck when outdoors
33. Is the child veiled? Yes ☐ No ☐ DK ☐
If yes, please tick the appropriate category below (tick one only): DK ☐
☐ consistently covered – covered up, including arms, hair and neck, when outdoors
☐ inconsistently covered – did not usually cover fully in her own backyard/garden
☐ uncovered – did not generally cover up arms, hair and neck when outdoors
If yes, from what age? ________
CLINICAL PRESENTATION AND DIAGNOSTIC STUDIES (ideally obtained prior to treatment)

34. a) What were the child’s presenting signs and symptoms? (tick as many as apply)
   - Seizures ☐
   - Limb deformity ☐
   - Fracture ☐
   - Motor delay ☐
   - Respiratory disease ☐
   - Poor growth ☐
   - Hypotonia ☐
   - Bone pain ☐
   - Other: ______________________

b) Was the child diagnosed due to screening because of affected sibling/s?  
   - Yes ☐
   - No ☐

35. Were there radiological signs of rickets? 
   - Yes ☐
   - No ☐
   - Not Done ☐
   - DK ☐

   If yes, (tick as many as apply)
   - distal ulna ☐
   - distal femoral epiphysis ☐

36. Biochemical data (please indicate whether results were obtained prior to treatment (Rx) or once Rx was initiated, along with the appropriate units and normal ranges for your laboratory)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results prior to Rx</th>
<th>Results during Rx*</th>
<th>Units</th>
<th>Normal range</th>
<th>DK</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-hydroxyvitamin D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ionized calcium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total calcium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Only necessary if biochemistry was not performed before starting vitamin D therapy.

37. Was 25-hydroxyvitamin D obtained in the mother?  
   - Yes ☐
   - No ☐
   - DK ☐

   If yes, what was the result? ______________________

MEDICAL TREATMENT

38. Was the child commenced on treatment?  
   - Yes ☐
   - No ☐
   - DK ☐

   If yes, what was prescribed?

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dose (units)</th>
<th>Frequency</th>
<th>Duration (days/weeks/months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MISCELLANEOUS

39. In your opinion, please check the risk factors for Rickets: (tick as many as apply)

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Child</th>
<th>Mother</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark-skinned</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inadequate dietary intake of vitamin D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inadequate vitamin D supplementation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inadequate sun exposure - Lifestyle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inadequate sun exposure - Southern latitude</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other (please specify): ______________________

Thank you for completing this form
Appendix 3. NZPSU - Ethics approval Vitamin D deficiency rickets study

Health and Disability Ethics Committees

21 June 2012

Dr Ben Wheeler
Department of Women’s and Children’s Health
Dunedin School of Medicine
PO Box 913
Dunedin 9054

Dear Dr Wheeler

Ethics ref: LRS/10/EXP/016 (please quote in all correspondence)
Study title: Vitamin D deficiency rickets (VDDR)

Thank you for submitting the annual progress report relating to the above named study. This report has been reviewed and approved by the Chairperson of the Lower South Ethics Committee under delegated authority.

Approved Document:

- Ethics Committee Progress Report signed and dated 13 June 2012 by Ben Wheeler

Ongoing ethical approval is reconfirmed until 11 June 2013. Please remember to submit the next annual progress report before this date. From 1 July 2012 all report forms must be submitted through Online Forms, please see www.ethics.health.govt.nz.

Please do not hesitate to contact me should you have any queries.

Yours sincerely

[Signature]

AWHINA RANGIWAI
ADMINISTRATOR
Lower South Ethics Committee
INCIDENCE AND CHARACTERISTICS OF VITAMIN D DEFICIENCY RICKETS IN NEW ZEALAND CHILDREN: A NZPSU STUDY

Wheeler BJ\(^1\), Dickson NP\(^2\), Houghton L\(^3\), Ward LM\(^4\), Taylor BJ\(^1,2\)

1Department of Women’s and Children’s Health, Dunedin School of Medicine, University of Otago, New Zealand
2NZ Paediatric Surveillance Unit, Department of Women’s and Child Health, Dunedin School of Medicine, University of Otago, NZ
3Department of Human Nutrition, University of Otago, Dunedin, New Zealand
4Department of Paediatrics, University of Ottawa, Ottawa, Ontario, Canada

Background
- Vitamin D critical for calcium homeostasis & skeletal mineralization
- Rickets is a mineralisation defect at growth plate & the most profound manifestation of vitamin D deficiency

Methods
- Prospective surveillance of Vitamin D Deficiency Rickets by the NZ Paediatric Surveillance Unit
- 36 months, from July 2010 – June 2013 inclusive.
- Inclusion criteria:
  - Children & adolescents ≤15 years with rickets
    (Defined by low 25-hydroxyvitamin D and elevated ALP, and/or radiological rickets)
- Questionnaire to obtain de-identified clinical, biochemical, management & demographic data

Results
- 58 cases of rickets confirmed and included
- Overall estimated annual incidence 2.2/100,000
- For children <3 years annual incidence 10.5/100,000
- Majority Indian (41%) or African ethnicities (24%)
- 95% children born in NZ (compared to 22% mothers)
- 81% Dark/Intermediate skin colour
- 93% Exclusive breastfeeding (past and/or current)

Table 1: Reaso for presentation of rickets

<table>
<thead>
<tr>
<th>Reason for presentation</th>
<th>No. of cases (%) Total n=58†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal abnormality/DELAY</td>
<td>17 (29.3%)</td>
</tr>
<tr>
<td>Poor growth</td>
<td>15 (25.9%)</td>
</tr>
<tr>
<td>Developmental Delay</td>
<td>12 (20.7%)</td>
</tr>
<tr>
<td>Symptomatic hypocalcaemia – Seizure (E)</td>
<td>9 (15.5%)</td>
</tr>
<tr>
<td>Bone pain</td>
<td>7 (12.1%)</td>
</tr>
<tr>
<td>Respiratory disease</td>
<td>5 (8.6%)</td>
</tr>
<tr>
<td>Fracture</td>
<td>5 (8.6%)</td>
</tr>
<tr>
<td>Incidental finding</td>
<td>4 (7%)</td>
</tr>
<tr>
<td>Eczema</td>
<td>3 (5.2%)</td>
</tr>
<tr>
<td>Screening (as sibling with rickets)</td>
<td>2 (3.4%)</td>
</tr>
<tr>
<td>Cardiacopathy</td>
<td>1 (1.7%)</td>
</tr>
</tbody>
</table>

Acknowledgements
Prof. Craig Murra and the Canadian and Australian Paediatric Surveillance Units
We would also like to thank all clinicians who participated by notifying cases
The NZPSU is funded by the NZ Ministry of Health

Table 2: Incidence of rickets in NZ by province

<table>
<thead>
<tr>
<th>Age group, years</th>
<th>Under 3</th>
<th>3 – 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined Provincial Region</td>
<td>Total n=58†</td>
<td>No. of confirmed cases</td>
</tr>
<tr>
<td>Auckland/Southland</td>
<td>10 (19)</td>
<td>9 (15)</td>
</tr>
<tr>
<td>Wellington/Manaia</td>
<td>14 (24)</td>
<td>13</td>
</tr>
<tr>
<td>Canterbury/Nelson</td>
<td>5 (9)</td>
<td>5</td>
</tr>
<tr>
<td>Otago/Southland</td>
<td>11 (19)</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>58 (100)</td>
<td>55</td>
</tr>
</tbody>
</table>

Table 3: Case biochemical analysis and x-ray status

<table>
<thead>
<tr>
<th>Biochemical analysis</th>
<th>Number of Children (n)</th>
<th>Reference range</th>
<th>Median</th>
<th>Range (cm)</th>
<th>Number abnormal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-Hydroxyvitamin D (nmol/L)</td>
<td>50</td>
<td>80 - 150</td>
<td>120</td>
<td>110 - 90</td>
<td>50.70 (100%)</td>
</tr>
<tr>
<td>Alkaline phosphatase (UI/L)</td>
<td>58</td>
<td>80 - 300</td>
<td>78</td>
<td>35 - 100</td>
<td>50.58 (100%)</td>
</tr>
<tr>
<td>Parathyroid hormone (pmol/L)</td>
<td>43</td>
<td>1.0 - 5.0</td>
<td>2.7</td>
<td>1.0 - 6.0</td>
<td>34.50 (79%)</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>55</td>
<td>2.1 - 2.65*</td>
<td>2.3</td>
<td>2.1 - 2.65</td>
<td>20.55 (36.5%)</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>55</td>
<td>1.0 - 2.2*</td>
<td>1.3</td>
<td>1.0 - 2.2</td>
<td>19.10 (35%)</td>
</tr>
<tr>
<td>X-ray changes</td>
<td>41</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>41.53 (75.4%)</td>
</tr>
</tbody>
</table>

*Laboratory ranges varied by age and location, leading to 100% incidence of elevated levels. Reference ranges vary based on age. Categorised according to age appropriate ranges, this phosphate can elevated based on age appropriate cut-points.

Figure 1: Cases of rickets diagnosed by month

Conclusion
- Vitamin D deficiency rickets remains a health problem for New Zealand children
- Key risk factors remain similar to those previously identified
- Increasing awareness/implementation of current public health policies for targeted maternal, infant, & child supplementation is required
Incidence and characteristics of vitamin D deficiency rickets in New Zealand children: a prospective New Zealand paediatric surveillance unit study

Benjamin Wheeler\textsuperscript{1,2*}, Nigel Dickson\textsuperscript{3}, Lisa Houghton\textsuperscript{4}, Leanne Ward\textsuperscript{5}, Barry Taylor\textsuperscript{1,3}

From 8th APPES Biennial Scientific Meeting

Darwin, Australia. 29 November – 1 November 2014

Vitamin D deficiency rickets is the most significant manifestation of vitamin D deficiency in growing children. Concerns have been raised in New Zealand (NZ), and worldwide, that cases continue to present, and may possibly increase. We undertook a prospective study to investigate the incidence and characteristics of vitamin D deficiency rickets in NZ children.

Prospective surveillance of Vitamin D Deficiency Rickets was conducted by the NZ Paediatric Surveillance Unit (NZPSU), for 36 months, from July 2010 – June 2013 inclusive. Inclusion criteria were: children aged <15 years with vitamin D deficiency rickets (defined by low 25-hydroxyvitamin D and elevated alkaline phosphatase levels, and/or radiological rickets).

56 children with confirmed vitamin D deficiency rickets were identified. Median age was 1.4 years (range 0.3 – 11), male gender 47%, 95% of children were born in NZ, as opposed to 22% of mothers. Overall annual incidence in those aged <15 years was 2.2/100,000, while incidence in the south of NZ peaked at 6.8/100,000. Overall NZ incidence in children aged <5 years was higher at 6.6/100,000. Skeletal abnormalities, poor growth and developmental delay were the most common presenting features, with hypocalcaemia and convulsion in 16%. Key risk factors identified were: dark skin pigmentation, Indian/South Asian and African ethnicity, age ≤2 years, exclusive breast feeding, and southern latitude, particularly when combined with season (winter/spring).

Vitamin D deficiency rickets remains a health problem for New Zealand children, with significant associated morbidity. Public health policy, utilising infant supplementation, for at minimum the above identified risk factors, should be considered to reduce the incidence of this disease among those at high risk.

Authors’ details
1Department of Women’s and Children’s Health, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand. 2Paediatric Endocrinology, Southern District Health Board, Dunedin, New Zealand. 3Department of Paediatrics and Child Health, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand. 4Department of Human Nutrition, University of Otago, Dunedin, New Zealand. 5Department of Pediatrics, University of Ottawa, Ottawa, Ontario, Canada.

Published: 28 April 2015

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Appendix 6. RCT - Protocol – vitamin D and breastfeeding study

Department of Women’s and Children’s Health

Dunedin School of Medicine

University Of Otago

Clinical Protocol:

The Vitamin D and Breast Feeding Study:

Intermittent maternal vitamin D supplementation to prevent vitamin D deficiency in the breast feeding infant and lactating mother.

ANZCTR: ACTRN12611000108910

June 2011

Primary Investigator: Dr Ben Wheeler

Co-Investigators: Dr Lisa Houghton, Prof. Barry Taylor, Prof. Peter Herbison, Dr Adel Mekhail
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List of Abbreviations

1,25OHD2 1,25-DiHydroxyvitamin D
25OHD 25-Hydroxyvitamin D
LMC Lead Maternity Carer
NZ New Zealand
UVB Ultraviolet B
SCL Southern Community Laboratories
Study Protocol:
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**Birth**

(X) Mum- only if IV line in or samples being collected

(X) Baby – Cord Blood
1. Key Roles

Investigators:

Principle Investigator: Dr Ben Wheeler

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Dr Lisa Houghton, Human Nutrition
Prof. Peter Herbison, Preventive and Social Medicine
Dr Adel Mekhail, Women’s and Children’s Health

Research Nurse:

Mrs Shirley Jones, Women’s and Children’s Health
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Off site Randomisation / Supplement-Placebo provision:

Mrs Kirsten Simonsen, Dunedin Hospital Pharmacy

Funding:

Healthcare Otago Charitable Trust
Dunedin School of Medicine - New Researcher Start-up grant
Deans Bequest Grant
2. Background Information and Scientific Rationale

Vitamin D is critical for calcium homeostasis and for mineralization of the skeleton. The most severe consequence of vitamin D deficiency in childhood is rickets (a mineralization defect at the epiphyseal growth plates) and osteomalacia (a mineralization defect of bone tissue). These effects can be associated with pain, fractures, skeletal deformity, growth retardation, dental defects, delayed developmental milestones and, in severe cases, seizures. Deficiency in children, without rickets, is also associated with low bone mineral density (Cheng et al., 2003) which has potential long-term implications particularly in adulthood. It has been recognised that Vitamin D plays a major role in the immune system. Low levels during foetal life, early infancy and adulthood potentially have a major effect on response to infection, the development of autoimmune disease as well as cardiovascular disease, and possibly cancer (M. F. Holick, 2004; Urashima et al., 2010).

Vitamin D is unique among vitamins as its main source is not dietary, but direct synthesis in the skin following exposure to UVB radiation, from sunlight. UVB radiation exposure varies based on latitude, skin colour, sunscreen use and clothing (McKenzie et al., 2009). Changes in human lifestyle including sun avoidance practices, indoor occupations and recreation, added to NZ’s geographical location at 35ºS to 47ºS, mean that children and adults cannot depend on adequate skin exposure to sunlight for vitamin D synthesis, especially during winter months (McKenzie et al., 2009; Rockell et al., 2008). At latitude 45 ºS the Otago region is particularly at risk.

Dietary intake is a secondary source, but without additional supplementation there is little in foods most humans normally ingest. As opposed to many countries NZ
currently has no mandatory food supplementation. Compounding this, human milk, which is advocated as the ideal fluid source for infants, has been criticised as being generally low in vitamin D and this has led to recommendations for universal supplementation to breast feeding infants in some countries (Wagner et al., 2008). One of the main reasons breast milk may not be a rich source of vitamin D is that many breast feeding mothers are themselves vitamin D deficient (Judkins & Eagleton, 2006; Nozza & Rodda, 2001). Preliminary work from the United States suggests that daily supplementation of the breast feeding mother with high dose vitamin D can safely lead to not only improved vitamin D levels for her, but also in her milk and subsequently her nursing infant (Hollis & Wagner, 2004). This suggests that an attractive alternative to universal supplementation of breast fed infants may be their indirect supplementation via effective supplementation of their mothers and hence their breast milk. This would support current WHO recommendations on the importance of breast feeding and also avoid the issue of direct supplementation of exclusively breast-fed infants which is controversial and poorly complied with by families (Taylor et al., 2010).

We propose, to investigate for the first time, the effect of high dose intermittent maternal vitamin D supplementation e.g. monthly on vitamin D levels of both the mother and nursing infant. This will be a safe (Hollis & Wagner, 2004; Vieth, 1999), easy and potentially more acceptable alternative to both daily maternal and/or infant dosing. In NZ we have a logical option in cholecalciferol 50,000 IU tablets (vitamin D3). Giving this fortnightly or monthly will be safe for mothers and provide a similar monthly vitamin D dose to that already shown to be of benefit in raising vitamin D levels in mothers, maternal breast milk and indirectly in the nursing infant.

3. Study Hypothesis and Objectives

3.1 Overall Goals:

To inform policy of effective and feasible interventions to combat vitamin D deficiency in exclusively breast fed infants. The testing of our hypotheses will allow policy makers to determine the value of providing high dose intermittent maternal vitamin D supplementation as an alternative to direct infant supplementation with vitamin D.
during periods of exclusive breastfeeding, thus encouraging and supporting true exclusive breast feeding.

3.2 **Hypothesis:**

It is proposed that monthly maternal supplementation with Cholecalciferol may improve the vitamin D status of the exclusively breast fed baby, the human milk and the lactating mother. We, therefore hypothesize that the serum 25OHD level of the exclusively breast fed infant and mother will be greater when supplemented with monthly 100,000IU vitamin D > 50,000IU vitamin D > placebo.

3.2 **Objectives:**

The main objective of this study is to evaluate the impact of maternal monthly vitamin D supplementation in the form of Cholecalciferol D3 on the 25OHD status of her exclusively breast fed infant. Secondly we will evaluate the maternal 25OHD status, the Cholecalciferol D3 status of human milk and a variety of bone markers.

3.3 **Primary Outcome Measure:**

Change in Infant 25OHD (nmol/L) at 20 weeks /proportion achieving serum 25OHD >50

3.4 **Secondary Outcome Measures:**

Change in Maternal 25OHD (nmol/L) at 20 weeks /proportion achieving serum 25OHD >50

Change in other bone/vitamin D markers at 20 weeks (PTH, Ca, P, ALP)

Change in Breast milk vitamin D3 at 20 weeks

4. **Subject Selection**

4.1 **Research Site:**

Dunedin and its surrounds is the single site for this study. Home visits will comprise the bulk of the investigator/subject contact. Some contact will be had within two locations in the Dunedin Public Hospital: Queen Mary maternity unit (98% of Otago’s
1800 deliveries/year); and the paediatric outpatient department (as a potential site for phlebotomy).

4.2 Number of Subjects/Power Calculation:

Calculated on basis of detecting a 15 nmol/L difference in mean serum 25(OH)D at study end [approximately 90% of the observed decline from the 2002 National Children’s Nutrition Survey data, while controlling for baseline 25OHD levels]. To have 80% power to detect such an effect with a standard deviation of 20 nmol/L (Rockell et al., 2005), 28 children would be required in each arm. Allowing for an estimated 20% loss to follow-up, 105 families will be recruited at baseline.

4.3 Recruitment:

Recruitment will occur via a variety of avenues.

4.3.1 With the receipt of maternal booking letter (sent via the DHB to confirm booking at Queen Mary), an introduction to the study will be sent out. This has our contact details but is intended as only a warning of future contact.

4.3.2 Identification of potential participants from antenatal registration. A weekly list of new registrations will be obtained from the Queen Mary IT staff (Darren Klemp (Darren.klemp@southerndhb.govt.nz)). At 20-24 weeks gestation - Potential participants will then be contacted by mail, and offered participation by an opt out (from January 2012, was opt in prior to this) design (to contact us by email, phone to participate/for further information). Families who do not opt-out will be contacted by phone a week later to discuss.

4.3.3 Directly approaching LMCs via email, breast feeding education sessions, and in person. LMCs may then recruit or refer directly

4.3.4 Direct recruitment from Dr Adel Mikail and A.Prof. Mike Stitley’s antenatal clinics

4.3.5 Pamphlets at breastfeeding antenatal classes

4.3.6 Posters at antenatal classes/Queen Mary/other

4.4 Inclusion Criteria:
4.4.1 Healthy pregnant women from Dunedin
4.4.2 Planning to exclusively breast fed for 5 months
4.4.3 Willing to take intermittent vitamin D supplementation
4.4.4 Ability to obtain written informed consent

4.5 Exclusion Criteria

4.5.1 Unwilling to stop taking additional postnatal supplements that contain vitamin D
4.5.2 Not intending to exclusively breast feed for at least 5 months
4.5.3 Delivery prior to 37 weeks gestation
4.5.4 Low or elevated calcium levels/urine Ca/Cr ratio at baseline
4.5.5 Not willing to take vitamin D supplementation
4.5.6 Health conditions potentially affecting vitamin D metabolism e.g. on anticonvulsants, malabsorption problems
4.5.7 Potential subjects will also be excluded if they are planning to travel to a sunny climate during the study period.

4.6 Premature Withdrawal

Participants are free to withdraw from the study at any time. If the subject prematurely withdraws, every effort will be made to collect all data up to and including the day of study discontinuation.

5. Study Supplementation

5.2 Description and formulation

The study uses Cholecalciferol 50,000 IU (1.25gram) tablets (Cal.D.Forte). This is a white Sugar coated 8mm biconvex tablet. This is manufactured along with identical placebo tablet by Optimus Healthcare limited, Auckland. Tablets come in individual bottles. Pharmacy will allocate tablets following randomisation.

5.3 Dosage regimen and rationale
All supplementation is provided monthly. There are three arms to the study: Placebo, 50,000 IU and 100,000IU given once per month. Dosing starts at 1 month postnatal and continues for 4 months total. Two capsules will be taken per month comprising of: two x placebo, two x 50,000IU or one x placebo and one x 50,000IU.

5.4 Placebo

Placebo is a white sugar coated tablet. It is identical in size and shape to the treatment arm. It is also manufactured by Optimus Healthcare limited.

5.5 Safety and Activity

Vitamin D can be toxic in large doses. This can cause a variety of adverse effects largely due to hypercalcaemia. The doses provided in this study (1666 IU & 3333 IU/day) are less than upper safe dosing levels as set by the Institute of Medicine (A. Catharine Ross & Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium., 2011) (4000 IU/d) and well under expected toxic doses as suggested by the literature on this topic. There are no documented overdoses in the literature with doses under 10,000 IU/day (Vieth, 1999).

Monitoring for toxicity will be undertaken. This is primarily looking for manifestations of hypercalcaemia. Maternal urine and serum monitoring will be undertaken from the first antenatal assessment, prior to the first dose of study supplementation, and at completion of the study (20 weeks).

Hypercalciuria or hypercalcaemia during intervention will be indications to stop study intervention and to investigate for possible vitamin D toxicity. Hypercalciuria prior to intervention will be repeated to confirm.

6. Study Procedures

6.1 Study Time Schedule: (See pages iii/iv/v above)

6.2 Study Visits

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Women are first visited at recruitment to get baseline data. This is via home visit or at hospital at preference of women. Phone call reminders antenatally are given prior to blood tests at 28 and 36 weeks (a week prior).

Following delivery, phone contact is made at 3 week postnatal. Congratulations are given and arrangements made for 4 week home visit. Randomisation will occur at this time if all is on track (or exclusion from study if not breast feeding or any other exclusion criteria met).

Further monthly reminder phone calls will occur, pre-monthly dose. Final visit at 20 weeks (phone call 1 week prior to arrange).

6.3 Randomisation:

Following delivery, breast feeding mothers will be randomly allocated to one of the three arms of the trial by research support at the Dunedin Public Hospital Pharmacy.

Randomisation will be done via randomisation list provided by Peter Herbison (statistician). This has been provided to pharmacy research unit. Participants will be block randomised in groups of 15. This is to ensure equal distribution by season. All investigators (including statistician) will be blinded to intervention. All will receive monthly medication in blister packs appropriate to one of the three arms of the intervention e.g. placebo/placebo, placebo/50,000 IU Cholecalciferol or 2 x 50,000 IU Cholecalciferol.

6.4 Socio-Demographic information:

Socio-demographic data is collected by questionnaire, at baseline enrolment (maternal only), and at 4 and 20 weeks postnatal (both mother and baby). Questionnaire will be conducted and completed as a joint process with research team during home visit. (See appendices 1, 2 and 3 for Questionnaires)

6.5 Concomitant Medications:

Information on all concomitant medications taken by either mother or infant will be documented. Postnatal supplements containing vitamin D are required to be stopped for the duration of the study. The investigators are happy to assist in arranging for ongoing pre-natal vitamins such as iodine and folic acid (see 6.9). Ongoing postnatal
maternal or infant vitamin D is an exclusion criterion. See above for further medication based exclusion criterion.

6.6 Blood/Urine Collection and Analysis:

Order of testing priority:

1) 25OHD  2) ALP/Ca/P/Ferritin  3) PTH

For each blood test: (minimum volume whole blood for all tests ~ 5ml)

- Mother + cord = 8ml red top

- Infant

Human Nutrition Lab:

25OHD - 0.5ml serum (~1ml whole blood) - LCMS
PTH - 0.5ml serum - Chemilum

Southern Community Labs:

ALP/Ca/Phos/Ferritin - 0.5ml serum

Maternal urine for Calcium creatinine ratio will be collected prenatally at 28 and 36 weeks, and postnatally at 4 and 20 weeks. To avoid falsely elevated results we will follow a protocol from the Canterbury Health Laboratory titled - “Collecting Overnight Fasting Urine for Hydroxyproline and Calcium Creatinine Ratios” (see appendix 7). Appendix 8 details our lab request form.

Maternal bloods are prospectively collected for the first phase of the study at 28 and 36 weeks gestation. As we strive to recruit most participants prior to 28 weeks our wish is >50% of total participants will be involved in this first phase of the study. As part of this consent is obtained for access to antenatal 1st trimester booking bloods. These are collected prior to recruitment and are stored in freezer space within SCL for 1 year from date taken. Currently the system in SCL is a large plastic bag without any
more formal storage system. Currently we will go through this bag 1 year from the booking of each patient and remove our sample prior to them being destroyed. To more formalise this we are exploring the option of making an additional storage bag labelled with our study into which participant booking bloods can be placed once recruited to avoid any risk of destruction.

6.7 Breast milk collection:

Expressed breast milk will be collected prior to monthly intervention at 4 and 20 weeks.

Milk will be collected with a “Medela™ Lactina plus” hospital grade breast pump (adjustable speed) in most instances. We have two units available. Those women with their own pump may use their own equipment (Dunedin hospital lactation consultants in their experience predict up to 50% of women may have their own equipment). Medela disposable sterile expressing sets ($212 excl. GST/30 units or $7.10/unit) will be allocated to each woman. These can be used for both episodes of expressing. For the minority of women requiring a larger cup size, the hospital lactation service is happy to provide this (personal communication with Anne Jenkins and Stefanie Kalmafoff - 29/6/11).

Expressed milk collection is recommended an hour after first morning feed (this is the time when milk supply is at its peak). Volume for collection is 100ml. As the average volume expressed in the morning following as feed is approx. 30-50ml, milk will need to be collected over two mornings in most cases and then frozen (some women will get 80-100ml in one go). Pump should be used at its lowest suction setting initially and then suction increased based on need and comfort.

Milk will be frozen and stored in pre-provided plastic 120ml specimen containers. Containers will be labelled with study ID and date collected. Milk can be stored in subjects’ freezer till pick up or drop off arranged. Frozen specimens will be transferred to freezer space at department of Human Nutrition laboratory as available. The samples are to only be <24h hours old prior to freezing and transported to -80 freezer in Human Nutrition without thawing.
The LC and Queen Mary are happy for a minority of women to use hospital pumps, in hospital, if requested by study participants or our machines are in use/under repair. A pump on an as available basis for short periods may also be available from the hospital LC team if the need arises (no formal provision for this has been made). Pumps can also be hired directly from Fisher & Paykel NZ (direct communication from Sandra Mackay (Sandra.mackay@fphcare.co.nz)

Lactation support:
We will provide a lactation support and referral service to all women participating in the study. Lactation services available for referral or self-referral are as follows.

Following delivery (while still inpatient in Queen Mary):
Women will be advised to request contact with the lactation consultants (LC) before discharge as part of the standard service. Women are advised to inform LC that they are part of study. Local LC have been briefed on study details. Specific advice is offered at this time regarding:
- Lactation advice and support
- Pumping and an opportunity to explore the hospital Medela™ pump

During the first 6 weeks postnatal there are three options for support for lactation:
- Lead Maternity Carer (LMC) support is available, and regularly provided as part of standard postnatal care over the first 6 weeks. We can advocate for mothers or advise they contact their LMC.
- During the first 6 weeks the hospital LC team is contracted and available by referral and drop in (may be a wait)
- Plunket breast feeding support person, Jill Moore, is available for community support.

From week 7 to month 5 there are two options for lactation support:
- Plunket breast feeding support person, Jill Moore, is available in the community. We can refer or advise mothers to self refer.

Dr Wheeler (PI) and Shirley Jones (Research Nurse) have been educated on pump procedures by the hospital lactation service.
Early Cessation of Breast feeding:

Women will be encouraged, and provided with all available support, to continue exclusively breast feeding till at least 20 weeks. If for any reason a woman ceases to breast fed we will request that they inform us of this. All future data and sampling (other than breast milk) will be collected as planned. Date of breast feeding cessation will be recorded.

1.1. Skin Colour Assessment:

Changes in skin colour are a marker of sun exposure during the course of the study. This is an important confounding factor in final 25OHD outcomes. Participant skin colour will be determined by spectrophotometer (CM2006d, Minolta Co. Ltd, Osaka, Japan). Maternal measures will be taken initial enrolment to study, 4 weeks post natal and at completion of the study (20 week assessment). Baby will also have measures taken at 1 and 5 months. Skin colour measurements are taken from the medial aspect of the upper arm (target sample – natural skin colour)) and dorsal aspect of forearm (sample – sun exposed surface). A calculation is made (appendix 9) and this number is then converted to a final skin colour assessment of “very light”, “light”, “intermediate”, “tan” and “brown”.
Appendix 7. RCT - Ethics approval Vitamin D and breastfeeding study

9 May 2011

Dr Ben Wheeler
Department of Women's and Children's Health
Dunedin School of Medicine
PO Box 913
Dunedin 9054

Dear Dr Wheeler -

Re: Ethics ref: LRS/11/02/007 (please quote in all correspondence)
Study title: Intermittent maternal vitamin D supplementation to prevent vitamin D deficiency in the breast feeding infant and lactating mother.
Investigators: Dr Ben Wheeler, Professor Barry Taylor, Dr Lisa Houghton, Dr Celia Devenish, Professor Peter Herbison

This study was given ethical approval by the Lower South Regional Ethics Committee on 9 May 2011. This approval is valid until 30 June 2012, provided that Annual Progress Reports are submitted (see below).

Access to ACC
For the purposes of section 32 of the Accident Compensation Act 2001, the Committee is satisfied that this study is not being conducted principally for the benefit of the manufacturer or distributor of the medicine or item in respect of which the trial is being carried out. Participants injured as a result of treatment received in this trial will therefore be eligible to be considered for compensation in respect of those injuries under the ACC scheme.

Amendments and Protocol Deviations
All significant amendments to this proposal must receive prior approval from the Committee. Significant amendments include (but are not limited to) changes to:
- the researcher responsible for the conduct of the study at a study site
- the addition of an extra study site
- the design or duration of the study
- the method of recruitment
- information sheets and informed consent procedures.

Significant deviations from the approved protocol must be reported to the Committee as soon as possible.

Annual Progress Reports and Final Reports
The first Annual Progress Report for this study is due to the Committee by 9 May 2012. The Annual Report Form that should be used is available at www.ethicscommittees.health.govt.nz. Please note that if you do not provide a progress report by this date, ethical approval may be withdrawn.
NGĀI TAHU RESEARCH CONSULTATION COMMITTEE
TE KOMITI RAKAHU Ki KĀi TAHU

19/04/2011 - 07
Tuesday, 19 April 2011

Dr Wheeler
Women’s and Children’s Health
Dunedin

Tēnā koe Dr Wheeler

Title: Intermittent maternal vitamin D supplementation to prevent vitamin D deficiency in the breastfeeding infant and lactating mother.

The Ngāi Tahu Research Consultation Committee (The Committee) met on Tuesday, 19 April 2011 to discuss your research proposition.

By way of introduction, this response from the Committee is provided as part of the Memorandum of Understanding between Te Rūnanga o Ngāi Tahu and the University. In the statement of principles of the memorandum, it states "Ngāi Tahu acknowledges that the consultation process outlined in this policy provides no power of veto by Ngāi Tahu to research undertaken at the University of Otago". As such, this response is not "approval" or "mandate" for the research, rather it is a mandated response from a Ngāi Tahu appointed committee. This process is part of a number of requirements for researchers to undertake and does not cover other issues relating to ethics, including methodology; they are separate requirements with other committees, for example the Human Ethics Committee, etc.

Within the context of the Policy for Research Consultation with Māori, the Committee base consultation on that defined by Justice McGeehan:

"Consultation does not mean negotiation or agreement. It means: setting out a proposal not fully decided upon; adequately informing a party about relevant information upon which the proposal is based; listening to what the others have to say with an open mind (in that there is room to be persuaded against the proposal); undertaking that task in a genuine and not cosmetic manner. Reaching a decision that may or may not alter the original proposal."

The Committee considers the research to be of importance to Māori health.

The Ministry of Health website

The Ngāi Tahu Research Consultation Committee has membership from:
Te Rūnanga o Ōtākou Incorporated
Kāti Huia Rūnaka ki Paketaraiki
Te Rūnanga o Moeraki
NGĀI TAHU RESEARCH CONSULTATION COMMITTEE

The Committee notes the information provided by Hine Forsyth.

The Committee suggests dissemination of the research findings to Māori health organisations regarding this study.

We wish you every success in your research and the Committee also requests a copy of the research findings.

This letter of suggestion, recommendation and advice is current for an 18 month period from Tuesday, 19 April 2011 to 19 October 2012.

The Committee notes and commends that ethnicity data is to be collected as part of the research project and recommends the use of the questions on self-identified ethnicity and descent, these questions are contained in the 2006 census.

The recommendations and suggestions above are provided on your proposal submitted through the consultation website process. These recommendations and suggestions do not necessarily relate to ethical issues with the research, including methodology. Other committees may also provide feedback in these areas.

Nāhaku nou, nā

Mark Brunton
Kaitakawaenga Rangahau Māori
Facilitator Research Māori
Research Division
Te Whare Wānanga o Otago
Ph: +64 3 479 8738
email: mark.brunton@otago.ac.nz
Web: www.otago.ac.nz

The Ngāi Tahu Research Consultation Committee has membership from:
Te Rūnanga o Ōtākou Incorporated
Kāti Huirapa Rūnaka ki Puketākā
Te Rūnanga o Moeraki
Collecting Overnight Fasting Urine for Hydroxyproline and Calcium Creatinine Ratios

Indication:

In patients with metabolic bone disease, malabsorption and/or disturbance in calcium handling.

Procedure:

The patient fasts from food and fluids for 12 hours from 8 pm the night before. At 8 am the bladder is emptied and the patient drinks 250 mL of distilled water over 5-10 minutes. Thirty to sixty minutes later, the bladder is again emptied and urine is saved for analysis. In some cases, fasting blood will be drawn at the time of voiding for fasting calcium etc.

Analysis:

Urine samples are sent to Canterbury Health Laboratories for determination of hydroxyproline/creatinine and calcium/creatinine ratio.

For further information please contact Special Biochemistry, phone 3640326, Trevor Walmsley.

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Version 1 Issued by: on 22-April-1997
Appendix 10. RCT - Consent form

The vitamin D and breast feeding study

Intermittent maternal vitamin D supplementation to prevent vitamin D deficiency in the breast feeding infant and lactating mother

CONSENT FORM FOR PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage. I know that:

1. My participation in the project is entirely voluntary;

2. I am free to withdraw from the project at any time without any disadvantage;

3. Personal identifying information will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage for 10 years after participants turn 16 years old (i.e. aged 26 years).

4. I am aware that I/my child may experience pain during the drawing of blood, and in some cases minor bruising may occur but this will generally disappear in about one to two days;

At the end of the study I would like left over biological specimens: (circle your preferred option)

RETURNED TO ME / DESTROYED / TRADITIONAL BLESSING AND DESTROYED

5. In the future, if the PI would like to examine your samples (blood, milk or urine) for other nutrition research purposes outside of those stated in this vitamin D research study, please indicate your preference:
Option A I consent to my sample being used in research outside the purposes stated here as long as the research is approved by the University of Otago Ethics Committee and is supported by the Māori Research Consultation Committee.

Option B. I wish to be contacted for permission to use my sample for research purposes outside of those stated above. I can be contacted at (email and permanent address):

Option C. I do not wish my samples used outside of the stated purposes.

6. I am aware that I will receive no payment for participation in this study.

7. The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve my anonymity.

I agree to take part in this project.

I allow access to my antenatal booking bloods for study sampling.

I agree to my GP being informed of my participation in this study.

..........................................................................................................

(Signature of participant) (Date)

..........................................................................................................

(Name)
An Invitation to take part in a research study

The Vitamin D and Breastfeeding Study

Intermittent maternal vitamin D supplementation to prevent vitamin D deficiency in the breast feeding infant and lactating mother.

Department of Women’s and Children’s Health University of Otago Dunedin
The Vitamin D Study

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. Your participation is entirely voluntary (your choice). If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you or your child of any kind.

WHAT IS THE STUDY ABOUT AND WHY ARE WE DOING IT?

Vitamin D is essential for bone health. It also has an important role in immune function, with low levels now linked to a variety of illnesses including diabetes, cancer and heart disease. Vitamin D is sometimes known as the “sunshine vitamin” as most of our body’s vitamin D is produced in our skin when it is exposed to sunlight. In New Zealand at least 40% of all children and adults have low vitamin D levels, due partly to an increased use of sun protection and other lifestyle factors, but mostly because ultraviolet B radiation from the sun is not strong enough during winter to make enough vitamin D.

Babies are at particularly high risk of vitamin D deficiency. Many mothers have low vitamin D levels during pregnancy and therefore their breast milk has a low vitamin D concentration. Their babies also have low vitamin D stores at birth. Because of this, in the USA and Canada, it is recommended that all breast fed babies are given daily vitamin D supplements.

We wish to supplement the breast feeding mother instead. This is an alternative approach to a daily supplement for babies. We propose that this will increase the vitamin D level of the mother, her milk and therefore her baby. Conveniently this supplement can be given once a month. By supplementing the mother we support her right to exclusive breast feeding.
WHAT'S INVOLVED FOR MY CHILD AND ME?

All going well we will meet with you once before delivery, and twice after your baby is born. Most study visits can take place in your home (unless you do not wish this). The first visit will take approximately 30 minutes and your height and weight will be measured, and you will be asked to complete a questionnaire. A monitor will also be used to assess the colour of your skin (and later your baby’s). We require blood samples from you in each of the three trimesters of pregnancy. These tests do not require extra blood sampling as we would like to collect these during your routine antenatal blood tests (at booking, 28 weeks and 36 weeks gestation). It is possible your booking bloods may have already been collected (and stored), if this is the case, we would like your consent to access a sample of this blood from the lab.

When you go into labour, or shortly after your delivery, we would like to collect further samples from you. These will include samples of blood, urine and breast milk. For most people an additional needle (for the blood test) will not be needed as this can be combined with standard blood testing at the time of your delivery. After your baby is born, to minimise infant distress we would like to arrange for a specimen of umbilical cord blood to be taken; this means no sampling directly from your baby or pain. You will then be randomly assigned into one of three groups:

1. 50,000 IU vitamin D once/month
2. 100,000 IU vitamin D once/month
3. a placebo pill (no vitamin D) once/month

The researchers and you will not know which supplement you have been assigned until the end of the study.

Over the following five months we will visit you at home twice. At these visits we will complete a similar questionnaire to previously, weigh and measure your baby, and take samples of your blood, urine and breast milk (two blood tests in total). At the final visit we would also like to take a blood sample from the heel of your baby (in a similar fashion to the newborn screening test).

Over the study period, we will ask that you maintain your regular diet and sun exposure habits, but after delivery avoid the intake of supplements containing vitamin D.

The vitamin D provided will not be available to you at the completion of the study. We are very happy to provide advice and put you in contact with your GP to arrange an ongoing supply if you wish.
PARTICIPANT CRITERIA
We are seeking healthy pregnant women planning to exclusively breastfeed their babies for the first five months of life. Some people may not be able to participate, particularly those in one or more of the categories listed below (these may affect the results of the study):

- Those taking additional vitamin D following delivery
- Not intending to exclusively breastfeed for at least 4 months
- Premature delivery (prior to 37 weeks gestation)
- Abnormally low or high calcium levels
- Taking medication known to affect vitamin D e.g. for epilepsy
- A chronic disease such as Ulcerative Colitis or Diabetes
- Planning a holiday (immediately following delivery) outside of New Zealand (if so, please discuss with the researchers).

POTENTIAL HARM OR DISCOMFORT
There is no known harm associated with taking supplements containing vitamin D at the study dose of approximately 1500 IU – 3000 IU per day. Current guidelines suggest the safe upper limit for daily dosing is 4000 IU. Excessive intakes of vitamin D can produce a syndrome known as vitamin D intoxication, which is characterized by high blood calcium, renal stones, and possible renal failure. This generally occurs during prolonged oral intakes in excess of 10,000 IU per day.

You and your baby may experience pain during the drawing of blood samples, and in some cases minor bruising after the sample has been taken, but this generally disappears over a few days. Skilled staff will draw your and your baby’s blood.
DATA COLLECTION / CONFIDENTIALITY

Some personal information will be collected e.g. your age, medical history, education level, ethnicity, skin colour, diet, weight, and height. The purpose of collecting this information is so that we are able to describe the overall characteristics of the study population.

You will be identified throughout the study with the use of an ID number. Only the researchers will be able to link your personal information to your ID number. All study data collected will be securely stored in such a way that only the research team will be able to gain access to it. At the end of the project any personal information will be destroyed immediately, except that, as required by the University's research policy, any raw data on which the results of the research project depend will be retained in secure storage for 10 years, after which it will be destroyed.

Most of each biological specimen (blood, urine, breast milk) will be utilised during the study. There are two options available to you for any specimens left over once testing is complete. These can be destroyed, following blessing by traditional Māori methods or returned to you.

Results of this project may be published and will be available in the University of Otago Library (Dunedin, New Zealand). No material which could personally identify you or your child will be used in any reports on this study.

COMPENSATION

In the unlikely event of a physical injury as a result of your participation in this study you will be entitled to ACC cover under the Injury Prevention, Rehabilitation and Compensation Act. If you have any questions about ACC, you should contact your nearest branch office for further information. These are listed in the blue section at the front of the phone book, or you could phone one of the Researchers.

If you have any queries or concerns about your rights as a participant in this study you may wish to contact a Health and Disability Advocate, telephone (03) 479 0265 or 0800 37 77 66.

ARE YOU AND YOUR CHILD KEEN TO BE INVOLVED?

Remember that your participation in this study is entirely voluntary (your choice). If you would like to take part in this study please phone 03 474 7644 (or email the investigators). Please leave a message if no answer.
RESEARCH TEAM

Dr Ben Wheeler  Paediatrician
Principal Investigator:  Women's & Children's Health
Dunedin School of Medicine
University of Otago
Tel:  474 0999 Ext 8154
Email:  ben.wheeler@otago.ac.nz

Shirley Jones  Research Nurse
Women's & Children's Health
Tel:  474 7644
Email:  shirleyjones@otago.ac.nz

This study has been approved from the Lower Southern Regional Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator on Ph 479 8256. Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

If you have any questions about our project either now or in the future, or you feel that you maybe experiencing side effects from the intervention, please feel free to contact either of the researchers above.

Department of Women's and Children's Health
University of Otago
Dunedin
The Vitamin D study

Principal Investigator: Dr Ben Wheeler
Department of Women’s & Children’s Health,
PO BOX 913, Dunedin, New Zealand
Research Nurse: Shirley Jones
Telephone: 64 03 474 7644  email: ben.wheeler@otago.ac.nz
Cell: 021 211 4113   shirley.jones@otago.ac.nz

Date *Day/Month/Year*

LMC Name

Address

DUNEDIN

Dear LMC Name

I would like to inform you of our research study. We believe Breast Milk is the ideal food for children less than 6 months. Our research project is aimed at improving both maternal and infant vitamin D status during periods of exclusive breast feeding, using monthly maternal vitamin D supplementation. By providing vitamin D supplementation to the mother, we can increase the vitamin D levels in her breastmilk. If we can provide evidence that this simple technique works then we strengthen our goal of exclusive breast feeding and can offer this as an alternative option to the currently available approach, which is infant supplementation.

Full details of the study are provided in the enclosed information sheet. As a brief outline, recruitment will take place in the antenatal period and will use a blinded randomised trial with three arms (50,000 IU or 100,000IU vitamin D administered monthly or placebo). This treatment will commence at 1 month following birth and be complete at 5 months. Mothers will be visited at home or in our clinic (depending on maternal preference) at 1 & 5 months to record baby growth/general health, complete a questionnaire, have a skin colour assessment and have samples of blood, urine and breast milk collected. At the final meeting a blood sample is required from the baby – either a venous sample or via heel prick. We will also appreciate your assistance to take a cord blood sample at delivery.

There is a second antenatal component to our study where we will be collecting maternal bloods alongside the standard antenatal collections, at 28 weeks (polycose screening) and 36 weeks (iron status screening). Consent will also be obtained to sample stored booking bloods. For women currently in the third trimester this will not be an option, however, we still wish to
offer them access to the main study above. Overall our hope is at least 50% of women will be available to participate. The aim of this second study is to increase our understanding of vitamin D metabolism and bone health during pregnancy.

The University of Otago supports the need for high quality research in order to prevent health problems. Thank you for taking the time to read this letter and the enclosed brochure. If you have any questions or have any women who you feel may be interested in the above study please contact myself or my Research Nurse; Shirley Jones at the above contact details. We will also be inviting women by letter once they have booked into Queen Mary so they may be aware of the study. We realise that you will have a busy schedule ahead but appreciate your assistance in recruiting for this important research.

Yours sincerely

Dr Ben Wheeler
Paediatrician / Senior Lecturer

ben.wheeler@otago.ac.nz / 027 4701980
Appendix 13. RCT – Mother invitation letter

The Vitamin D study

**Principal Investigator: Dr Ben Wheeler**
Department of Women’s & Children’s Health,
PO BOX 913, Dunedin, New Zealand
Research Nurse: Shirley Jones
Telephone: 64 03 474 7644   email: ben.wheeler@otago.ac.nz
Cell: 021 211 4113   shirley.jones@otago.ac.nz

Date *Day/Month/Year*

Mother’s Name

Address

DUNEDIN

Dear ........

I would like to inform you of our research study. We believe Breast Milk is the ideal food for children less than 6 months. Our research project is aimed at improving both the mother’s and babies vitamin D status during periods of exclusive breast feeding, using monthly maternal vitamin D supplementation. By providing vitamin D supplementation to the mother, we can increase the vitamin D levels in her breastmilk. If we can provide evidence that this simple technique works then we strengthen our goal of exclusive breast feeding and can offer this as an alternative option to the currently available approach, which is supplementation to baby.

Full details of the study are provided in the enclosed information sheet. As a brief outline, recruitment will take place during your pregnancy and you will be randomly placed in one of the 3 options (Vitamin D in two different monthly doses or placebo). This treatment will commence at 1 month following birth and be complete at 5 months. Mothers will be visited at home or in our clinic (depending on your preference) at 1 & 5 months to record baby’s growth/general health, complete a questionnaire, have a skin colour assessment and you will have samples of blood, urine and breast milk collected. At the final meeting (Month 5) a blood sample is required from the baby – either a venous sample or via heel prick (like the standard newborn screening sample). We will also need a cord blood sample from your placenta following delivery. Your Midwife/Obstetrician will organise and collect this for you.

There is a second pre-delivery component to our study where we will be collecting your blood alongside the standard pre-delivery collections, at 28 weeks (polycose screening) and 36 weeks
(iron status screening). This part of the study is optional. We also wish to obtain a sample of your stored booking bloods taken at the start of your pregnancy. Overall, our hope is at least 50% of women will be available to participate in this second study. The aim of this second study is to increase our understanding of vitamin D metabolism and bone health during pregnancy.

The University of Otago supports the need for high quality research in order to prevent health problems. Thank you for taking the time to read this letter and the enclosed brochure.

To participate or ask any questions, please contact us by phone or email (as above).

Yours sincerely

Dr Ben Wheeler
Paediatrician / Senior Lecturer

ben.wheeler@otago.ac.nz / 027 4701980
Appendix 14. RCT – GP information letter

Date *Day/Month/Year*

Dr

DUNEDIN

Dear Dr

«MotherName» «Surname»

DOB: «DOB»

«Address_1» «Address_2» «Address_3»

The «Surname» family have agreed to be part of a randomised controlled trail looking at intermittent supplementation of vitamin D to lactating mothers. The trial has three arms; 50,000 IU, 100,000 IU or placebo administered monthly. The aim of the study is to assess if intermittent vitamin D supplementation prevents vitamin D deficiency in the mother and breast feeding infant as an alternative to daily infant supplementation. Maternal bloods will be collected along with standard antenatal collection at booking, 28 and 36 weeks. Further samples including blood, urine and breast milk will be taken at 1 month and 5 months. Bloods for baby include: cord blood, and a venous sample at 5 months.

We would appreciate if during your normal post delivery care you refrain from giving any vitamin D supplements. Please let us know if you feel these will be clinically indicated over and above what we are doing.

We will also contact you if any abnormal results occur during the course of the study.

We look forward to having «MotherName» and her baby assisting us in our research. If you have any queries please do not hesitate to contact one of the research team on 474 7644, or Shirley.jones@otago.ac.nz.

Yours sincerely

Dr Ben Wheeler

Senior Lecturer / Paediatrician
Appendix 15. RCT – Recruitment questionnaire – baseline

The Vitamin D and breastfeeding study

University of Otago
Departments of Women’s and Children’s Health
and Human Nutrition

Principle investigator:
Dr Ben Wheeler

Research Nurse:
Shirley Jones

The answers from all women surveyed will be gathered together, and no individual answers will be published or passed on. If you do not wish to answer any of the questions, leave them blank.

- Please note that all questions relate to your **RECENT PREGNANCY**, not previous pregnancies.

- Most answers require a tick in a box, for example:
  - □ yes
  - ☑ no
  - □ not sure

- Some questions require a written answer in the space provided.
Study Recruitment Questionnaire

Date: ______________ 

Study ID: ______________

PERSONAL INFORMATION:

Name: __________________________

Home address: ____________________________

Tel: ____________________________

Midwife: ____________________________ Phone: ____________________________

G.P: ____________________________ Practice: ____________________________

1. What was your age at your last birthday? ____________________

2. What is your height? ____________________

3. What is your current weight? ____________________

4. What was your weight before pregnancy? ____________________

5. How many times have you been pregnant?
   ☐ once, including my recent pregnancy
   ☐ more than once (Please specify number of times) ____________________

6. How many birth children do you have? ____________________

7. How many weeks pregnant are you? ____________________

8. What is your estimated due date? ____________________

9. Are you experiencing any pregnancy complications?
   ☐ No
   ☐ Yes, please specify:
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________

10. Which country were you born in? ____________________

    If you were NOT born in New Zealand, in what year did you arrive?
    ____________________
11. **Which ethnic group do you belong to?**

*Please tick the box or boxes that apply to you.*

☐ New Zealand European

☐ Māori

☐ Samoan

☐ Cook Island Maori

☐ Tongan

☐ Niuean

☐ Chinese

☐ Indian

☐ other such as Dutch, Japanese, Tokelauan *(Please specify)*

12. **Which ethnic group does the father of your baby belong to?**

*Please tick the box or boxes that apply to him.*

☐ New Zealand European

☐ Māori

☐ Samoan

☐ Cook Island Maori

☐ Tongan

☐ Niuean

☐ Chinese

☐ Indian

☐ other such as Dutch, Japanese, Tokelauan *(Please specify)*

13. **Which country was the father of your baby born in?**

If he was NOT born in New Zealand, in what year did he arrive? ____________
14. **What is your marital status?**
   - ☐ married
   - ☐ de facto (living with a partner)
   - ☐ separated / divorced
   - ☐ widowed
   - ☐ in a relationship, but **NOT** living with a partner
   - ☐ single

15. **What is the highest education qualification you have completed?**
   - ☐ left before high school
   - ☐ some high school
   - ☐ completed high school to year 12 (Sixth Form Certificate)
   - ☐ completed high school to year 13 (University Bursary)
   - ☐ further training (for example, apprenticeship)
   - ☐ tertiary (diploma or degree)
   - ☐ postgraduate qualification (Master’s or PhD)
   - ☐ other (Please specify) _______________________________________

**SUN EXPOSURE INFORMATION:**

17. **Do you wear any veiling?**
   - ☐ No
   - ☐ Yes, *please tick the appropriate category below* (tick one only):
     - ☐ consistently covered – covered up, including arms, hair and neck, when outdoors
     - ☐ inconsistently covered – did not usually cover fully in her own backyard/garden
☐ uncovered – did not generally cover up arms, hair and neck when outdoors

18. In the LAST MONTH, on average, how many hours are you outside per day between 10 AM and 4 PM... on WEEKDAYS (Monday to Friday)?
   ☐ 30 minutes or less
   ☐ 31 minutes to 1 hour
   ☐ 2 hours
   ☐ 3 hours
   ☐ 4 hours
   ☐ 5 hours
   ☐ 6 hours

19. In the LAST MONTH, on average, how many hours are you outside per day between 10 AM and 4 PM... on WEEKENDS (Saturday & Sunday)?
   ☐ 30 minutes or less
   ☐ 31 minutes to 1 hour
   ☐ 2 hours
   ☐ 3 hours
   ☐ 4 hours
   ☐ 5 hours
   ☐ 6 hours

For the following question, think about what you do when you are outside during the LAST MONTH

NEVER RARELY SOMETIMES OFTEN

20. How often do you wear SUNSCREEN?... ☐ ☐ ☐ ☐

21. How often do you wear a SHIRT WITH SLEEVES that cover your shoulders?... ☐ ☐ ☐ ☐

22. How often do you wear a HAT?...  ☐ ☐ ☐ ☐
23. How often do you stay in the SHADE or UNDER AN UMBRELLA? ...

☐ ☐ ☐ ☐ ☐

24. How often do you wear SUNGLASSES? ☐ ☐ ☐ ☐ ☐

☐

25. How often do you spend time in the sun in order to get a tan?...

☐ ☐ ☐ ☐ ☐

☐
26. **Are you taking any vitamin or mineral tablets?**

☐ No

☐ Yes, please specify which vitamin or mineral tablet(s) you are taking?

*Please answer in the table below. Use a separate row for each tablet you took, fill in as many rows as you need to, and please tell the interviewer if you need more space.*

<table>
<thead>
<tr>
<th>name and brand-name</th>
<th>cost, if known</th>
<th>number of tablets per day</th>
<th>month before pregnancy</th>
<th>1st month of pregnancy</th>
<th>2nd month of pregnancy</th>
<th>3rd month of pregnancy</th>
<th>months 4–9 of pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EXAMPLE 1:</strong> Blackmores Pregnancy &amp; Breast-Feeding Gold</td>
<td>$51.30</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><strong>EXAMPLE 2:</strong> Thompson's Vitamin C</td>
<td></td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>YOUR ANSWER(S):</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

181
MEDICAL HISTORY:

18. **Do you have any medical conditions (including allergies)?**
   - □ No
   - □ Yes, please specify:
     
     _______________________________________________________________
     _______________________________________________________________
     _______________________________________________________________
     _______________________________________________________________

19. **Are you on any medications currently?**
   - □ No
   - □ Yes, please specify:
     
     _______________________________________________________________
     _______________________________________________________________
     _______________________________________________________________
     _______________________________________________________________

20. **BEFORE you became pregnant, did you smoke cigarettes?**
    - □ yes, but I stopped because I was trying to become pregnant
    - □ yes
    - □ no, I don’t smoke cigarettes *(Please go to question XX)*

33. **Since you have become pregnant, have you smoked cigarettes?**
    - □ yes, but I stopped when I knew I was pregnant
    - □ yes
    - □ no
    - □ not sure
Appendix 16. RCT – Recruitment questionnaire – after delivery

The Vitamin D and breastfeeding study
(2nd Visit)

University of Otago
Departments of Women’s and Children’s Health
and Human Nutrition

Principle investigator:
Dr Ben Wheeler

Research Nurse:
Shirley Jones

The answers from all women surveyed will be gathered together, and no individual answers will be published or passed on. If you do not wish to answer any of the questions, leave them blank.

- Please note that all questions relate to your RECENT PREGNANCY, not previous pregnancies.

- Most answers require a tick in a box, for example:
  - ☐ yes
  - ☑ no
  - ☐ not sure

- Some questions require a written answer in the space provided.

Study Recruitment Questionnaire                                      Date:  
_________________________________________  

183
Mothers Study ID: ___________________

PERSONAL INFORMATION:
Name: ____________________________________
Home address: __________________________________
Tel: _______________________________________

1. What was your age at Delivery? ___________________
2. What is your height? ___________________
3. What is your current weight? ___________________

4. Did you experience any pregnancy/birth complications?
   ☐ No
   ☐ Yes, please specify:
   __________________________________________________________________________
   __________________________________________________________________________
   __________________________________________________________________________
   __________________________________________________________________________

SUN EXPOSURE INFORMATION: Mother

5. Do you wear any veiling?
   ☐ No
   ☐ Yes, please tick the appropriate category below (tick one only):
      ☐ consistently covered – covered up, including arms, hair and neck, when outdoors
      ☐ inconsistently covered – did not usually cover fully in her own backyard/garden
      ☐ uncovered – did not generally cover up arms, hair and neck when outdoors
6. In the LAST MONTH, on average, how many hours are you outside per day between 10 AM and 4 PM... on WEEKDAYS (Monday to Friday)?

☐ 30 minutes or less
☐ 31 minutes to 1 hour
☐ 2 hours
☐ 3 hours
☐ 4 hours
☐ 5 hours
☐ 6 hours

7. In the LAST MONTH, on average, how many hours are you outside per day between 10 AM and 4 PM... on WEEKENDS (Saturday & Sunday)?

☐ 30 minutes or less
☐ 31 minutes to 1 hour
☐ 2 hours
☐ 3 hours
☐ 4 hours
☐ 5 hours
☐ 6 hours

For the following question, think about what you do when you are outside during the LAST MONTH

<table>
<thead>
<tr>
<th>NEVER</th>
<th>RARELY</th>
<th>SOMETIMES</th>
<th>OFTEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALWAYS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. How often do you wear SUNSCREEN? ☐ ☐ ☐ ☐ ☐

9. How often do you wear a SHIRT WITH SLEEVES that cover your shoulders?... ☐ ☐ ☐ ☐ ☐

10. How often do you wear a HAT?... ☐ ☐ ☐ ☐ ☐

11. How often do you stay in the SHADE
or UNDER AN UMBRELLA? ...

12. How often do you wear SUNGLASSES?

13. How often do you spend time in the sun in order to get a tan?...
14. **Are you taking any vitamin or mineral tablets?**

- [ ] No

- [ ] Yes, please specify which vitamin or mineral tablet(s) you are taking?

*Please answer in the table below. Use a separate row for each tablet you took, fill in as many rows as you need to, and please tell the interviewer if you need more space.*

<table>
<thead>
<tr>
<th>name and brand-name</th>
<th>cost, if known</th>
<th>number of tablets per day</th>
<th>month before pregnancy</th>
<th>1st month of pregnancy</th>
<th>2nd month of pregnancy</th>
<th>3rd month of pregnancy</th>
<th>months 4–9 of pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EXAMPLE 1:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Blackmores</td>
<td>$51.30</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Pregnancy &amp; Breast-Feeding Gold</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EXAMPLE 2:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thompson's</td>
<td></td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**YOUR ANSWER(S):**
13. MEDICAL HISTORY:

15. Do you have any medical conditions (including allergies)?
   □ No
   □ Yes, please specify:
   _______________________________________________________________
   _______________________________________________________________
   _______________________________________________________________
   _______________________________________________________________

16. Are you on any medications currently?
   □ No
   □ Yes, please specify:
   _______________________________________________________________
   _______________________________________________________________
   _______________________________________________________________
   _______________________________________________________________

17. Since you have become pregnant, have you smoked cigarettes?
   □ yes, but I stopped when I knew I was pregnant
   □ yes
   □ no
   □ not sure

18. Since you delivered have you smoked cigarettes?
   □ yes
   □ no
1. Infant name: __________________________
4. Infant Date of Birth: __________________________
5. What was your estimated due date? __________________________
6. Infant Gestational Age (e.g. 40 weeks)? __________________________
7. Birth Weight (kg): __________
8. Birth length: __________
9. Birth Head Circumference: __________
10. Current Age: __________________________
11. Current weight: __________________________
12. Current Length: __________________________
13. Current Head Circumference: __________
14. Exposure to formula?
   □ No
   □ Yes, please specify:
       __________________________
       __________________________
       __________________________
       __________________________

SUN EXPOSURE INFORMATION: Baby

14. In the LAST MONTH, on average, how many hours is your baby outside per day between 10 AM and 4 PM... on WEEKDAYS (Monday to Friday)?
   □ 30 minutes or less
   □ 31 minutes to 1 hour
   □ 2 hours
☐ 3 hours
☐ 4 hours
☐ 5 hours
☐ 6 hours

15. **In the LAST MONTH, on average, how many hours is your baby outside per day between 10 AM and 4 PM... on WEEKENDS (Saturday & Sunday)?**

☐ 30 minutes or less
☐ 31 minutes to 1 hour
☐ 2 hours
☐ 3 hours
☐ 4 hours
☐ 5 hours
☐ 6 hours

The following question refers to sun exposure habits for your baby: when outside during the **LAST MONTH**

<table>
<thead>
<tr>
<th>NEVER</th>
<th>RARELY</th>
<th>SOMETIMES</th>
<th>OFTEN</th>
<th>ALWAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

16. **How often do you use SUNSCREEN?...** ☐ ☐ ☐ ☐ ☐

17. **How often does he/she wear a SHIRT WITH SLEEVES that covers shoulders/arms?** ☐ ☐ ☐ ☐

18. **How often do you use a HAT?...** ☐ ☐ ☐ ☐

19. **How often does he/she stay in the SHADE or UNDER COVER? ...** ☐ ☐ ☐ ☐

20. **Does your baby have any medical conditions (including allergies)?**
21. Is your baby on any medications/supplements currently (or at any time since delivery)?

☐ No

☐ Yes, please specify:

_______________________________________________________________
_______________________________________________________________
_______________________________________________________________
_______________________________________________________________

_______________________________________________________________

_______________________________________________________________

_______________________________________________________________
Appendix 17. RCT – Recruitment questionnaire – final visit

The Vitamin D and breastfeeding study

University of Otago

Departments of Women’s and Children’s Health

and Human Nutrition

Principle investigator:

Dr Ben Wheeler

Research Nurse:

Shirley Jones

The answers from all women surveyed will be gathered together, and no individual answers will be published or passed on. If you do not wish to answer any of the questions, leave them blank.

- Please note that all questions relate to your RECENT PREGNANCY, not previous pregnancies.

- Most answers require a tick in a box, for example:

  □ yes

  ☒ no

  □ not sure

- Some questions require a written answer in the space provided.
Study Recruitment Questionnaire

Date: __________________________

Study ID: ______________________

PERSONAL INFORMATION:

Name: ______________________________________

Home address: ______________________________________

Tel: ______________________________________

Midwife: __________________________ Phone: ______________________

G.P. __________________________ Practice: ______________________

1. What was your age at your last birthday? ______________________

2. What is your height? ______________________

3. What is your current weight? ______________________

4. What was your weight before pregnancy? ______________________

5. How many times have you been pregnant?
   ☐ once, including my recent pregnancy
   ☐ more than once (Please specify number of times) ______________________

6. How many birth children do you have? ______________________

7. How many weeks pregnant are you? ______________________

8. What is your estimated due date? ______________________

9. Are you experiencing any pregnancy complications?
   ☐ No
□ Yes, please specify:

_____________________________________________________________________________
_____________________________________________________________________________
_____________________________________________________________________________

10. Which country were you born in? ______________________________

If you were NOT born in New Zealand, in what year did you arrive? ____________

11. Which ethnic group do you belong to?

Please tick the box or boxes that apply to you.

☐ New Zealand European

☐ Māori

☐ Samoan

☐ Cook Island Maori

☐ Tongan

☐ Niuean

☐ Chinese

☐ Indian

☐ other such as Dutch, Japanese, Tokelauan (Please specify) ____________________

12. Which ethnic group does the father of your baby belong to?

Please tick the box or boxes that apply to him.

☐ New Zealand European

☐ Māori
☐ Samoan

☐ Cook Island Maori

☐ Tongan

☐ Niuean

☐ Chinese

☐ Indian

☐ other such as Dutch, Japanese, Tokelauan (Please specify)

13. Which country was the father of your baby born in? ______________________

If he was NOT born in New Zealand, in what year did he arrive? __________

14. What is your marital status?

☐ married

☐ de facto (living with a partner)

☐ separated / divorced

☐ widowed

☐ in a relationship, but NOT living with a partner

☐ single

15. What is the highest education qualification you have completed?

☐ left before high school

☐ some high school
☐ completed high school to year 12 (Sixth Form Certificate)

☐ completed high school to year 13 (University Bursary)

☐ further training (for example, apprenticeship)

☐ tertiary (diploma or degree)

☐ postgraduate qualification (Master’s or PhD)

☐ other (Please specify) ________________________________

SUN EXPOSURE INFORMATION:

17. Do you wear any veiling?

☐ No

☐ Yes, please tick the appropriate category below (tick one only):

☐ consistently covered – covered up, including arms, hair and neck, when outdoors

☐ inconsistently covered – did not usually cover fully in her own backyard/garden

☐ uncovered – did not generally cover up arms, hair and neck when outdoors

18. In the LAST MONTH, on average, how many hours are you outside per day between 10 AM and 4 PM... on WEEKDAYS (Monday to Friday)?

☐ 30 minutes or less

☐ 31 minutes to 1 hour

☐ 2 hours

☐ 3 hours

☐ 4 hours
19. In the LAST MONTH, on average, how many hours are you outside per day between 10 AM and 4 PM... on WEEKENDS (Saturday & Sunday)?

☐ 5 hours
☐ 6 hours

☐ 30 minutes or less
☐ 31 minutes to 1 hour
☐ 2 hours
☐ 3 hours
☐ 4 hours

☐ 5 hours
☐ 6 hours

For the following question, think about what you do when you are outside during the

LAST MONTH
20. How often do you wear SUNSCREEN?...

☐ ☐ ☐ ☐ ☐ ☐

21. How often do you wear a SHIRT WITH SLEEVES that cover your shoulders?...

☐ ☐ ☐ ☐ ☐ ☐

22. How often do you wear a HAT?...

☐ ☐ ☐ ☐ ☐ ☐

23. How often do you stay in the SHADE or UNDER AN UMBRELLA? ...

☐ ☐ ☐ ☐ ☐ ☐

☐

24. How often do you wear SUNGLASSES? ☐ ☐ ☐ ☐ ☐ ☐

25. How often do you spend time in the sun in order to get a tan?...

☐ ☐ ☐ ☐ ☐ ☐
### VITAMIN AND MINERAL SUPPLEMENT INFORMATION

26. Are you taking any vitamin or mineral tablets?

- [ ] No

- [ ] Yes, please specify which vitamin or mineral tablet(s) you are taking?

*Please answer in the table below. Use a separate row for each tablet you took, fill in as many rows as you need to, and please tell the interviewer if you need more space.*

<table>
<thead>
<tr>
<th>Tablet 1</th>
<th>Tablet 2</th>
<th>Tablet 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>name and brand-name</td>
<td>cost, if known</td>
<td>number of tablets per day</td>
</tr>
<tr>
<td>---------------------</td>
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<td>Blackmores</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>EXAMPLE 2:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thompson’s Vitamin C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YOUR ANSWER(S):</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
14. MEDICAL HISTORY:

18. Do you have any medical conditions (including allergies)?

☐ No

☐ Yes, please specify:
_____________________________________________________________________________
_____________________________________________________________________________
_____________________________________________________________________________

19. Are you on any medications currently?

☐ No

☐ Yes, please specify:
_____________________________________________________________________________
_____________________________________________________________________________
_____________________________________________________________________________

20. BEFORE you became pregnant, did you smoke cigarettes?

☐ yes, but I stopped because I was trying to become pregnant

☐ yes

☐ no, I don’t smoke cigarettes (Please go to question XX)

33. Since you have become pregnant, have you smoked cigarettes?

☐ yes, but I stopped when I knew I was pregnant

☐ yes

☐ no

☐ not sure