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Vitamin D Status of Preterm Infants at 4 Months Post Hospital Discharge

A thesis presented in partial fulfilment of the requirements for the degree of Masters of Science in Nutrition and Dietetics

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Abstract

Preterm birth and survival rates are increasing in New Zealand and around the world. Preterm infants are subject to shorter gestational lengths and subsequently suffer from decreased nutrient accretion in *utero*. Vitamin D is one nutrient that is accrued in the final stages of gestation. At birth preterm infants rely on an exogenous source of this nutrient to achieve and maintain adequate stores. The vitamin D status of preterm infants after hospital discharge in New Zealand was previously unknown.

The aim of this study was to investigate the serum 25-hydroxyvitamin D (25(OH)D) status of preterm infants at 4 months post hospital discharge, and describe the factors affecting these concentrations.

An observational study of 49 preterm infants (<37 weeks gestation) at 4 months post hospital discharge was undertaken. A capillary blood sample was obtained from infants. Serum 25(OH)D was analysed using ADIVA Centaur Vitamin D Total immunoassay. Questionnaires were used to assess sun exposure behaviours and feeding and supplement use.

In this sample of 49 preterm infants, 28.6% were classified as having insufficient vitamin D status (25(OH)D \leq 50 nmol/L), of these 8.2% were further classified as having mild to moderate vitamin D deficiency (25(OH)D \leq 25 nmol/L). The mean 25(OH)D concentration was 73.8 nmol/L, the range was 16 nmol/L – 314 nmol/L. Vitadol C supplementation had the most significant effect on infant 25(OH)D concentrations. All (n=14) exclusively breastfed infants who did not receive Vitadol C supplements were vitamin D insufficient or deficient on analysis. All infants who received Vitadol C or infant formula were vitamin D sufficient.

Vitamin D deficiency is prevalent in exclusively breastfed preterm infants not receiving vitamin D supplements. Vitamin D supplementation should be considered for all preterm infants as part of New Zealand's child health policy.

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Abbreviations

Abbreviation	Term
1,25(OH) ₂ D ₃	1 α ,25-dihydroxyvitamin D ₃ or Calcitriol
25(OH)D	25-hydroxyvitamin D
25(OH)D D- D-1-hydroxylase	25-hydroxyvitamin D D-1-hydroxylase
IU	International Units
Kg	Kilogram
L	Litres
μ g	Micrograms
Mg	Milligrams
ml	Millilitres
nmol//L	Nanomol per Litre
ng/ml	Nanograms per millilitre
AI	Adequate Intake
AAP	American Academy of Paediatrics
ALRI	Acute lower respiratory infection
ASPEN	American Society of Parenteral and Enteral Nutrition
BMF	Breast milk fortifier
CRP	C-Reactive Protein
DOB	Date of birth
GA	Gestational age
EAR	Estimated Average Requirement
EBM	Expressed breast milk
ELBW	Extremely low birth weight
EN	Enteral Nutrition
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
FEBM	Fortified expressed breast milk
INF γ	interferon- γ
IOM	Institute of Medicine
LBW	Low birth weight
MED	Minimal erythematous dose
MOH	Ministry of Health
NHMRC	National Health and Medical Research Council
NICU	Neonatal intensive care unit
Nm	Nanometres
PHARMAC	Pharmaceutical Management Agency New Zealand
PN	Parenteral nutrition
RANZCOG	Australian and New Zealand College of Obstetricians and Gynaecologists
RDA	Recommended daily allowance
RDI	Recommended daily intake
SGA	Small for gestational age
SPF	Sunscreen protection factor
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TNF α	Tumour necrosis factor- α
UL	Upper limit

Abbreviation

UVA
UVβ
VDR
VLBW
WHO

Term

Ultraviolet Alpha
Ultraviolet Beta
Vitamin D Receptors
Very low birth weight
World Health Organisation

Chapter 1.0 Introduction

1.1 Introduction

The incidence of preterm birth and survival rates is increasing across the globe (Blencowe, Cousens, Oestergaard, Chou, Moller, Narwal, Adler, Garcia, Rhode, Say, Lawn, 2012). Preterm birth is defined as birth prior to 37 weeks gestation. Being born too soon is associated with poorer health outcomes and is universally the most significant contributor to infant morbidity and mortality rates (Blencowe et al., 2012). A shorter time in *utero* can result in underdevelopment of fetal organ systems and reduced accretion of energy and nutrient stores placing the neonate at high risk of poor outcomes at birth and beyond (Beck, 2010).

There has been a substantial rise in preterm birth and survival rates in New Zealand since 1990 with an approximate 2.1% increase per annum, equating to a current rate of 7.4% of infants being born too soon (Beck, 2010; Ministry of Health, 2012). Rates of preterm birth in New Zealand are similar to other developed countries which are in the range of 5-7% (Beck, 2010; Blencowe et al., 2012). Increased survival rates reflect vast improvements in knowledge and technology available to support the preterm infant. In New Zealand, moderate to late preterm infants, who are defined as those more than 32 weeks gestation, make up the highest percentage of preterm birth. In 2009 6% of all infants born were moderately preterm, accounting for a total of 3771 infants (Ministry of Health, 2010).

Preterm infants have higher rates of nutritional deficiency in comparison to those born at term, because the accretion of macro and micronutrients which usually occurs in the third trimester of pregnancy has not taken place. Subsequently preterm infants have much higher requirements of all major nutrients (Blencowe et al., 2012).

Vitamin D is one of the nutrients which may be deficient in preterm infants (Bowyer et al., 2009; Monangi, 2013). Transfer of vitamin D is apparent across all trimesters of pregnancy, although transfer is most rapid in the final stages of pregnancy (Bowyer et al., 2009; Marwaha et al., 2011; Monangi, 2013; Salle, 1983). It is thought that this is due to the close association of vitamin D with adipose tissue and calcium, which are primarily passed to the fetus in the final trimester (Bowyer et al., 2009; Dror, 2011; Marwaha et al., 2011). Transfer of vitamin D to the fetus is also highly dependent on maternal vitamin D stores (Bowyer et al., 2009; Dawodu et al., 2013; De-Regil, 2012). Maternal vitamin D insufficiency (25(OH)D <50 nmol/l) substantially reduces vitamin D transfer to the fetus (Dawodu et al., 2013; De-Regil, 2012).

Vitamin D is an essential micronutrient which has several important roles; primarily it is known to be involved in calcium and phosphate homeostasis and is important for bone, muscle and nervous system health (Holick, 1996). Vitamin D receptors (VDR) are present in nearly all cells of the body, and through this mechanism vitamin D may also have an essential function in cellular proliferation, differentiation, and immunomodulation (Holick, 2008b).

There is currently no consensus on the vitamin D recommendations for preterm infants (Agostoni, 2010; Wagner & Greer, 2008). The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) revised nutrition guidelines in 2010 and set specific recommendations for the

vitamin D intake in preterm infants. They recommend that the daily intake (DI) is 20-25µg (800-1000IU) per day for all infants born preterm (Agostoni, 2010). The American Academy of Paediatrics (AAP) recommends 10µg (400IU) of vitamin D per day for preterm infants with a birth weight of more than 1,500g (Abrams and the Committee on Nutrition, 2013). There are no specific recommendations for preterm infants in New Zealand, however the adequate intake (AI) set for term infants is 5µg (200IU) of vitamin D per day (NHMRC, 2006).

As with vitamin D recommendations, deficiency cut off values in all population groups remain controversial. Vitamin D deficiency levels have been set for term infants in New Zealand, however there are no specific cut off values set for those born preterm (NHMRC, 2006). In New Zealand and Australia the level considered as vitamin D deficiency and insufficiency in term infants is plasma 25(OH)D levels ≤ 25 nmol/L and < 50 nmol/L, respectively. These levels are consistent with those set for children, adolescents and adults (NHMRC, 2006). These cut off levels have been utilised in this study to determine the percentage of preterm infants classed as vitamin D sufficient and deficient, however, it remains unclear whether such values are appropriate to use in this group. Other countries also lack deficiency cut off values for preterm infants and instead provide population wide recommendations. The Institute of Medicine (IOM) and AAP have similar values to define vitamin D deficiency as New Zealand (IOM, 2011; Wagner, 2008). However, other vitamin D experts recommend extremely varied guidelines. Holick et al. (2011) suggests that 25(OH)D levels below 75 nmol/L indicate vitamin D insufficiency, whereas Heaney et al. (2008) suggests levels below 80 nmol/L indicate insufficiency. Variation in these levels adds to the confusion when determining the percentage of a specific population group deemed vitamin D deficient or insufficient.

1.2 Justification

At birth the provision of an exogenous source of vitamin D is necessary to prevent vitamin D deficiency in the preterm infant. Vitamin D is unique; its primary source is attained via cutaneous synthesis (Holick, 1996b). The action of ultraviolet- β (UV β) radiation with 7-dehydrocholesterol in the skin allows the formation of pre-vitamin D (Holick, 1996). This reaction requires exposure to light in the UV β range. Whilst indoors during hospital stay this synthesis is not possible. Preterm infants may spend their first few days to months of life in an intensive care unit (Ministry of Health, 2012). The cutaneous synthesis of vitamin D is impossible over this period. Of further concern is that once infants are discharged from hospital the likelihood of correcting vitamin D status through sun exposure alone is minimal. In New Zealand it is recommended that all infants (0-6 months) are not exposed to direct sunlight (Ministry of Health, 2013). If exposure occurs protection is recommended (Ministry of Health, 2013). Therefore the cutaneous route of vitamin D synthesis in preterm and term infants (0-6 months) should not be relied on as a viable source of vitamin D in this group.

These infants therefore rely on other sources of vitamin D. Human breast milk is universally accepted as the best feed for preterm and term infants (World Health Organization, 2007). As well as nutrition it provides many non-nutritive components. The AAP on their policy statement on breastfeeding and the use of human milk recommend breastfeeding or the provision of expressed breast milk for all preterm infants (American Academy of Pediatrics, 2012). Breast feeding preterm infants is associated with the same benefits as those seen in term infants, and is further linked with a reduced incidence of necrotising enterocolitis (NEC), sepsis and diarrhoea (Schanler et al., 1999; Silva et al., 2004). Despite these benefits, breast milk is a poor source of vitamin D. Even in a vitamin D sufficient women (25(OH)D ≥ 50 nmol/L) vitamin D content in breast milk is low; approximately 0.55µg (22-30IU) per litre (Thiele, Senti & Anderson, 2013). Based on a low vitamin D content of

0.04µg (1.6IU) per 100ml of breast milk, 25L of breast milk would be required daily to meet the AAP recommended vitamin D intake of 10µg (400IU) set for preterm infants (Wagner & Greer, 2008).

Whereas 50L would be required to meet the minimum recommendation of 20µg (800IU) per day for preterm infants set by ESPGHAN (Agostoni, 2010). Such levels in breast milk make obtaining recommended vitamin D levels from maternal milk alone impossible. The addition of a commercial breast milk fortifier (BMF) is one method of increasing the vitamin D content of maternal milk. However, this is only routinely provided to preterm infants at Auckland City Hospital's neonatal intensive care unit (NICU) who are ≤32 weeks gestation or weigh ≤1,800g at birth, unless otherwise clinically indicated (Cormack, 2013). All preterm infants who do not fit these criteria will not receive vitamin D supplementation (Cormack, 2013).

If breast milk is unavailable the provision of a breast milk substitute is necessary. All infant formula in New Zealand is supplemented with vitamin D₃ (Greer, 2004). Preterm infant formula is supplemented with a higher dose of vitamin D in comparison to term formula to meet the higher requirements and smaller volumes ingested by the preterm infant (Klein, 2002). Preterm infants are eligible to receive a preterm infant formula if they are ≤32 weeks gestation or weigh ≤1,800g at birth (Cormack, 2013). Preterm infants that do not meet these criteria are routinely provided with standard term formula, unless otherwise clinically indicated (Cormack, 2013). A preterm infant formula containing 2.0µg (80IU) per 100ml would require the ingestion of 500ml and 1000ml per day to meet the vitamin D recommendations set by the AAP (10µg/400IU) and ESPGHAN (20µg/800IU) per day, respectively (Abrams and the Committee on Nutrition, 2013; Agostoni, 2010). Whereas term infant formula containing 1.0µg per 100ml would require the ingestion of 1000ml and 2000ml per day to meet the AAP and ESPGHAN recommendations, respectively.

Vitamin D supplements are another source of vitamin D for preterm infants. However, again like breast milk fortifier and preterm infant formula, preterm infants must meet the criteria previously described to receive these supplements. In New Zealand hospitals Vitadol C is the subsidised liquid preparation of vitamin D and contains vitamins D, A and C (Cormack, 2013; PHARMAC; Pharmaceutical Management Agency New Zealand, 2013).

The standard dose of Vitadol C whilst in Auckland City Hospital's NICU is 0.2ml. This is given once per day for all infants that weigh between 1,500 and 1,800g. Infants weighing less than 1,500g receive 0.2ml twice per day (Cormack, 2013). A 0.2ml dose provides 7.7µg (311IU) of vitamin D. A 0.4ml dose provides 15.5µg (622IU) of vitamin D (PHARMAC; Pharmaceutical Management Agency New Zealand, 2013). In addition to supplements, these infants will receive either BMF or preterm infant formula - depending on mode of feeding. The addition of these will increase vitamin D intake further, the extent of which is dependent on volume provided.

Optimal supplementation dose for preterm infants remains controversial (Agostoni, 2010; Ministry of Health, 2013; Ross, 2011; Wagner & Greer, 2008). Of concern is that a dose of 0.3ml of Vitadol C per day provides 667µg of vitamin A, which is above the upper limit set for all ages in New Zealand and Australia (NHMRC, 2006). Considering this is the routine dose for preterm infants after hospital discharge (who previously met the criteria) is alarming. It is also recognised as one of the barriers for not routinely implementing Vitadol C in all preterm and term infants (Ministry of Health, 2013).

Considering that all preterm infants who meet the criteria for vitamin D supplementation will also receive BMF or preterm infant formula, it is unlikely that these infants will suffer from vitamin D deficiency. Preterm infants who do not receive vitamin D supplementation and who are exclusively

breastfed are at the highest risk of vitamin D deficiency (Dawodu & Nath, 2011; McCarthy et al., 2013).

Evidence of this deficiency is becoming available (Dawodu & Nath, 2011; McCarthy et al., 2013). A recent study by McCarthy et al. (2013) concluded that vitamin D deficiency was prevalent in a group of primarily Caucasian (89%) preterm infants. Dawodu and Nath (2011) found high levels of severe vitamin D deficiency in a group of 34 Arabian preterm infants (26-34 weeks gestation). Cord blood samples were taken at birth and 25(OH)D levels were measured, results indicated that almost half of the group (44%) were severely vitamin D deficient (25(OH)D <12.5 nmol/L). Whilst there has been a recent increase in the reporting of vitamin D deficiency in preterm infants, this is not a new issue. Earlier studies also document high levels of deficiency (Rosen, 1974; Salle, 1983).

Vitamin D status in preterm infants after hospital discharge in New Zealand is unknown. A recent study in New Zealand by Wall et al. (2013) found that vitamin D deficiency (25(OH)D \leq 27.5 nmol/L) was prevalent in healthy term breast fed infants at 2-3 months of age. These infants received no supplemental vitamin D source and were exclusively breastfed until this age; 24% had established vitamin D deficiency. High levels of observed deficiency in term infants indicate that even higher levels may be observed in the preterm population due to shorter gestational lengths resulting in reduced vitamin D stores at birth (Tsang et al., 2005).

Vitamin D deficiency is linked with several poor health outcomes in preterm infants including hypocalcemia, bone disease, decreased immune function and a subsequent increased risk of infection (Koo, Gupta, Nayanar, Wilkinson, & Posen, 1982; Ross, 2011; Wagner, 2008). Vitamin D deficiency has long been associated with inadequate mineralisation of the skeleton, and is commonly referred to as metabolic bone disease of prematurity, the implications of which may have lifelong effects on skeletal health (Koo et al., 1982; Ross, 2011; Wagner, 2008). More recently however vitamin D deficiency during infancy has been associated with an immediate increased risk of infection (Leis et al., 2012; Mohamed & Al-Shehri, 2013).

There is grave concern that preterm infants who are exclusively breast fed and do not receive vitamin D supplementation are at a high risk of vitamin D deficiency. These infants suffer from reduced vitamin D accretion in *utero* and subsequently have higher requirements in infancy. Breast milk is a poor source of vitamin D and alone is unable to satisfy the requirements of the preterm infant. Considering the crucial roles of vitamin D in the body and its potential for adverse effects at insufficient levels, it seems prudent that immediate research is carried out to determine whether the vitamin D supplementation practices for preterm infants in New Zealand are appropriate.

The current study will be the first to document the vitamin D status of preterm infants after hospital discharge in New Zealand. This research will enable us to find whether supplementation efficacy in those being supplemented is appropriate or whether levels and criteria need to be reviewed. The current study is being carried out as part of a larger longitudinal observational study aimed at assessing the micronutrient status of preterm babies at 4 months post hospital discharge and then to follow the babies up at 6, 9 and 12 months corrected age to assess feeding practices over the first year of life.

This thesis will present a situation analysis of the vitamin D status of preterm infants at 4 months after hospital discharge and describe the factors which influence vitamin D status including mode of feeding, any supplementation practices as well as skin colour and sun exposure.

1.3 Purpose of the Study

1.3.1 Aim

To investigate the vitamin D status of preterm infants living in Auckland, New Zealand at 4 months after hospital discharge and the factors which influence vitamin D status.

1.3.2 Objectives

- To assess vitamin D status of preterm babies at 4 months post hospital discharge and determine the factors affecting vitamin D status.
- Determine the effect of breastfeeding and formula feeding on vitamin D status at 4 months post hospital discharge.
- Determine the effect of vitamin D supplementation on vitamin D status at 4 months after hospital discharge.
- To determine compliance with prescribed supplements for preterm babies after hospital discharge.
- To determine sun exposure behaviours in preterm infants after hospital discharge.

1.3.1 Hypothesis

H₁: It is hypothesised that preterm infants who do not receive vitamin D supplements and are exclusively breastfed will be vitamin D insufficient (25(OH)D <50nmol/L) at 4 months post hospital discharge.

1.2 Structure of Thesis

The literature regarding the contributors to vitamin D status in preterm infants and the effects of deficiency will be reviewed in Chapter 2.0. Following this Chapter 3.0 provides a detailed description and justification of the materials and methods used within this study. Chapter 4.0 will cover the results and findings of the study. Chapter 5.0 will provide a detailed discussion of these findings, and will conclude with strengths, limitations and recommendations from the current study.

Chapter 2.0: Literature Review

2.1 Preterm Infants

2.1.1 Prematurity

A preterm infant is defined as one that is born before 37 completed weeks (259 days) of gestation. The extent of prematurity of an infant is also further defined into extremely preterm – born before 28 weeks of gestation, very preterm – born between 28 and 32 weeks of gestation, and moderate to late preterm – 32 to less than 37 weeks gestation (World Health Organization, 2012).

Preterm infants are often further classified according to their birth weight. Infants born less than 2,500g are considered low birth weight (LBW), less than 1,500g very low birth weight (VLBW) and less than 1000g extremely low birth weight (ELBW). Term infants; those born between 37 and 42 weeks gestation can also be classified as LBW, VLBW and ELBW, however this is more commonly observed in preterm infants (Groh-Wargo, 2009).

Extent of prematurity and birth weight are important classification systems as they often guide feeding and supplementation practices of preterm infants (Cormack, 2013).

2.1.2 Premature Birth

Many factors can contribute to preterm birth. In New Zealand a high percentage of preterm births are documented as 'spontaneous' (Ministry of Health, 2010). Environmental, social and genetic factors contribute to the complex interplay of events that lead up to spontaneous preterm birth, with several factors known to considerably increase risk (Ananth & Vintzileos, 2006; Gardner, 1995; Savitz, 2011).

Previous spontaneous preterm delivery increases maternal risk for delivering preterm in following pregnancies (odds ratio 3.6, CI 95%) (Ananth, Getahun, Peltier, Salihu & Vintzileos, 2006). Multiple pregnancies also notably increase risk, with twins accounting for a disproportionate number of preterm births (Gardner, 1995). Approximately 60% of all twins worldwide are born before 37 weeks gestation (Blencowe, Cousens, Oestergaard, Chou, Moller, Narwal, Adler, Garcia, Rhode, Say, Lawn, 2012). This number is greatest in higher income countries, in part due to an upward trend in the use of assisted fertility treatments. Other maternal factors that contribute to an increased risk of delivering prematurely include older age, short inter-pregnancy intervals, and low body mass index (Ananth & Vintzileos, 2006; Savitz, 2011; Smith, 2003). Intrauterine infection is also documented as a significant contributor to extremely preterm birth in high income countries, whilst in lower income countries intrauterine infection is a common cause of spontaneous preterm birth across the spectrum. Causes commonly include malaria, bacterial vaginosis, human-immunodeficiency virus (HIV) and syphilis (Ananth & Vintzileos, 2006).

In New Zealand the greatest risk factors documented for moderate preterm delivery are previous preterm birth, cervical weakness, uterine abnormality, multiple pregnancies, haemorrhage and intrauterine growth restriction with the highest numbers suffered by Maori and Pacific people (Ministry of Health, 2012).

Provider initiated preterm birth is also common in New Zealand, as well as around the world (Blencowe et al., 2012; Ministry of Health, 2012). Provider initiated preterm birth is often a life saving medical intervention which initiates preterm birth either through induction or elective

caesarean section (Ananth & Vintzileos, 2006). The reasons for provider initiated preterm birth are highly variable, and can be related to both maternal and fetal complications. Severe maternal pre-eclampsia is often cited as the most common cause of provider initiated preterm birth; left untreated it can result in life threatening eclampsia (Ananth & Vintzileos, 2006; Meis, 1998). Fetal distress and fetal intrauterine growth restriction also commonly require inducing preterm birth (Ananth & Vintzileos, 2006).

Multiple complex aetiologies contributing to spontaneous preterm birth and factors requiring provider initiated preterm birth, make prevention extremely difficult. This is reflected by the continuous rise in premature birth rates (Blencowe et al., 2012). Nutrition becomes of key importance in contributing to the improved health of these infants. Rising numbers mean that if nutrition practices are not appropriate there will be a continuous rise in the number of preterm infants who develop nutritional deficiencies.

2.1.3 Preterm Birth Rates

Preterm birth and survival rates are increasing substantially across the world (Blencowe et al., 2012). Fifteen million infants are born preterm annually, equating to 1 in every 10 infants being born too soon (Beck, 2010). The highest rates of preterm birth with poor survival are seen in Africa, with the contrary observed in high income areas of Europe (Blencowe et al., 2012).

New Zealand has also experienced an escalation in preterm birth rates in the last two decades. In 2010 it was documented that 7.4% of all births were preterm (Ministry of Health, 2012). Within New Zealand disparity is also experienced by certain ethnic groups; 8.1% of all Maori babies are born preterm annually (Ministry of Health, 2010). This is consistent with an increased rate of preterm birth being experienced by those who are affected by increasing levels of socioeconomic deprivation (Ministry of Health, 2010).

Technological advances in neonatal care are primarily responsible for increased survival rates in New Zealand and across the world (Beck, 2010). The late 1960's saw the beginning of this development when Sir Graham Liggins discovered the beneficial effects of corticosteroids on fetal lung maturation in lambs, which was followed by extensive trials and now the regular use of steroids in preterm infants (Liggins, 1969; Liggins, 1972).

Dissimilarities in technological advances nearly 30 years later however are apparent between countries. The impact of which can be fully recognised when comparing differing preterm birth and survival rates across countries (Beck, 2010). In poorer countries survival rates of extremely preterm infants are less than half of that seen in higher income countries; in fact levels are almost immeasurable (Beck, 2010).

Increasing rates of preterm birth and survival have social and economic implications for the entire population. It is estimated that preterm infants incur approximately triple the medical costs of a term infant. In the United States the economic burden per preterm infant is an estimated \$51,600 per lifetime (Galson, 2008).

The continuous rise in preterm birth and survival rates combined with difficulty in prevention make research into all aspects of preterm infant care, including in the nutritional arena imperative to continuing to improve outcomes in this group (Beck, 2010).

2.1.4 Health Consequences of Prematurity

Worldwide more than 1 million infants die each year as a consequence of preterm birth (Blencowe et al., 2012). Preterm infants who do survive will often suffer a significant number of health challenges due to being born too soon, the consequences of which can last a lifetime (Blencowe et al., 2012). Health challenges suffered by preterm infants often require them spending the first few days to months of life in a neonatal intensive care unit (NICU), the extent is often dependent on the degree of their prematurity and preterm-related health consequences (Saigal, 2008). With decreasing gestational age and birth weight the risk of prematurity related health consequences is substantially increased. However, moderate to late preterm infants are still subject to poor health outcomes, and in comparison to term infants suffer much higher rates of morbidity (Saigal, 2008).

Preterm-related health consequences are a primary contributor to high rates of infant morbidity and mortality (Beck, 2010). The second and third trimesters of pregnancy represent the stage of life where growth, development and nutrient accretion are most rapid. Missing out on such an imperative period can give rise to a plethora of outcomes (Beck, 2010).

Short term consequences commonly suffered by the preterm infant include respiratory distress syndrome (RDS), temperature deregulation, physiological jaundice, apnoea of prematurity, anemia of prematurity, hypoglycaemia, seizures, bronchopulmonary dysplasia, necrotising enterocolitis (NEC), short bowel syndrome (SBS), osteopenia, cholestasis, feeding difficulties and nutrient deficiencies (Blencowe et al., 2012; Galson, 2008; Saigal, 2008). Morbidities associated with prematurity will often extend to later life resulting in significant physiological and psychological burdens for both the infants and their families. Barker's 'Foetal Origins of Adult Disease Hypothesis' suggests that inadequate nutrition in *utero* may program the development for chronic disease in later life (Barker, 2002). Barker (2002) showed an association of LBW with an increased risk of developing chronic heart disease in adulthood. A large longitudinal Norwegian study showed that the risk of long term health consequences including cerebral palsy, mental retardation, blindness and hearing increased markedly with decreasing gestational age (Moster, 2008). Preterm birth has also been associated with higher incidence of osteoporosis, hypertension and type 2 diabetes mellitus (T2DM) in later life (Blencowe et al., 2012).

The higher health burden imparted on preterm infants and the association between inadequate nutrition in early life and poor health outcomes with ageing necessitate the importance of nutrition for preterm infants. Nutrition is one area that can be modified easily and economically and has the potential for dramatic improvements in the lifelong health of infants affected by preterm related health consequences (Beck, 2010).

2.2 Nutrition for Preterm Infants

Nutrition is a crucial part of care in preterm infants. Nutritional needs of preterm infants are much greater in comparison to term infants. This is due to reduced gestational length which reduces nutrient accretion periods in *utero* (Groh-Wargo, 2009). The second and third trimesters of pregnancy represent a stage of life where growth, development and nutrition accretion are most rapid (Groh-Wargo, 2009). Missing out on this critical time necessitates catch up growth both at birth, and possibly lifelong (Groh-Wargo, 2009).

Consequently at birth preterm infants commonly have inadequate nutrient stores and experience altered nutrient absorption and increased growth rates (Embleton, 2013; Groh-Wargo, 2009). As a result a significant number of preterm infants are dependent on the provision of nutritional support both during hospital stay and after discharge (Groh-Wargo, 2009).

Vitamin D is one nutrient that is primarily accrued in the final stages of pregnancy. Vitamin D is a fat soluble vitamin that requires deposition into adipose tissue (Holick, 1996). Decreased accretion of adipose stores in preterm infants, therefore limits in *utero* vitamin D accretion. Reduced length of gestation experienced thus renders preterm infants more likely to be vitamin D insufficient at birth (Tsang et al., 2005).

Correction of vitamin D insufficiency and deficiency is best achieved with supplementation. Cutaneous synthesis of vitamin D requires the action of ultraviolet beta (UV β) light with 7-dehydrocholesterol within the epidermis, which forms a series of reactions that creates calcitriol or 1 α ,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) the active metabolite of vitamin D (Holick, 1996). However, depending on the infant's extent of prematurity and associated complications, the first few days to months of life may be spent in a NICU, within which exposure to light in the UV β range is not possible.

Furthermore, in New Zealand and Australia as well as in a number of other countries all infants up to the age of 6 months are recommended to avoid all direct sun exposure and to utilise shaded areas, protective clothing, hats and sunglasses when outside (American Academy of Pediatrics, 1999; Ministry of Health, 2013). Therefore even after hospital discharge cutaneous synthesis of vitamin D is still not a reliable source of vitamin D in these infants (Ministry of Health, 2013).

2.3 Vitamin D Overview

Vitamin D is a unique fat soluble vitamin that has several different physiological forms. The term vitamin D essentially encompasses vitamins D₂-D₇. Vitamins D₂ and D₃ are those that are physiologically pertinent to humans, including the preterm neonate (Battersby, 2012; Holick, 1996). Vitamin D₂ also known as ergocalciferol is produced by UV irradiation of ergosterol, a steroid in plants, and is subsequently found naturally in a small number of foods (Holick, 1996). Vitamin D₃ also known as cholecalciferol is formed through the action of UV β light on the skin (Holick, 2006; Lips, 2006). Subsequent reactions then produce calcitriol or 1 α ,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) the active metabolite of vitamin D (Holick, 2006; Lips, 2006). Both cholecalciferol and ergocalciferol are used in vitamin D supplement preparations and in fortified foods. Within New Zealand cholecalciferol is more widely used and is commonly found in breast milk fortifier, infant formula and Vitadol C. Both forms of vitamin D are regarded as being similar in terms of their effects within the body. However cholecalciferol is deemed more effective in raising serum 25(OH)D concentrations (IOM, 2010; National Institute of Health, 2011;).

Vitamin D acts as a hormone and has numerous roles within the body (Brown, 1999). Vitamin D has a pertinent role in the homeostasis of calcium and phosphate. Vitamin D influences skeletal growth and has an integral role in bone modelling and remodelling over the lifecycle. This role begins in the uterine environment (Brown, 1999; Thorne-Lyman & Fawzi, 2012). Adequate vitamin D is also essential for muscle contraction, normal cellular function and nervous system activities (Czech-Kowalska et al., 2012; Thorne-Lyman & Fawzi, 2012). Non-calcitropic functions of vitamin D may include acting as an immunomodulator, including having an important role in the modulation of substances including monocytes, one of which – cathelicidin an antimicrobial peptide has a key role in innate immunity (Bagnoli et al., 2011).

The presence of vitamin D substantially increases the intestinal absorption of calcium and phosphorous (Brown, 1999; Holick, 1996). However, in the preterm neonate, the intestinal absorption of these minerals does not appear to be affected by vitamin D (Salle, 1983). It is not completely clear when vitamin D becomes pertinent in this role. However this does not translate

into reduced vitamin D requirements in the preterm neonate; exact requirements in this group are debatable (Agostoni, 2010; Ross, 2011; Wagner & Greer, 2008). However, as previously mentioned decreased vitamin D accretion in *utero*, prolonged hospital stay and the recommendation to avoid direct sun exposure during infancy make the provision of exogenous sources during this time crucial (Ministry of Health, 2013).

Within this thesis vitamin D will refer to both vitamins D₂ and D₃. Dietary forms of vitamin D are measured in micrograms (µg) or international units (IU). One microgram of vitamin D is equivalent to 40 international units. Plasma levels of vitamin D are expressed as ng/mL and nmol/L. One ng/mL of 25-hydroxyvitamin D (25(OH)D) is equivalent to 2.496 nmol/L 25(OH)D (NHMRC, 2006).

2.3.1 Vitamin D Metabolism

Calcitriol, the active metabolite of vitamin D is formed through a series of processes and reactions. Calcitriol can be produced from cholecalciferol (vitamin D₃) which is produced from the action of UVβ radiation on the skin as well as ergocalciferol (vitamin D₂) which is obtained from food and supplemental sources (Brown, 1999).

Cholecalciferol and ergocalciferol undergo the same series of reactions to become the active metabolite calcitriol. However, ergocalciferol from food and supplemental sources must first be absorbed in the small intestine and incorporated into chylomicrons prior to entering the circulation. Chylomicrons then transport ergocalciferol into the lymphatic system (Holick, 1996). Whereas cholecalciferol enters the extracellular space and is drawn into the dermal capillary bed by a vitamin D binding protein (DBP).

Within the circulation ergocalciferol and cholecalciferol are bound to a DBP - α₂-globulin and are transported in the blood to the liver where they undergo a hydroxylation step catalysed by vitamin D-25-hydroxylase, which involves the insertion of a carboxyl group at carbon 25, yielding 25-hydroxyvitamin D₃ (25(OH)D₃) (Brown, 1999).

When calcitriol is required a second hydroxylation step takes place; 25(OH)D₃ is bound to a DBP and is transported to the mitochondria of the renal cortex where the second hydroxylation step catalysed by renal 25(OH)D₃-1α-hydroxylase (1αOHase) occurs, forming the biologically active metabolite calcitriol also known as 1,25 dihydroxyvitamin D (1,25(OH)₂D) (Brown, 1999).

2.3.2 Vitamin D Receptor

The vitamin D receptor (VDR) mediates the actions of calcitriol upon binding. The VDR is a 427 amino acid peptide containing several different domains which mediate the effects of vitamin D (Brown, 1999; DeLuca, 2004).

Almost all body cells contain a VDR within their nuclei (DeLuca, 2004). The functions of these however are poorly understood. Tissues which contain VDRs are numerous and include even those that do not have a primary role in calcium and phosphate homeostasis. Such cells include those of the immune system, heart, brain, lungs, skin, pancreas and several other organs. The presence of VDR in such cells is thought to mediate the autocrine effects of vitamin D and potentially have a significant role in mediating the therapeutic effects of vitamin D (Brown, 1999; DeLuca, 2004).

2.3.3 Determination of Vitamin D status in Preterm Infants

While calcitriol is the active metabolite of vitamin D, it is not routinely used as a clinical indicator of vitamin D status. Vitamin D deficiency often results in a state of secondary hyperparathyroidism, which results in increased stimulation of the kidneys to produce calcitriol. Thus using calcitriol as an

indicator of vitamin D status has the ability to make vitamin D status appear falsely elevated in deficiency states (Holick, 2006b). Instead 25(OH)D₃ is used as levels are not falsely affected by secondary hyperparathyroidism, furthermore it has a reasonably long half life in the circulation of 2-3 weeks making it a useful nutritional indicator of vitamin D status (Holick, 2006b).

In all infants, the major circulating form of vitamin D is often represented by a C3 epimer of the 25(OH)D molecule known as 3-epi-25(OH)D₃ (Battersby, 2012). The primary difference between the two molecules is their asymmetrical arrangement; 3-epi-25(OH)D₃ has a hydroxyl group in the C3 position and is thought to be physiologically less effective in calcium mediated bone metabolism, which may be a result of the immature vitamin D metabolism in these infants (Battersby, 2012). In infants because a significant amount of cholecalciferol or ergocalciferol is converted into 3-epi-25(OH)D₃ the efficacy of supplementation has been questioned (Battersby, 2012). Precise measurement of vitamin D status in infants would require the 3-epi-25(OH)D₃ proportion of 25(OH)D to be measured, however due to cost and time constraints this is not commonly practiced. The measurement of 3-epi-25(OH)D₃ can only be reliably carried out through liquid chromatography-tandem mass spectroscopy method which effectively deciphers the proportion of vitamin D that is 3-epi-25(OH)D₃ (Battersby, 2012). Therefore 25(OH)D levels are also commonly used to determine vitamin D status in infants.

2.3.4 Vitamin D Recommendations in Preterm Infants

There is currently no consensus on the vitamin D recommendations for preterm infants (Agostoni, 2010; Wagner & Greer, 2008). The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) revised nutrition guidelines in 2010 and set specific recommendations for the vitamin D intake in preterm infants. They recommend that the daily intake (DI) is 20-25µg (800-1000IU) per day for all infants born preterm (Agostoni, 2010). Other recommendations for preterm and VLBW infants are shown in Table 2.1.

Vitamin D recommendations are available for term infants. Whilst these vary between countries; they remain within a similar range of between 5-10µg (200-400IU) per day. In New Zealand and Australia recommended adequate intake (AI) set for term infants is 5µg (200IU) of vitamin D per day. Table 2.2 displays term vitamin D recommendations set in different countries. There are no New Zealand or Australian recommendations for preterm infants. However the latest companion statement released by the Ministry of Health (MOH) on vitamin D and sun exposure during pregnancy and infancy recognises preterm infants as an at risk group for vitamin D deficiency (Ministry of Health, 2013). This document suggests that before routine vitamin D supplementation can be commenced an appropriate supplement needs to be made available; the current vitamin D supplement used in preterm infants (Vitadol C) provides 667µg of vitamin A when a dose of 0.3ml or more is given; which is above the upper limit set for vitamin A for all ages in New Zealand and Australia (Ministry of Health, 2006; Ministry of Health, 2013; NHMRC, 2006).

Countries including the United States, Canada, United Kingdom, Austria, Switzerland and Germany all recommend intakes higher than New Zealand and Australia for term infants (Agostoni, 2010; IOM, 2011; Wagner & Greer, 2008). Indeed some of these countries have recommendations for routine vitamin D supplementation in all infants and children (Wagner & Greer, 2008).

In 2008 the American Academy of Paediatrics (AAP) issued a revised statement for the recommendations of vitamin D for term infants, children and adolescents to be increased to 10µg (400IU) per day, which should commence in the first few days after birth (Wagner & Greer, 2008). This is an additional 5µg (200IU) per day above the previous recommendation for these age groups

(Wagner & Greer, 2008). These recommendations are consistent with those from the Institute of Medicine (IOM) for term infants which were revised in 2011 (IOM, 2011; Ross, 2011). The recommendations were changed in light of emerging evidence about the potential health benefits of vitamin D and historical evidence showing this amount can be safely consumed. Some of the evidence noted by the AAP includes improving innate immunity and associations with vitamin D sufficiency and reduced risk of diabetes and certain cancers lifelong (Garland, 2006; Holick, 2008; Holick, 2006; Hypponen, 2001; Hypponen, 2004; Wagner & Greer, 2008).

In 2013, the AAP published a clinical report on specific mineral requirements of preterm infants; they advised that 10µg (400IU) of vitamin D per day is appropriate for preterm infants with a birth weight of more than 1,500g - until further research is available to determine the safety of higher levels in this population group (Abrams and the Committee on Nutrition, 2013). Whilst the IOM also provided revised USA and Canadian recommendations for term infants in 2011, as mentioned above, specific recommendations were not provided for preterm infants; the IOM considered them as a unique population group for which there was not sufficient research to support their requirements (IOM, 2011). However, they suggested that a vitamin D intake ranging from 4-10µg (160-400IU) daily seemed sufficient (Abrams and the Committee on Nutrition, 2013; IOM, 2011; Ross, 2011). Recently Ireland has convened a working party on vitamin D supplementation which concluded that a vitamin D supplement of 5µg (200IU) per day should be given to all infants regardless of feed type from birth. However they also advise that this should not be commenced in all infants until a supplement containing only vitamin D is available - as they are in a similar situation to New Zealand; the current subsidised vitamin D supplement (Abidec) contains vitamin A, levels of which are above the recommended upper level when combined with 500ml or more of infant formula.

Universal consensus for vitamin D requirements and recommendations urgently needs to be established in preterm infants. Without such recommendations this vulnerable group is placed at further risk of vitamin D deficiency as well as potential risk of toxicity.

Table 2.1: Vitamin D Recommendations in Preterm and VLBW infants

Vitamin D Recommendations for Preterm and VLBW infants	
Author	Vitamin D (per day)
ESPGHAN (Agostoni,2010)	20-25µg 800-1000IU
AAP (Clinical Report, 2013)	5-10µg 200-400IU
Tsang (2005)	3.75-10µg 150-400IU
Klein (2002)	2.25-3.12µg/kg/day (3.37-8.45µg/kg/day for ≤1.5kg) 90-125IU/kg/day (135-338IU/day for >1.5kg)

Table 2.2: Vitamin D Recommended Intakes for Term Infants

Vitamin D Recommended Intakes for Term Infants (0-6 months)				
Country/Institute	AI (per day)	EAR (per day)	RDI/RDA (per day)	UL (per day)
Australia and NZ (NHMRC 2006)	5µg 200IU	*	*	25µg 1000IU
USA (AAP, 2008)	10µg 400IU	*	*	25µg 1000IU
USA, Canada (IOM, 2011)	10µg 400IU	*	*	25µg 1000IU
UK (Department of Health, 1991)	7-8.5µg 280-340IU	*	*	*
Germany, Austria and Switzerland (Deutsche Gesellschaft für Ernährung, 2012)	10µg 400IU	*	*	*
* Level not set				

2.3.5 Vitamin D Deficiency Levels in Preterm Infants

Vitamin D deficiency levels have been set for term infants in New Zealand, however there are no specific values set for those born preterm (NHMRC, 2006). In New Zealand and Australia the level considered as vitamin D deficiency and insufficiency in term infants is plasma 25(OH)D levels ≤ 25 nmol/L and < 50 nmol/L respectively. These levels are consistent with those set for children, adolescents and adults (NHMRC, 2006). Other countries also lack specific deficiency cut off values for preterm infants and instead provide population wide recommendations (AAP, 2008; Braegger, 2013; IOM, 2011).

As with recommended intakes of vitamin D there is much debate about the exact level that should be used to define vitamin D deficiency, insufficiency, sufficiency and upper levels in all population groups. Experts are yet to reach consensus on what these levels should be (Braegger, 2013; Brouwer-Brolsma, 2013; Heaney, 2008; Holick et al., 2011; NHMRC, 2006). The IOM and AAP have similar values to define vitamin D deficiency as New Zealand (Table 2.3) (IOM, 2011; Wagner, 2008). However, some vitamin D experts recommend much higher values. Holick et al. (2011) suggests that 25(OH)D concentrations below 75 nmol/L indicate vitamin D insufficiency, whereas Heaney et al. (2008) suggests levels below 80 nmol/L indicate insufficiency.

It is important to recognise that current vitamin D deficiency cut off points do not necessarily correlate with specific health conditions, but instead are associated with an increased risk of poor health outcomes (IOM, 2011). Lower vitamin D deficiency levels, including those recommended by the AAP, IOM and National Health and Medical Research Council (NHMRC) commonly only consider the effects of vitamin D deficiency on bone health (AAP, 2008; IOM, 2011; NHMRC, 2006). Whereas higher cut off points including those recommended by Holick et al. (2011) and Heaney et al. (2008) take into consideration the potential effects of vitamin D levels on overall health (Braegger, 2013; Brouwer-Brolsma, 2013). The values that should be used are highly debated and hence the reason for such varied recommendations. The cut off points for vitamin D deficiency suggested by various organisations and researchers can be found in Table 2.3.

Table 2.3: Vitamin D Deficiency Levels in Infants and Adults

Vitamin D Deficiency Levels (25(OH)D) in Infants and Adults (nmol/L and ng/ml)					
	Severe Deficiency	Mild-Moderate Deficiency	Insufficient	Sufficient	High levels
New Zealand and Australia (NHMRC, 2006)	≤12.5 nmol/L (≤5 ng/ml)	>12.5-25 nmol/L (>5-10 ng/ml)	≥25.0-49.9 nmol/L (≥10-19.9 ng/ml)	≥50 nmol/L (≥20 ng/ml)	≥125 nmol/L (≥50 ng/ml)
USA (AAP, 2008)	*	*	*	≥50 nmol/L (≥20 ng/ml)	≥125 nmol/L (≥50 ng/ml)
USA (IOM, 2011)	*	*	<40 nmol/L (<16 ng/ml)	≥50 nmol/L (≥20 ng/ml)	≥125 nmol/L (≥50 ng/ml)
USA Vitamin D Council (Vitamin D Council 2013)	*	0-75 nmol/L (0-30 ng/ml)	≥77.5-97.5 nmol/L (≥31-39 ng/ml)	≥100-200 nmol/L (≥40-80 ng/ml)	>375 nmol/L (>150 ng/ml)
Europe (Braegger, 2013)	<25 nmol/L (<10 ng/ml)	*	≥25-49.9 nmol/L (≥10-19.9 ng/ml)	≥50 nmol/L (≥20 ng/ml)	*
Holick Endocrine Society (Holick, 2011)	*	<50 nmol/L (<20 ng/ml)	≥50-72.5 nmol/L (≥21-29 ng/ml)	>75-250 nmol/L (>30 ng/ml)	*
Heaney (Heaney, 2008)	*	<20 nmol/L (<8 ng/ml)	≥20-80 nmol/L (≥8-32 ng/ml)	>80 nmol/L (>32 ng/ml)	*
*Level of deficiency, sufficiency or upper level not set					

2.4 Nutrition Provision for Preterm Infants during Hospital Stay

In comparison to other population groups, feeding mode and supplement use are the most important sources of vitamin D in preterm infants. Provision of nutrition to the preterm infant is however dependent on several factors, some of which include gastrointestinal development and maturation as well as the presence of feeding reflexes (Berse, 1993; Neu, 2007). Such factors depict the safety of receiving nutrition and the route by which it can be provided (Neu, 2007). Gestational age and birth weight are frequently used as a guide to such development. These are therefore commonly used to guide feeding regimes, including the provision of parenteral, enteral and oral feeding. All of which contribute to the vitamin D status of the infant.

2.4.1 Feeding Reflexes

Preterm infants commonly lack feeding reflexes required to obtain sufficient nutrition safely (Ingham, 2008; Thoyre, 2005). Reflexes including seeking out a breast, sucking and swallowing whilst breathing in a coordinated manner are imperative to competent breast and bottle feeding (Thoyre, 2005). These feeding reflexes develop from approximately 32 weeks gestation, with the majority of preterm infants being unable to breastfeed competently until 34-35 weeks gestation (Ingham, 2008).

When such reflexes are not present intervention is required to ensure the provision of adequate fluid and nutrition (Ingham, 2008; Thoyre, 2005).

2.4.2 Gastrointestinal Maturation

As well as underdeveloped feeding reflexes the preterm infant commonly suffers from an immature gastrointestinal tract (Berseth, 1993; Ingham, 2008; Neu, 2007). In the third trimester of pregnancy the fetal digestive system undergoes significant development, with a momentous increase in size, surface area and growth of villi and microvilli; all of which are essential to optimal function (Berseth, 1993). Preterm infants will often suffer from intestinal dysmotility, immature mucosal barrier function, increased permeability and have immature host defences, including lower levels of intestinal immune factors such as secretory immunoglobulin A (Berseth, 1993). These factors together can make the digestive system of the preterm infant incapable of obtaining adequate nutrition safely (Berseth, 1993). Such factors increase susceptibility for infection, substantially increasing the risk of developing life threatening NEC (Neu, 2007).

Extent of prematurity, presence of coordinated feeding reflexes, level of gastrointestinal development and maturation as well as associated complications are therefore factors that must be considered when feeding the preterm infant (Berseth, 1993; Ingham, 2008; Neu, 2007).

2.4.3 Parenteral/Intravenous Nutrition

Parental nutrition is the provision of nutrition through a central or peripheral intravenous catheter (American Society for Parenteral and Enteral Nutrition, 2013). Intravenous nutrition completely bypasses the digestive system and instead provides nutrition directly into the blood stream (American Society for Parenteral and Enteral Nutrition, 2013). It is essential in preterm infants who have functional immaturity of the gastrointestinal tract and is recommended for preterm infants who are unable to start feeds or obtain sufficient enteral or oral nutrition within the first 1-2 days of life (Cormack & Battin, 2008; North American Society for Pediatric Gastroenterology Hepatology and Nutrition, 2011).

The exact combination of nutrients provided by parenteral nutrition is individually guided by the infant's weight and gestational age as well as any conditions or complications experienced (Cormack, 2013). At Auckland City Hospital's NICU a standard intravenous nutrition solution is available; this is provided in Appendix 28. A lipid solution is also given to which fat soluble vitamins and minerals are added (Cormack & Battin, 2008; North American Society for Pediatric Gastroenterology Hepatology and Nutrition, 2011). Vitamin D is provided in a solution known as Vitalipid which contains 1µg of vitamin D per 1ml (Cormack, 2013). In Auckland City Hospital's NICU preterm infants receive 4ml of Vitalipid per kilogram of body weight (Cormack & Battin, 2008). Thus a 1.5kg infant would receive 6ml of Vitalipid providing them with 4µg (160IU) of vitamin D per day. This amount is sufficient to meet the upper level of recommended intravenous vitamin D intake in preterm infants of 0.75µg (30IU) per kilogram per day set by ESPGHAN or 1-4µg (40 – 160IU) per day (Tsang et al., 2005). Table 2.4 shows the recommendations for administration of Vitalipid in parenteral nutrition.

It has however been suggested that parenteral vitamin D requirements are less in comparison to enteral nutrition requirements in preterm infants (Tsang et al., 2005). This is due to vitamin D being provided directly into the blood stream, therefore bypassing digestion and not being involved in aiding calcium absorption. However, as already described the role of vitamin D in aiding calcium absorption in preterm neonates does not appear to be as efficient in comparison to term neonates (Tsang et al., 2005). Therefore whether parenteral nutrition translates into reduced vitamin D requirements is unclear.

Table 2.4: Vitalipid Recommendations for Preterm Infants

	Vitamin D Recommendation in Parenteral nutrition per kg/day	Amount of Vitalipid added to formulae	Composition of vitamin D in 1ml Vitalipid
Preterm Infants (Cormack, 2013)	1-4µg 40-160IU	4ml/kg (up to 5kg)	1µg
Term Infants (Agostoni, 2010)	0.8µg 32IU	2ml/kg (up to 5kg)	1µg

2.4.4 Enteral Nutrition

Enteral nutrition (EN) is the provision of nutrition through a feeding tube directly into an area of the gastrointestinal tract (American Society for Parenteral and Enteral Nutrition, 2013). It is essential in preterm infants who lack coordinated feeding reflexes necessary to obtain sufficient nutrition (North American Society for Pediatric Gastroenterology Hepatology and Nutrition, 2011).

In preterm infants, breast milk is the first choice of enteral nutrition (Agostoni, 2010). However, if this is unavailable an appropriate preterm infant formula will be used. The vitamin D content of infant formula in New Zealand ranges from 7-15µg (280-600IU) per 1000ml (Cormack, 2013). Enteral feeds are often started at 1ml every 4 – 12 hours and increased when the baby is tolerating the feeds by approximately 20ml per kilogram per day. Intravenous nutrition is titrated with enteral nutrition until the goal rate (180ml/kg/day) is reached (Cormack, 2013). During this stage the baby's vitamin D requirements are being met by a combination of enteral and intravenous nutrition. When intravenous lipid is stopped, the only source of vitamin D is the enteral feed.

2.4.5 Breast Milk and Vitamin D Content

Human breast milk is universally accepted as the best feed for preterm and term infants (World Health Organization, 2007). As well as nutrition it provides many non-nutritive components including a source of host resistance factors and antibodies including immunoglobulin A, lysozyme and interleukins, as well as enzymes, hormones and growth factors (Anderson, 1981). The AAP on their policy statement on breastfeeding and the use of human milk recommend breastfeeding or the provision of expressed breast milk (EBM) for all preterm infants (American Academy of Pediatrics, 2012). Breast feeding preterm infants is associated with the same benefits as those seen in term infants, and is further linked with a reduced incidence of NEC, sepsis and diarrhoea (Schanler et al., 1999; Silva et al., 2004).

Whilst breast milk is considered the best feed for preterm infants, the nutritional content of breast milk alone is insufficient to meet all nutritional requirements of the preterm infant (Anderson, 1981; Atkinson, 1980). Preterm breast milk has a different composition to mature milk; over the first month of lactation the composition of preterm breast milk is similar to colostrum (Anderson, 1981; Atkinson, 1980). It contains higher amounts of important non nutritive components as well as higher

amounts of protein, sodium, chloride and iron, and is lower in fat, carbohydrate, potassium and some vitamins (Anderson, 1981; Atkinson, 1980).

The vitamin D content of preterm and mature breast milk is similar; however documented levels vary (Gross, 1981). Maternal vitamin D status is the primary determinant of vitamin D content in breast milk. However, 25(OH)D transfer into breast milk is minimal (Thiele, Senti & Anderson, 2013). Thus, even in a vitamin D sufficient women (25(OH)D \geq 50 nmol/L) vitamin D content in breast milk is low; approximately 0.55 μ g (22-30IU) per litre. One study measured vitamin D concentrations directly from breast milk in twenty-one vitamin D sufficient (25(OH)D \geq 90 nmol/L) Malawian mothers and found levels were below the limit of detection $<$ 0.1 nmol/L (Amukele et al., 2013). However, it should be noted that all mothers were infected with HIV. Conversely another study by Seth et al. (2009) showed that whilst maternal vitamin D status in breastfeeding mothers was low, it was directly reflected in the vitamin D status of the infants; levels were 27.2 nmol/L and 28.9 nmol/L in mothers and infants, respectively.

Maternal vitamin D supplementation during lactation increases vitamin D transfer into maternal milk, resulting in improved vitamin D status in the breastfed infant (Thiele et al., 2013; Wagner, Hulseley, Fanning, Ebeling & Hollis, 2006). A supplement of 10 μ g (400IU) per day increases vitamin D content in maternal milk to approximately 2 μ g (80IU) per litre. A higher dose supplement is shown to increase levels further; women supplemented with 160 μ g (6400IU) of vitamin D per day over a 6 month period saw an increase in breast milk vitamin D concentrations from 2 to 22 μ g (80 to 880IU) per litre (Wagner et al., 2006). This dramatic increase in vitamin D content would meet the vitamin D requirements of the preterm infant, however the safety of such doses are not well understood (NHMRC, 2006). Ambiguity regarding dosage and timing of maternal supplementation combined with differing definitions of vitamin D sufficiency and deficiency are a barrier to implementing vitamin D supplementation guidelines for lactating women (NHMRC, 2006).

In New Zealand vitamin D supplementation during lactation is not part of current policy for healthy women. The NHMRC currently recommend an AI of 5 μ g (200IU) of vitamin D per day during lactation, which is consistent with recommendations for infants, children and adults (NHMRC, 2006). However for women at risk of vitamin D deficiency, for example for those with dark skin, or with limited access to sunlight a 10 μ g (400IU) supplement of vitamin D per day is recommended (NHMRC, 2006; The Royal Australian and New Zealand College of Obstetricians and Gynaecologists RANZCOG, 2009). However, whether this amount is sufficient is controversial. Based on expert reviews, the Endocrine Society clinical practice guideline recommends 15 μ g (600IU) per day for pregnant and lactating women. They also suggest that upwards of 38-50 μ g (1500IU-2000IU) per day would be required to maintain circulating 25(OH)D levels of \geq 75 nmol/L (Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney, Murad & Weaver, 2011).

Assuming a low breast milk vitamin D content of 0.04 μ g (1.6IU) per 100ml of breast milk, 25L of breast milk would be required daily to meet the AAP recommended vitamin D intake of 10 μ g (400IU) set for preterm infants (Wagner et al., 2006). Whereas 50L would be required to meet the minimum recommendation of 20 μ g (800IU) per day for preterm infants set by ESPGHAN (Agostoni, 2010; NHMRC, 2006). Such levels in breast milk would make obtaining recommended vitamin D levels from maternal milk alone impossible.

Unless vitamin D levels in breast milk are in the upper range seen with high dose maternal supplementation, breast feeding alone will not meet the recommended vitamin D intake for preterm infants (Agostoni, 2010). Exclusively breastfed preterm infants are therefore at an even greater risk

of vitamin D deficiency. In New Zealand the World Health Organisation (WHO) policy to exclusively breast feed infants until 6 months of age was adopted in 2008 (World Health Organization, 2011). Whilst breastfeeding is acknowledged as the best feed for preterm and term infants, the implications of such recommendations on vitamin D status in term infants has already been identified. Wall et al. (2013) found that 24% of term exclusively breastfed New Zealand infants had vitamin D deficiency (<27.5 nmol/L), with no significant differences between ethnicities. The potentially greater effects of exclusive breastfeeding to 6 months on preterm infants have not yet been studied.

Table 2.5: Vitamin D Content in Breast Milk

Vitamin D Content in Differing Volumes of Breast Milk											AAP 400IU	ESPGHAN 800IU – 1000IU
Volume (ml)	100	200	300	400	500	600	700	800	900	1000 1L	25,000 25L	50,000 50L
Vitamin D (IU)	1.6	3.2	4.8	6.4	8	9.6	11.2	12.8	14.4	16	400 (10µg)	800 (20µg)
Cormack, 2013												

2.4.6 Fortification of Breast Milk

Breastfeeding in term and preterm infants is recommended and in accordance with current health policy in New Zealand (World Health Organization, 2011). However, fortification of breast milk is necessary for preterm infants to ensure it meets nutrient and energy requirements (American Academy of Pediatrics, 2012; O'Connor et al., 2008).

Commercial breast milk fortifier increases the vitamin D content of breast milk; along with vitamin D, it provides additional energy, macro and micro nutrients (Cormack, 2013). It commonly comes in a powder form that can be added into EBM, effectively increasing the nutritional content without substantially increasing volume. The vitamin D content of breast milk fortifier is in the range of 2.5-4.0µg (100-160IU) per serve (2.2g) (Cormack, 2013). Some of the different fortifiers available in New Zealand and their vitamin D content per serve can be seen in Table 2.6.

Whilst providing breast milk fortifier to all preterm infants may be beneficial to their vitamin D status, this is not universal practice in all NICUs. In Auckland City Hospitals NICU breast milk fortifier is added to expressed breast milk for preterm infants who meet the following criteria; ≤32 weeks gestation or who weigh ≤1,800g at birth (Cormack, 2013). Breast milk fortifier is added when feed volume reaches 5ml per feed. All preterm infants who are ≥33 weeks gestation or who weigh >1,800g at birth do not receive breast milk fortifier, unless individually prescribed (Cormack, 2013). The AAP recommends that all preterm infants weighing ≤1,500g at birth should receive breast milk fortification in addition to all preterm infants (and term infants) receiving a 10µg (400IU) supplement of vitamin D per day (American Academy of Pediatrics, 2012).

Table 2.6: Concentration of Vitamin D in Commercial Breast Milk Fortifier

Concentration of Vitamin D in Commercial Breast Milk Fortifier		
Breast Milk Fortifier	Serving Size	Amount of vitamin D per serving size
Nutricia Breast Milk Fortifier	2.2g (1 sachet)	2.5µg 100IU
Wyeth S-26 Human Milk Fortifier	2.2g (1 sachet)	3.8µg 152IU
Nestle preNAN FM 85	5g	4µg 160IU

2.4.7 Provision of Infant Formula in Hospital

All infant formula in New Zealand is supplemented with vitamin D₃; this began in New Zealand and around the world in the middle of the 20th century due to an outbreak of rickets in infants and children (Greer, 2004).

Standard term formulas provide vitamin D in the range of 0.76-1.2µg (30-48IU) per 100ml (Cormack, 2013). A range of term formulas provided in New Zealand and their vitamin D content can be seen in Table 2.7. Preterm infant formula is supplemented with a higher dose of vitamin D in comparison to term formulas to meet the higher requirements and smaller volumes ingested by the preterm infant (Klein, 2002). These are generally in the range of 3-4µg (120-160IU) per 100ml (Cormack, 2013). Preterm infant formulas available in New Zealand and their vitamin D content can be seen in Table 2.8.

If breast milk is unavailable preterm infants receive a preterm infant formula. At Auckland City Hospital's NICU the criteria is identical for receiving breast milk fortifier (preterm infants who are ≤32 weeks gestation or who weigh ≤1,800g at birth) (Cormack, 2013). Preterm infants that do not meet these criteria are routinely provided with a standard term formula, unless individually prescribed (Cormack, 2013).

There is no set brand of preterm infant formula used at Auckland City Hospital's NICU, as with other hospitals in New Zealand these operate on a rotation scheme which ensures no one formula brand is favoured. This is in compliance with the WHO 'International Code of Marketing of Breast-Milk Substitutes', which requires all infant formula purchased to be rotated on a regular basis (World Health Organization, 1981).

A preterm infant formula containing 3µg (120IU) of vitamin D per 100ml would require the ingestion of 660ml per day to meet the minimum vitamin D requirements set by ESPGHAN of 20µg (800IU) per day, whilst 330ml would be required daily to meet the recommendations set by the AAP of 10µg (400IU) per day (Abrams & the Committee on Nutrition, 2013; Agostoni, 2010).

Table 2.7: Vitamin D Content of Term Infant Formula

Term Infant Formula	Amount of Vitamin D per 100ml
Karicare Gold Plus Infant Formula from birth (0-6 months)	0.76µg 30.4IU
Karicare Gold Plus Infant Formula from birth ready to feed	1.2µg 48IU
Aptimil Gold Plus Infant Formula from birth (0-6 months)	0.74µg 29.6IU
Wyeth/Nestle S-26 Infant Formula Gold from birth (0-6 months)	1.2µg 48IU
Wyeth/Nestle S-26 Infant Formula Original from birth (0-6 months)	1µg 40IU
A2 Platinum Premium Infant Formula from birth (0-6 months)	0.90µg 36IU
Heinz Nurture Gold Starter Infant Formula from birth (0-6 months)	0.74µg 29.6IU
Aptimil Gold De-Lact Infant Formula from birth (0-12 months)	1.2µg 48IU
Karicare HA Gold Plus Infant Formula from birth	1.2µg 48IU
Karicare Goat from birth (0-6 months)	1.0µg 40IU
Karicare Soy all ages	1.1µg 44IU
Karicare Pepti-Junior Gold all ages infant formula from birth	1.3µg 52IU

Table 2.8: Vitamin D Content of Preterm Infant Formula

Preterm Infant Formula	Amount of Vitamin D per 100ml
Nestle PreNAN Gold	3.7 µg 148IU
Nutricia Aptamil Gold Plus	3 µg 120IU
Wyeth S-26 Gold LBW	3.4µg 136IU

2.4.8 Vitamin D Supplementation Procedures in Hospital

Vitadol C is the subsidised liquid preparation of vitamin D provided to infants and children in New Zealand hospitals (PHARMAC; Pharmaceutical Management Agency New Zealand, 2013). Vitadol C contains vitamins D, A and C. It is provided to preterm infants in Auckland City Hospitals NICU provided they are ≤32 weeks gestation at birth, or have a birth weight of ≤1,800g (Cormack, 2013). Vitamin D supplementation is also provided to infants at high risk of, or who have established vitamin D deficiency (Cormack, 2013).

The standard dose of Vitadol C whilst in hospital is 0.2ml; this is given once per day for all infants that weigh between 1,500g and 1,800g. For infants weighing less than 1,500g this dose is given twice per day (Cormack, 2013). A standard 0.2ml dose provides 7.7µg (311IU) of vitamin D, a 0.4ml dose provides 15.5µg (622IU) of vitamin D (PHARMAC; Pharmaceutical Management Agency New

Zealand, 2013). A 0.2ml dose alone does not meet the intake recommended by the AAP for preterm and term infants (10µg/400 IU per day), or the AI set for term infants in New Zealand and Australia (5µg/200IU per day) (NHMRC, 2006; Wagner & Greer, 2008). A 0.4ml dose does meet these recommendations (NHMRC, 2006; Wagner & Greer, 2008). However, neither dose meets the recommendations for preterm infants set by ESPGHAN (20-25µg/800-1000IU per day) (Agostoni, 2010). However, if the infant is receiving a 0.2ml dose of Vitadol C in addition to receiving approximately 500ml of preterm infant containing 3µg (120IU) of vitamin D per 100ml, or term infant formula containing 0.76µg (30IU) of vitamin D per 100ml, total vitamin D intake will increase to 22.7µg (911IU) and 11.5µg (461IU), respectively. Both of these vitamin D intakes would meet the AAP recommended intake, in addition infants who are fed preterm infant formula would also meet ESPGHAN recommendations. If infants receive a 0.2ml dose of Vitadol C in addition to approximately 500ml of breast milk containing 0.04µg (1.6IU) per 100ml, total vitamin D intake would increase to 7.9µg (316IU) per day. This value does not meet the recommended intake set by AAP or ESPGHAN. Optimal supplementation dose for preterm infants however remains controversial (Agostoni, 2010; Ministry of Health, 2013; Ross, 2011; Wagner & Greer, 2008).

Recent research showed that a vitamin D intake of 10µg (400IU) per day (5µg/200IU from both feed and vitamin D supplements) was sufficient to meet target vitamin D levels (≥ 50 nmol/L) in 87% of stable preterm VLBW infants in a cohort of 148 who were previously vitamin D insufficient (≤ 50 nmol/L) (McCarthy et al., 2013). Earlier evidence corresponds with these results, further suggesting a vitamin D intake of higher than 10µg (400IU) per day confers no additional benefits in this group (Backstrom, 1999). Markestad et al. (1983) also showed that a vitamin D supplement of 12.5µg (500IU) per day in addition to a combination of breast and formula feeding was sufficient to normalise vitamin D levels (≥ 50 nmol/L) in preterm infants (mean age 32 weeks) by one month chronological age, even in those that were previously deficient.

Research on optimal vitamin D dose for term infants is also inconclusive. Atlas et al. (2013) compared vitamin D supplementation of 5µg (200IU) and 10µg (400IU) per day in two groups of exclusively breastfed stable term infants over a 4 month period. Authors concluded that the 10µg (400IU) supplement was adequate in all infants to reach vitamin D sufficiency ($25(\text{OH})\text{D} > 75$ nmol/L), whilst the 5µg (200IU) supplement was not; with 21.3% of infants having insufficient vitamin D status (< 75 nmol/L) on analysis.

More high quality research is required before the optimal vitamin D dose in preterm and term infants is determined (Ministry of Health, 2013; Ross, 2011). The safety of higher dose supplementation, such as that proposed by ESPGHAN also needs to be determined before it is universally implemented (Ministry of Health, 2013; Ross, 2011; Wagner & Greer, 2008). In New Zealand high dose supplementation with Vitadol C is not advised, due to the high vitamin A content. As already described a 0.3ml dose of Vitadol C daily provides 667µg of vitamin A - which is above the upper limit (600µg) set for all ages in New Zealand and Australia (NHMRC, 2006). However, this dose is routinely provided to preterm infants (provided they meet criteria) after hospital discharge, with no current contraindication (Cormack, 2013). However, Vitadol C is not recommended for routine use in term infants due to the vitamin A content (Ministry of Health, 2013; NHMRC, 2006).

2.4.9 Contraindications with Vitamin D Supplementation

There are certain instances where vitamin D supplementation is not recommended in preterm infants. If the infant is known to have hypervitaminosis D, hypercalcaemia or renal osteodystrophy supplementation is not recommended (Holick, 2006; Tsang et al., 2005).

Due to high levels of vitamin A in Vitadol C, routine supplementation in all infants may not be safe. Therefore before such practices can be implemented an appropriate supplement is required (Ministry of Health, 2013; NHMRC, 2006).

2.5 Feeding Practices and Supplement Use after Hospital Discharge

2.5.1 Vitamin D supplementation Procedures after Hospital Discharge

After hospital discharge a 0.3ml dose of Vitadol C daily is recommended in preterm infants who previously met the criteria for in hospital supplementation (Cormack, 2013). A 0.3ml dose of Vitadol C provides 11.7µg (467 IU) of vitamin D, 667µg of vitamin A and 33mg of vitamin C (PHARMAC; Pharmaceutical Management Agency New Zealand, 2013). However if an infant does not meet the criteria for supplementation and there is any risk of, or if vitamin D deficiency is established Vitadol C is prescribed (Cormack, 2013).

2.5.2 Feeding Practices after Hospital Discharge

After hospital discharge breast milk or infant formula are still likely to be the primary source of nutrition for the previously preterm infant. As already described the WHO recommends exclusive breastfeeding until 6 months of age (Ministry of Health, 2002). Therefore vitamin D intake will only differ marginally with differing volumes of breast milk or infant formula consumed.

Post discharge preterm infant formula is also available for the previously preterm infant. This is provided to preterm infants who were less than 33 weeks gestation at birth and post term (provided breast milk is not available) (Cormack, 2013). As with preterm infant formula, post discharge preterm infant formula contains a higher amount of vitamin D in comparison to standard term infant formula (Cormack, 2013).

2.5.3 Complementary Feeding and Vitamin D

The time at which complementary feeds should be introduced to infants born preterm is unknown. It has been suggested that the introduction of complementary foods should be considered between 5 and 8 months uncorrected age (King, 2009). Auckland DHB recommends introduction between 16 weeks after the infants due date (earliest) and before 7 months uncorrected age (latest) (Cormack, 2007). All recommendations emphasise the importance of assessing the infant's readiness, by observing developmental cues (Cormack, 2007; King, 2009). At this stage breast milk or infant formula are still going to be the primary source of nutrition in infants (Ministry of Health, 2002).

When infants do begin to consume larger volumes of food it is unlikely that this will have a significant effect on vitamin D status. In New Zealand, vitamin D fortification of food (other than infant formula) is not mandatory, therefore food sources are not considered to be a major contributor to vitamin D status in infants and all other age groups (NHMRC, 2006; Sivakumaran, 2012).

In 1966 New Zealand permitted the voluntary vitamin D fortification of a small number of food products (Sivakumaran, 2012). Items include margarine and other oil based spreads, skim and reduced fat milks and food products derived from cereal grains (Sivakumaran, 2012). Vitamin D is also found naturally in a limited number of plant foods and yeast. However, these foods do not make a significant contribution to the vitamin D status of New Zealanders (Ministry of Health, 2011; NHMRC, 2006). Table 2.9 below, shows some food sources in New Zealand and their vitamin D content.

Table 2.9: Vitamin D Content of Foods in New Zealand

Vitamin D Content of Foods in New Zealand (Sivakumaran, 2012)		
Food	Serve	Amount of Vitamin D (µg and IU)
Portobello mushrooms	100g	0.3-0.4 µg/10-18 IU
Shitake mushrooms	100g	0.3-0.4 µg/10-18 IU
Egg yolk	1 egg/17g	0.8 µg/32IU
Canned fatty fish (pink salmon, mackerel and sardines)	100g	2.2-13.7µg/86-547 IU
Butter (salted/unsalted composite)	100g	5.2µg/208IU
Canola margarine (50% fat, composite)	100g	20µg/800IU
Assorted cheeses	100g	0.2-0.5µg/8-20IU
Beef liver	100g	1.2 µg/49 IU
Fortified foods (dairy products, margarine)	1000g/ml	5-10 µg/200-400 IU
High calcium trim milk (yellow top)	100ml	0.7µg/28IU
Low fat milk	100ml	0.31µg/12.4
Rice milk (assorted flavours)	250ml	2.8µg/112IU
Blue top milk	1000ml	0.3µg/12IU
Breads and Cereals	100g	0.2-0.5µg/8-20IU

2.5.4 Concerns with Current Feeding, Fortification and Supplementation Practices

In New Zealand the WHO policy to exclusively breast feed infants until 6 months of age is recommended (World Health Organization, 2011). Breastfeeding the infant has numerous benefits, however as already discussed exclusive breastfeeding without concurrent fortification or supplementation, substantially increases the risk of vitamin D deficiency (Ministry of Health, 2013; Wagner & Greer, 2008). The vitamin D intake of preterm infants based on different feeding scenarios according to birth weight and gestational age is provided in Table 2.10 below.

The addition of breast milk fortifier and vitamin D supplementation are only routinely provided to preterm infants who meet the set criteria or in whom there is a clinical need; as described earlier. Therefore preterm infants who do not meet these criteria will essentially miss out on all additional sources of vitamin D (Cormack, 2013). Consequently preterm infants who are greater than 32 weeks gestation and who weigh more than 1,800g at birth and are exclusively breastfed are at considerable risk of vitamin D deficiency (Dawodu & Nath, 2011; McCarthy et al., 2013; Wagner & Greer, 2008).

Formula fed infants who are more than 32 weeks gestation and who weigh less than 1,800g at birth are routinely provided with standard term infant formula and no Vitadol C supplements (Cormack, 2013). Risk of vitamin D deficiency is higher in these infants in comparison to preterm infants receiving preterm infant formula and Vitadol C supplements. However, due to the higher content of vitamin D in infant formula in comparison to breast milk vitamin D levels are likely to normalise in this group over time. However, at least 1315ml of standard infant formula containing 0.76µg (30IU) of vitamin D is required to be ingested daily to meet AAP recommendations of 10µg (400IU) per day and 2631ml is required to meet ESPGHAN requirements of 20µg (800IU) per day (Cormack, 2013). The smaller volumes ingested by this group as well as higher likelihood of vitamin D deficiency at birth indicate that normalisation of vitamin D levels may take upwards of 3 months (Tsang et al., 2005).

It is unlikely that preterm infants who receive infant formula or Vitadol C supplements will be vitamin D deficient after hospital discharge. This is indeed what recent research on supplementation procedures in preterm infants in New England suggests (McCarthy et al., 2013). Conversely however, research into similar nutritional strategies used across 3 different NICUs in Ohio show that such strategies may be inadequate to achieve the recommended minimum vitamin D intake in preterm infants (10µg/400IU per day) and normalise serum vitamin D concentrations of ≥ 50 nmol/L (Monangi, 2013).

It is crucial that the vitamin D feeding, fortification and supplementation practices in preterm infants in New Zealand are reviewed to ensure they are sufficient to prevent deficiencies in all preterm infants.

Table 2.10: Daily Vitamin D Intake of Preterm Infants Based on Different Feeding Scenarios

	Infant 1 (32 weeks, 1,500g) Breast milk		Infant 2 (32 weeks, 1,500g) Preterm infant formula		Infant 3 (33 weeks, 1,850g) Breast milk		Infant 4 (33 weeks, 1850g) Standard term formula	
	ml	Vitamin D	ml	Vitamin D	ml	Vitamin D	ml	Vitamin D
Vitadol C	0.4ml	15.5µg 622IU	0.4	15.5µg 622IU	0	0	0	0
Breast milk	270ml	0.1µg 4.32IU	0	0	350ml	0.14µg 5.6IU	0	0
Breast milk and fortifier	5.5 sachets (Nutricia BMF)	12.5µg 500IU	0	0	0	0	0	0
Preterm infant formula	0	0	270 ml	15µg 600IU	0	0	0	0
Standard term formula	0	0	0	0	0	0	350ml	3.5µg 140IU
TOTAL Vitamin D/day	28.1µg 1124IU		30.5µg 1220IU		0.14µg 5.6IU		4.56µg 140IU	

Values for volume of feed and the addition of Vitadol C supplements and BMF are based on recommendations for infants according to their birth weight and gestational age (Cormack, 2013).

2.6 Sun exposure and Cutaneous Synthesis of Vitamin D in Preterm Infants

2.6.1 Sun Exposure in Infants

Sun exposure is essential for vitamin D synthesis in humans (Brown, 1999; Holick, 1994). New Zealand experiences a relatively sunny climate with high ultraviolet radiation (UVR) levels optimal for vitamin D synthesis (NIWA, 2013). However, the cutaneous synthesis of vitamin D is thought to be a minimal contributor to total vitamin D status in preterm and term infants (Ministry of Health, 2013).

In New Zealand direct sun exposure is not recommended in infants from 0-6 months of age (Ministry of Health, 2013). This concurs with recommendations from the AAP (American Academy of Pediatrics, 1999). If infants are exposed to sunlight it is recommended that sunscreen, protective clothing and shady areas are utilised (Ministry of Health, 2013). Such advice is in place to ensure the safety of the infant; it is evident that the epidermal barrier remains immature for the first two years of life. Thus ultraviolet (UV) light may have a more damaging and possibly accumulative effect in infants (American Academy of Pediatrics, 1999). Furthermore, due to the immobility of infants at

this age they are unable to remove themselves from uncomfortable heat. Sweating capacity may also be reduced in this group, therefore significantly increasing the risk of heatstroke (American Academy of Pediatrics, 1999).

It has been suggested that incidental sun exposure in all infants may be sufficient to maintain normal vitamin D status. The amount of sun exposure required in infants to provide adequate vitamin D however is unclear. Holick et al. (2007) suggests that exposure to one minimal erythemal dose (MED) whilst in a bathing suit is equivalent to consuming between 250µg (10,000IU) and 625µg (25,000IU) of vitamin D. A study based in Australia showed that fair skinned adult subjects (Fitzpatrick skin type II; 19-50years) could obtain adequate vitamin D levels from 2-14 minutes sun exposure with fifteen percent of their body exposed during peak sun periods in summer. They also showed that one MED could occur in as little as 8 minutes in some subjects (Samaneck, 2006). Other research suggests that those with darker skin (Fitzpatrick skin type VI) require between 2-10 times more sun exposure to produce the same amount of vitamin D (NIWA, 2013). Longer sun exposure times are also required outside of peak sunshine times and in winter months (NIWA, 2013). Whether these sun exposure times are relatable to the infant population however are unclear.

There is a significant knowledge gap in relation to sun exposure practices in preterm and term infants after hospital discharge in New Zealand and around the world. Whether sun exposure is in fact a reliable source of vitamin D in this group is unknown. However, the current recommendations for infants to avoid direct sun exposure and to utilise protective clothing indicates that this route of vitamin D production should not be relied on as an appropriate source in these groups (American Academy of Paediatrics, 1999; Ministry of Health, 2013). This issue is even further exacerbated in preterm infants, who as alluded to earlier will often spend the first few weeks to months of their lives in a NICU, in which the cutaneous synthesis of vitamin D is not possible.

2.6.2 Cholecalciferol Production in Infants

Whilst it seems evident that sun exposure is a very minimal contributor to vitamin D status in preterm infants, cutaneous synthesis is still a possible route after hospital discharge and therefore will be discussed briefly.

Cholecalciferol is a precursor for the active metabolite of vitamin D (calcitriol). Cholecalciferol is formed through the action of Ultraviolet beta (UVβ) radiation (in the range of 290-315nm) on 7-dehydrocholesterol found within the layers of the human epidermis (Holick, 1994). Whilst all layers of the human epidermis have the capacity to produce vitamin D₃, the most inner layers; the strata spinosum and strata basale have the greatest capacity for this. Ultraviolet β radiation interacts with 7-dehydrocholesterol allowing its conversion to pre-vitamin D₃. Pre-vitamin D₃ then rapidly isomerises into cholecalciferol within the plasma membrane via a heat dependent reaction (Brown, 1999; Holick, 1994). Whilst this conversion can continue for up to 3 days following UVβ exposure, excess sun exposure does not result in an excess accumulation of cholecalciferol. Instead lumesterol and tachysterol, photoisomers of vitamin D₃ are formed (Holick, 1994). The production of these photoisomers is essential for preventing vitamin D toxicity, however their specific roles are unclear (Holick, 1994).

2.6.3 Factors Affecting the Cutaneous Synthesis of Vitamin D

Several factors are known to affect the efficacy of cutaneous vitamin D synthesis. Such factors include season, latitude, geographical location, time of day, extent of cloud cover, pollution, angle of the sun, skin pigmentation and protective factors for example clothing, hats and sunscreen (Holick, 1994). Season, skin melanin pigmentation, and protective factors will be discussed here.

2.6.3.1 Season

Season is a major determinant of cutaneous synthesis of vitamin D. During summer months less sun exposure time is required to obtain sufficient vitamin D in comparison to winter months. Individuals also tend to spend more time outside and wear fewer clothes thus increasing the chances of incidental vitamin D synthesis (Holick, 2008b). The opposite is commonly observed in winter months. While these practices are clearly observable in a number of New Zealand adults, such practices may not be generalisable to infants. Maternal preference to keep young infants indoors as well as recommendations to avoid sun exposure and wear protective clothing may be a large contributing factor (Ministry of Health, 2013).

Evidence for the association between season of birth and 25(OH)D concentrations in cord blood is abundant (Basile, 2007; Giapros et al., 2012; Jain et al., 2011; Marwaha et al., 2011). New Zealand researchers concluded that summer birth was the strongest predictor of vitamin D levels in cord blood of term infants (Camargo et al., 2010). In a recent study Wall et al. (2013) showed a significant difference in vitamin D levels in different seasons in term infants aged 2-3 months; analysis of 25(OH)D demonstrated a median level of 21 nmol/L for infants enrolled during winter and a median of 75 nmol/L in infants enrolled during summer.

However, what needs to be considered is the relationship between maternal and neonate vitamin D status. At birth the main predictor of infant vitamin D status is maternal vitamin D concentration (Dawodu et al., 2013; Delvin, Glorieux, Salle, David, & Varenne, 1982; Karras et al., 2013; Lee et al., 2007; Roth et al., 2013; Thomas, Fudge, Whiting, & Coates, 2011). Therefore, any relation between infant vitamin D status and season at birth will be primarily due to seasonal changes in maternal vitamin D status.

Data on the association between season and vitamin D status in later infancy is lacking. Research to determine whether season is a predictor of vitamin D stores in preterm and term infants after hospital discharge is imperative. Without such knowledge it is difficult to determine whether season does affect sun exposure practices in preterm infants and thus vitamin D status.

2.6.3.2 Skin Melanin Pigmentation

Increased skin melanin pigmentation dramatically reduces cutaneous production of cholecalciferol (Holick, Chen, Lu & Sauter, 2007). Melanin pigmentation is extremely efficient at absorbing UV β photons; it produces a barrier disabling the photons from penetrating the epidermal layer thereby efficiently reducing the amount available for the synthesis of cholecalciferol (Holick, Chen, Lu & Sauter, 2007). Darker skin pigmentation requires an individual to be exposed to UV β light for 2-10 times longer to generate the same amount of vitamin D₃ as individuals with lighter skin pigmentation (Holick, 2006a; NIWA, 2013).

Vitamin D deficiency is well documented in infants with dark skin pigmentation. However, the majority of evidence available again reports the significance of 25(OH)D status in cord blood at birth (Basile, 2007; Jain et al., 2011; Marwaha et al., 2011). Thus indicating the correlation is primarily due to maternal skin pigmentation and her resultant vitamin D status during pregnancy (Agarwal et al., 2012; Johnson et al., 2011). However, there is a small amount of research that shows a correlation between skin pigmentation and vitamin D status in later infancy (Ziegler, Hollis, Nelson, & Jeter, 2006).

Ziegler et al. (2006) measured 25(OH)D stores in cord blood of neonates; median levels were 35 nmol/L and 25 nmol/L for Caucasian and African-American infants, respectively. The 25(OH)D concentrations of this cohort were again measured at 280 days after birth, results again showed that

vitamin D levels were higher in lighter skinned infants in comparison to darker skinned infants. Another study assessed factors associated with infantile and childhood rickets; results showed of 104 cases 89% of these had intermediate to dark skin colouring (Ward, Gaboury, Ladhani & Zlotkin, 2007).

Whilst melanin pigmentation is known to reduce the cutaneous synthesis of vitamin D, it is unknown whether this is an issue in preterm and term infants after hospital discharge. As already discussed the sun exposure behaviours of infants after hospital discharge are unclear. If infants are not exposed to the sunshine then skin melanin pigmentation will not be of importance to their vitamin D status (Holick, 2006).

2.6.3.3 Sun Protection Behaviours

Sun protection behaviours are likely to be the most significant contributor to reducing incidental sun exposure and hence cutaneous vitamin D synthesis in all infants. Sun protection behaviours can include keeping indoors and in shady areas, wearing protective clothing and applying sunscreen (NIWA, 2013).

Clothing efficiently absorbs UV β photons therefore preventing epidermal absorption; thus if an infant is placed in full body clothing cutaneous vitamin D synthesis is not possible. Similarly if the infant is placed in a shady area outside of the sun's rays the absorption of UV β photons is not possible. As alluded to earlier UV β light is also unable to pass through glass and other surfaces therefore preventing epidermal synthesis of vitamin D, thus placing an infant inside by a window will have no effect on their vitamin D status. Sunscreen application also inhibits vitamin D synthesis; the accurate application of a sunscreen with a sunscreen protection factor (SPF) of only 15 can result in a 99% reduction in pre-vitamin D production (Holick, Chen, Lu & Sauter, 2007)

If one or more of the above protective factors are employed in infants when they are exposed to sunlight the cutaneous synthesis of vitamin D will be effectively inhibited (Holick, Chen, Lu & Sauter, 2007). Considering sun protection is recommended during infancy, endogenous synthesis should not be considered as a reliable source of vitamin D in all infants, especially in those born preterm.

2.7 Prevalence of Vitamin D deficiency in Preterm and Term Infants

Recent years have seen a worldwide increase in reported rates of vitamin D deficiency during infancy (Camargo et al., 2010; Dawodu & Nath, 2011; McCarthy et al., 2013). Evidence of deficiency in term infants is becoming increasingly abundant (Agarwal, Faridi, & Singh, 2010; Bowyer et al., 2009; Robinson, 2006; Wall, 2013; Jain, Gupta, Kalaivani, Sinha & Agarwal, et al., 2011). Data on the vitamin D status of preterm infants is lacking.

A small number of recent studies have reported vitamin D levels in preterm infants. McCarthy et al. (2013) concluded that vitamin D deficiency was prevalent in a group of primarily Caucasian (89%) preterm infants. Researchers examined the vitamin D status of these infants at a mean age of 18 days, and found that based on term deficiency cut off values 14% were deficient (25(OH)D <30nmol/L) and 59% had insufficient levels (25(OH)D >30 nmol/L - \leq 50 nmol/L). At this stage infants had not received any vitamin D supplementation, however they had received vitamin D in parenteral and enteral feeds, as well as in fortified expressed breast milk (FEBM) or preterm infant formula, thus suggesting higher levels of deficiency would have been observed in this group at birth. Dawodu and Nath (2011) found particularly high levels of severe vitamin D deficiency in a group of 34 Arabian preterm infants (26-34 weeks gestation). Cord blood samples were taken at birth and 25(OH)D levels were measured, results concluded that almost half of the group (44%) were severely vitamin D

deficient (25(OH)D <12.5 nmol/L). Much earlier studies also indicate that vitamin D deficiency in preterm infants is not a new problem (Rosen, 1974; Salle, 1983).

There are significant gaps within the research regarding the vitamin D status of preterm infants, whilst there are a small number of studies that report the vitamin D status of these infants at birth; very few report their vitamin D status after hospital discharge and beyond. Furthermore, no studies have looked at the vitamin D status of preterm infants who are exclusively breastfed and do not receive vitamin D supplements. This group of preterm infants are those who will be at significant risk of vitamin D deficiency and urgent research is required to determine vitamin D levels in this group.

In New Zealand no studies have determined the vitamin D status of preterm infants after hospital discharge; the current study will be the first to document these levels. Vitamin D status in breastfed term infants has been established. A recent study by Wall et al. (2013) found that vitamin D deficiency (25(OH)D \leq 27.5 nmol/L) was prevalent in healthy term breast fed infants at 2-3 months of age. These infants received no supplemental source of vitamin D, which is consistent with current health policy in New Zealand, and had been exclusively breastfed until this age (Ministry of Health, 2013; NHMRC, 2006). Results showed 24% of these infants were vitamin D deficient (25(OH)D \leq 27.5 nmol/L). These levels are fairly consistent with an earlier study conducted in a cohort of 922 healthy term New Zealand infants (73% New Zealand European). Analysis of 25(OH)D levels in cord blood found that 19% of infants were vitamin D deficient (25(OH)D \leq 27.5 nmol/L) with a further 57% insufficient (25(OH)D \leq 50 nmol/L) (Camargo et al., 2010).

Prevalence of deficiency in term infants is abundant in several other countries (Agarwal, Faridi, Aggarwal, & Singh, 2010; Robinson, 2006; Jain, Gupta, Kalaivani, Sinha & Agarwal, 2011; Zeghoud et al., 1997). A large study in Sydney, Australia analysed vitamin D status of term neonates via cord blood and found that 144 (15%) were deficient, with 25(OH)D levels less than 25 nmol/L. Likelihood of deficiency was significantly associated with maternal deficiency, which correlated with maternal birth place outside of Australia and cultural clothing including wearing a veil. Dark maternal skin phototype measured using the Fitzpatrick scale was not associated with vitamin D deficiency (Bowyer et al., 2009). An earlier longitudinal study based in Sydney, Australia demonstrated 126 cases of rickets from 1993 to 2003 due to vitamin D deficiency. Of these cases 25% occurred in infants less than 6 months of age. Again, cases were primarily associated with infants and children of parents who had a birth place outside of Australia (Robinson, 2006).

In India vitamin D deficiency in infancy also appears to be widespread (Agarwal, Faridi, Aggarwal, & Singh, 2010; Jain, Gupta, Kalaivani, Sinha & Agarwal, 2011). Jain et al. (2011) looked at the vitamin D status of breastfed infants at 2.5-3.5 months of age, whilst a number of these infants received a vitamin D supplement of 10 μ g (400IU) per day, prevalence of deficiency (25(OH)D \leq 38 nmol/L) was still observed in 66.7% of the infants. Additionally 19.8% had insufficient levels (>38- \leq 50 nmol/L). Radiological rickets was also found in 30.3% of the infants with severe deficiency (25(OH)D <25 nmol/L). Agarwal et al. (2010) also showed significantly high levels of vitamin D deficiency in a cohort of 220 LBW and 119 normal birth weight term Indian infants at birth. Vitamin D deficiency was defined as levels lower than 38 nmol/L and was observed at similar levels in both groups; 87.3% of LBW infants and 88.6% of NBW infants.

Zeghoud et al. (1997) found that 63.7% of term neonates based in Northern France had insufficient (25(OH)D \leq 30 nmol/L) vitamin D status at the end of winter. A retrospective cohort study based in Boston Massachusetts analysed vitamin D status in 376 neonates and found that 58% were vitamin D deficient (25(OH)D \leq 50 nmol/L), 38% of which were severely vitamin D deficient (25(OH)D \leq 38

nmol/L). Highest levels of deficiency were associated with both black race and winter birth (Merewood et al., 2010).

Whilst such research provides a snapshot of the vitamin D status of term and preterm infants, a larger number of robust studies are required to determine whether sun exposure, feeding and supplementation practices in all infants are suitable to attain and/or maintain adequate vitamin D status in these infants from birth to beyond. Of particular importance is the vitamin D status of preterm infants who are likely to be at an even higher risk of deficiency due to decreased vitamin D accretion in *utero* (Tsang et al., 2005).

2.8 Consequences of Vitamin D Deficiency in Preterm Infants

Vitamin D deficiency and insufficiency are linked with several poor health outcomes including hypocalcemia, bone disease, decreased immune function and a subsequent increased risk of infection (Ross, 2011; Wagner, 2008). Epidemiological studies also suggest chronic vitamin D insufficiency during childhood is associated with a higher incidence of diabetes, multiple sclerosis and certain cancers in later life (Hypponen, Laara, Reunanen, Jarvelin & Virtanen, 2001). Whilst such findings allude to the possible pleiotropic actions of vitamin D, evidence is inconclusive and fails to show causality (Ross, 2011). Consequently, vitamin D deficiency levels currently set in New Zealand and Australia (deficiency $25(\text{OH})\text{D} \leq 27.5$ nmol and insufficiency ≤ 50 nmol) are lower than what some vitamin D experts recommend (NHMRC, 2006). Holick et al. (2011) suggests $25(\text{OH})\text{D}$ concentrations below 75 nmol/L should be considered deficient and Heaney (2008) suggests any value below 80 nmol/L should be considered deficient.

Hypocalcemia, raised serum alkaline phosphatase and secondary hyperparathyroidism are all recognised as markers of vitamin D deficiency (Bosley, Verrier-Jones & Campbell, 1980; Holick & Chen, 2008; Taylor, Wagner, Fanning, Quinones & Hollis, 2006).

2.8.1 Hypocalcemia

Hypocalcemia is a presenting symptom in infants with vitamin D deficiency, and is commonly associated with seizures and tetany (Zeghoud et al., 1997). Rare cases have also been associated with heart failure and death (Maiya et al., 2008; Navas-Carretero et al., 2008).

One study reported 19 cases of symptomatic neonatal hypocalcemia in term infants with severe vitamin D deficiency ($25(\text{OH})\text{D} \leq 12.5$ - 37.5 nmol/L), with more than half of the infants suffering from hypocalcemic seizures (Teaema & Al-Ansari, 2010). Another study showed that hypocalcemia was prevalent in 13 exclusively breast fed preterm infants with vitamin D deficiency (Balasubramanian, Shivbalan & Kumar, 2006). A more recent study also reported 4 cases of symptomatic hypocalcemia secondary to vitamin D deficiency. In a 4 month old male infant hypocalcemia was associated with cardiogenic shock (Pedrosa, Ferraria, Limbert & Lopes, 2013). Hypocalcemia has also been associated with infant heart failure and death in some infants (Maiya et al., 2008).

2.8.2 Infection

During infancy insufficient vitamin D intake has been associated with an increased risk of infection and increased morbidity rates (Camargo et al., 2011; Leis et al., 2012; Mohamed & Al-Shehri, 2013).

Respiratory infections are one of the most common causes of illness and hospital admissions in infancy (Statistics New Zealand, 2007). Recent evidence suggests there may be an association between vitamin D deficiency and increased occurrence of acute lower respiratory infection (ALRI) (Camargo et al., 2011; Leis et al., 2012; Mohamed & Al-Shehri, 2013).

A follow up study in a cohort of 922 New Zealand term infants showed there was an inverse association between cord blood 25(OH)D status and risk of respiratory infection at 3 months of age; as well as an inverse association with wheezing at 15 months, 3 and 5 years of age (Camargo et al., 2011). Leis et al. (2012) found that infants and children with a vitamin D intake of less than 2µg (80 IU) per kilogram per day were significantly more likely to suffer from ALRI in comparison to those with intakes above this. Low 25(OH)D levels in cord blood have been associated with increased risk of ALRI in the first two years of life (Mohamed & Al-Shehri, 2013). Another study concurred with these results and concluded there was up to a six fold increased risk of developing ALRI in infants with 25(OH)D levels less than 50 nmol/L (Belderbos et al., 2011). Conversely, McNally et al. (2009) concluded that there was no significant difference in the vitamin D levels of young children with ALRI compared to controls. However, authors did conclude that vitamin D deficiency was significantly associated with increased admissions into a paediatric intensive care unit.

The Delhi Infant Vitamin D Supplementation (DIVDS) study involved supplementing 2079 term LBW infants with 35µg (1400IU) of vitamin D per week from birth to 6 months. Authors concluded that whilst supplementation improved vitamin D status and bone health of infants, there was no association with hospital admissions or death at 6 months of age (Trilok-Kumar, Sachdev, Chellani, Rehman, Singh, Arora & Filteau, 2011). In addition, another study involved analysing blood samples taken from the infants who participated in the DIVDS study; authors concluded that there was no association between vitamin D status and immunomodulating markers including plasma C-reactive protein (CRP), tumour necrosis factor-α (TNFα), interferon-γ (INFγ), interleukin 10 (IL-10) or IL-13 after 6 months of supplementation (Trilok-Kumar, Aroroa, Raiput, Chellani, Singh, Raynes, Arya, Aggarwal, Srivastava, Sachdev & Filteau, 2012).

2.8.3 Bone Health

Chronic vitamin D deficiency is the primary cause of rickets in infancy. The incidence of rickets peaks at 3-18 months of age (Ministry of Health, 2013). Rickets results in deformation of growing bones, and causes bone pain, knocked knees, bow legs, cranial bossing, enlarged wrist joints, anterior bowing of the femur, dental anomalies, hypotonia and delayed walking (Soliman et al., 2010).

Metabolic bone disease of prematurity is a multifactorial disease resulting in inadequate bone mineralisation; progression of which results in rickets (Brooke, 1985). However, unlike rickets, metabolic bone disease of prematurity is not primarily associated with vitamin D deficiency. Instead calcium and phosphorous deficiency play a more integral role in the progression of the disease (Brooke, 1985). Interruption of placental - fetal mineral transfer, namely of calcium, phosphorous and vitamin D, as well as higher requirements at birth puts preterm infants at a higher risk of developing metabolic bone disease, and subsequently a higher risk of developing rickets in later infancy (Bosley et al., 1980; Brooke, 1985; Pieltain, de Halleux, Senterre & Rigo, 2013). The risk of which is exacerbated even further with inadequate mineral supplementation (Bosley et al., 1980; Pieltain et al., 2013). Therefore vitamin D provision is still a crucial element in the treatment of metabolic bone disease of prematurity (Brooke, 1985).

2.8.4 Autoimmune Disease and Cancer

Vitamin D deficiency during infancy and childhood has been associated with an increased risk of certain autoimmune diseases during later life. Links were made early on in those living at higher latitudes where the cutaneous synthesis of vitamin D is limited. Evidence indicates that these individuals are at a greater risk of suffering from Crohn's disease, multiple sclerosis and type 1 diabetes mellitus (T1DM) (Holick, 2006a; Holick, 2008).

It is suggested that the presence of VDRs in nearly all cells of the body are responsible for the possible therapeutic effects of vitamin D. For example VDRs are found in pancreatic beta cells, and are thus thought to play a fundamental role in promoting beta cell insulin secretion and helping prevent the development of T1DM. The Northern Finland Birth cohort followed infants from birth through to adulthood; the relative risk (RR) for developing T1DM was significantly higher for those who were suspected to have rickets and did not receive vitamin D supplements versus those without suspected rickets who did receive supplements (3.0 versus 0.22 respectively) (Hypponen, Laara, Reunanen, Jarvelin & Virtanen, 2001). Conversely in the same cohort of infants vitamin D supplementation was associated with an increased risk of asthma, allergic rhinitis and atopy during adulthood (Hypponen et al., 2004). A meta analysis of 10 studies concluded that vitamin D supplementation significantly reduced the risk of developing T1DM compared to no supplementation (Zipitis, 2008). Whereas the ABIS study found no association between vitamin D supplementation during early infancy and the development of diabetes related auto antibodies at 1 and 2.5 years of life (Brekke, 2007).

It has been suggested that vitamin D sufficiency may reduce cancer risk (Garland, 2006). The mechanisms thought to produce these beneficial effects include the presence of VDRs in certain cells. A systematic review of 63 observational studies indicated that vitamin D sufficiency was significantly associated with reduced incidence of certain cancers including colon, breast, prostate and ovarian cancer (Garland, 2006). Evidence for the association of vitamin D deficiency during infancy and incidence of cancer in later life however is limited.

Whilst evidence suggests that vitamin D sufficient states and supplementation are beneficial for reducing the risk of a range of diseases, more evidence is required before a causal relationship can be confirmed. Such a relationship requires long term, high quality, randomised controlled trials in order to determine the supplementation dose and duration that is most beneficial without any undue side effects.

2.9 Vitamin D Toxicity

As with deficiency and sufficiency states, safe upper ranges of vitamin D also vary within the literature. The NHMRC, IOM and AAP define the upper level of vitamin D as 25(OH)D higher than 125 nmol/L, suggesting that levels above this may be associated with adverse affects (NHMRC, 2006; Ross, 2011; Wagner, 2008). However, levels at which symptoms of toxicity occur are highly variable. Holick & Chen suggest (2008) that vitamin D toxicity does not occur until 25(OH)D levels reach 375 nmol/L and above.

Hypercalcemia and hypercalciuria can both develop from vitamin D toxicity states; clinical symptoms of which can include faltering growth, polyuria and ectopic calcification (Tsang et al., 2005). Toxic levels with associated clinical symptoms are rarely observed with current prescribed levels of vitamin D. Even high levels recommended by ESPGHAN of up to 25µg (1000IU) of vitamin D per day have not been associated with toxicity (Agostoni, 2010; Markestad, 1987). Pharmacological doses with levels greater than 250µg (10,000IU) per kilogram per month have been associated with toxicity (Markestad, 1987). One study provided infants with different supplementation doses for the treatment of vitamin D deficiency induced rickets. Whilst a one off dose of 3750µg (150,000IU) was associated with improved symptoms and no toxicity, doses of 7500µg (300,000IU) and 15000µg (600,000IU) resulted in hypercalcemia (Cesur, Caksen, Gundem, Kirimi & Odabas, 2003).

Caution with high supplementation dose is warranted due to the storage of vitamin D in adipose tissue, as well as the severe side effects associated with toxic levels (Agostoni, 2010; NHMRC, 2006; Ross, 2011; Wagner, 2008).

According to the literature, current prescribed doses of vitamin D in preterm infants in Auckland City Hospitals NICU are within safe and recommended ranges (Agostoni, 2010; IOM, 2011; Tsang et al., 2005; Wagner, 2011; Holick & Chen 2008). However, determination of the vitamin D status of infants receiving supplemental doses of vitamin D is necessary to determine if the current dose maintains serum 25(OH)D concentrations within the current recommended range.

2.10 Conclusions

There are significant gaps within the research regarding the vitamin D status of preterm infants in New Zealand. Whilst there are a small number of studies that report the vitamin D status of these infants at birth; very few report their vitamin D status after hospital discharge. In addition there is a large knowledge gap on the sun exposure behaviours of preterm infants. Considering the recommendations for infants to be kept out of direct sunlight and utilise protective clothing, sunscreen and shady areas it seems extremely unrealistic to rely on sun exposure as the major source of vitamin D in this group. In addition if infants are exclusively breastfed until 6 months of age as recommended, their risk of being vitamin D deficient is high (Amukele et al., 2013; Holick, Binkley, Bischoff-Ferrari, Gordon, Hanley, Heaney, Murad & Weaver, 2011; Thiele, Senti & Anderson, 2013).

The aim of the current study is to determine the vitamin D status of preterm infants at 4 months post hospital discharge and to investigate the factors affecting status.

Chapter 3.0: Methods

3.1 Study Design

The Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge is a longitudinal observational study being conducted in Auckland, New Zealand. The observational study is run by the Human Nutrition Research Unit at Massey University in Auckland in collaboration with Professor Frank Bloomfield (specialist neonatologist) and Barbara Cormack (neonatal/paediatric dietitian) at Auckland City Hospital. The aim of the observational study is to assess micronutrient status of preterm babies at 4 months post hospital discharge and then to follow the babies up at 6, 9 and 12 months corrected age to assess feeding practices over the first year of life. This thesis will present a situation analysis of the vitamin D status of preterm babies at 4 months after hospital discharge and the factors which influence vitamin D status including mode of feeding, skin colour and sun exposure practices and any supplementation practices.

3.2 Ethical Approval

Massey University Ethics Committee

Ethical approval was granted from Massey University Human Ethics Committee (MUHEC) in February 2013, reference: HEC: Southern A Application 13/06, post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge.

Auckland District Health Board Research Committee

Research committee approval was obtained from Auckland District Health Board Ethics Committee in February 2013, reference: A+5810, Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge.

3.3 Setting

Auckland City Hospital

Participants were recruited from Auckland City Hospitals neonatal intensive care unit (NICU). Auckland City Hospital is located in central Auckland, New Zealand. Auckland City Hospital provides intensive care services to neonates from Central, North and West Auckland as well as Northland. It has forty six cots, of which sixteen provide level 3 intensive care services, twenty level 2 high dependency spaces and ten level 2 low dependency spaces. An estimated nine hundred neonates are admitted to the unit annually (Auckland District Health Board, 2013). More information about Auckland City Hospital NICU can be found on their website: <http://www.adhb.govt.nz/newborn/>.

3.4 Population

The population in the current study consists of preterm infants (born at less than 37 weeks gestation) at 4 months post hospital discharge who were previously admitted into Auckland City Hospitals NICU from October 1 2012 to April 30 2013.

3.5 Power Calculation

Population number required for statistical significance was determined as a part of the larger study; "Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge".

This required specifically analysing the numbers of preterm infants required to determine both vitamin D and iron status in a cohort. The available data on vitamin D status of preterm infants at approximately 3 months of age indicated that a sample of 32 infants was required to determine the proportion of infants that are vitamin D deficient ($\leq 25\text{nmol/L}$) (McCarthy et al., 2013). As a part of the study there was a plan to construct separate models for different categories of infants based on gestational age (≤ 32 weeks gestation versus $>32 - <37$ weeks gestation) and from this it was determined that at least 22 infants from each category would be required. Thus a total of 44 infants being the minimum requirement for multiple regression to be achieved (Faul, 2007). Therefore the number required to meet the current studies objectives was 44 infants. However as this was a part of a larger study the number required to meet the entire study's objectives was 76 infants, with the aim to recruit 100 preterm infants over a 12 month period, which would also allow for an attrition rate of 30%.

3.6 Consultation with Health Professionals at Auckland Hospital prior to Recruitment

Prior to recruitment all nurses, dietitians and other medical staff at Auckland City Hospital NICU were informed about the study via a PowerPoint Presentation.

The PowerPoint presentation was developed with a voice over, which allowed it to be viewed by health professionals at their convenience. The presentation provided a detailed explanation of the study, including recruitment procedures, timeframe and the importance of carrying out this research.

3.7 Recruitment

Retrieving Contact Details

Contact details including a unique National Health Index number (NHI), name, telephone number and address were retrieved from Auckland City Hospital NICU log book by the Ward Clerk. Patient labels of all surviving infants born at less than 37 weeks gestation and discharged out of the NICU from October 1 2012 to 30 April 2013 were provided to the lead researcher at Massey University.

Inclusion Criteria

The inclusion criteria followed:

- All infants with a gestational age of <37 weeks
- Living in a home (community) setting at time of participation in the study.
- Being as close to 4 months post hospital discharge at time of participation in the study
- Being located in the Auckland region (as far North as Whangaparoa, and as far South as the Bombay Hills).

Exclusion Criteria

The exclusion criteria followed:

- Any preterm infant requiring on-going specialist inpatient paediatric care.

All preterm infants who met the inclusion criteria and had been admitted in to Auckland City Hospitals NICU for any length of stay and their mothers were invited to participate in the study. This included preterm infants that were discharged out of the NICU home, and also to other wards within

Auckland City Hospital as well as other hospitals in Auckland, including Waitakere, North Shore and Middlemore hospitals.

Contacting Potential Participants

All mothers of infants were contacted in batches, with the mothers of infants who were discharged earliest (October 2012) being contacted first. Researchers sent out an information sheet (Appendix 1), contact letter (Appendix 2) contact details sheet (Appendix 3) and a freepost envelope. This gave parents the opportunity to learn more about the study and send in their contact details if they were interested in taking part.

Massey University researchers then proceeded to follow all letters with a phone call. This allowed us to address any questions, and discuss the study in more detail if necessary. Verbal consent was obtained in by telephone during recruitment from the mothers for themselves and their infants to participate in the study. Provided participants met the inclusion criteria and verbal consent was obtained, the demographics questionnaire was then completed (Appendix 7). At this stage participants were also booked in to be seen at as close to 4 months post hospital discharge as possible. The standard operating procedure (SOP) followed for the recruitment process can be found in Appendix 6.

Consent for Participation in the Study

As well as gaining verbal consent, two written consent forms were provided to mothers at the home visits, one was to obtain consent for the infant to take part in the study, and this was required to be filled out by the mother (Appendix 4). A second written consent form was provided to all mothers who were breastfeeding at the time of home visits; this provided consent from them to provide a sample of their blood for analysis (Appendix 5).

3.8 Booking in Visits

Researchers scheduled home visits with mothers and infants at as close as possible to 4 months post hospital discharge.

3.9 Home Visits

All visits took place at the participant's home. The paediatric trained phlebotomist and MSc Nutrition and Dietetic student conducted these.

Home visits were approximately 1 hour in duration for 1 infant.

The schedule followed at the home visits can be found in Appendix 8.

3.10 Data Collection

3.10.1 Questionnaires

There were 4 questionnaires in total. These included the demographics questionnaire (Appendix 7), sun exposure questionnaire (Appendix 10), supplement questionnaire (Appendix 11) and the micronutrient and feeding practices questionnaire (Appendix 12). The demographics questionnaire was interviewer administered at time of booking participants in and the others were self completed by the mother prior to home visits

3.10.1.1 Administration of Online Questionnaires prior to Home Visits

Pilot testing the questionnaires was initially carried out at home visits to determine acceptability by the mothers. Mothers commented that these would be easier to fill out at their own convenience. It was therefore decided to provide all questionnaires on Survey Monkey. Providing questionnaires online provided an elevated level of quality control. The programme has several features which ensure the questionnaires are completed. If a question is unanswered the programme prompts the user to answer this before being able to move onto the next question, thus ensuring all answers have a response. The programme also provides survey skip logic which skips sections that are not relevant to the user.

Online questionnaires as well as being shown to provide more accurate results also reduce interviewer bias which can be introduced when questionnaires are interviewer administered (Wright, 2006). Questionnaires could also be answered at the participant's convenience. Completing these in private may have also allowed them to provide information that they may not have been comfortable conveying in person.

Researchers also had access to all questionnaire responses. This allowed researchers to ensure these were accurately filled out prior to home visits. Any data that was missing, or was not clear to the researcher was clarified with the mother. Any mother that did not have access to the online questionnaires completed these at the home visits.

3.10.1.2 Demographics Questionnaire

The demographics questionnaire (Appendix 7) consisted of 9 questions. It was designed to determine the characteristics of the population being studied and ensure they met the inclusion criteria. Questions regarding the infant included gestational age, gender, ethnicity, hospital discharge date and hospital venue. Questions regarding the mother included DOB, ethnicity and parity. This questionnaire was interviewer administered at the time of recruitment.

3.10.1.3 Supplement Questionnaire

The supplement questionnaire (Appendix 11) consisted of 11 questions. It was designed to determine supplementation practices followed in infants after hospital discharge and at time of participation in the study.

Questions included; whether the infant was discharged home from hospital with any supplements and if so what these supplements were and the dose that was given, whether the dose of these supplements had changed since the infant was discharged from hospital and if so what the dose of these supplements currently was, after discharge whether these supplements were given to the infant daily or other, if there were any barriers or issues experienced with giving the infant supplements daily, whether these supplements were still being given to the infant, and if not when and why were these stopped.

This questionnaire provided prompts for supplements including Vitadol C, however also provided prompts for 'other' supplements that the mother may have chosen to give the infant.

3.10.1.4 Sun Exposure Questionnaire

The sun exposure questionnaire (Appendix 10) consisted of 7 questions, 2 of which were further broken down into 5 questions each. The sun exposure questionnaire was developed to determine sun exposure behaviours in infants and in the mothers if they were breastfeeding.

Initial questions included current season, whether the mother had received any advice on sun exposure for her infant since hospital discharge and if so what this was and who gave the advice.

Infant Related Questions

Questions including how often is your infant exposed to sunlight, do you apply sunscreen to your infant before going in the sun, do you put a hat or protective clothing on your infant when in the sun and do you usually keep your infant in the shade were asked.

Questions Related to the Breastfeeding Mother

The same questions detailed above were then required to be answered by all breastfeeding mothers. In addition mothers were also asked whether they wore covering such as a veil/burka/other for cultural/religious/other reasons. This was important to determine as such clothing dramatically reduces sun exposure.

3.10.1.5 Micronutrient and Feeding Practices Questionnaire

The micronutrient and feeding practices questionnaire (Appendix 12) consisted of 22 questions. Only those questions relevant to the vitamin D status of the infant are discussed here.

Infant Related Questions

The questionnaire specifically determined what and how the infants were fed when discharged from hospital as well as what they were currently being fed at time of participation in the study. The questionnaire also asked if any fortifiers were added to the infant's feeds.

Questions Related to the Breastfeeding Mother

The questionnaire also had questions specific to mothers that were breastfeeding or expressing breast milk at the time of participation in the study. Questions were to help determine whether the mother was vitamin D deficient, and whether she was currently taking any supplements including vitamin D, Elevit, multivitamins or other.

3.10.2 Blood Sampling and Analysis of 25(OH)D

Infant and maternal blood samples (if applicable) were obtained during home visits.

3.10.2.1 Infant Blood Sample Collection

Capillary blood was taken from infants using the heel prick method by the paediatric trained phlebotomist (SOP; Appendix 17).

Prior to the procedure mothers had been asked to keep the infants feet warm to increase blood flow. Both prior to the procedure and during, massage and gentle squeezing of the foot was also important to encourage blood flow. The outer side of the infant's heel was pricked using a sterilised lancet. The first drop of blood was wiped away to ensure the collected blood was not contaminated with tissue fluid, the remaining drops were collected. It was also ensured that no more than very gentle pressure was applied to ensure that excess discomfort was not experienced by the infant and to ensure that that sample collected was not diluted by tissue fluid. Approximately 10-15 drops of blood equating to 600µL was required for accurate sample analysis. Blood was collected in a gold capped pre labelled collection tube that contained an additive to prevent clotting. Blood samples were stored upright in a cool (approximately 4-8°C) polystyrene box for safe transporting. The box was kept cool with the use of a small ice pack and was kept out of direct light at all times. The

samples were transported back to Massey University's Human Nutrition laboratory for processing within 2 hours.

3.10.2.3 Maternal Blood Sample Collection

If the mother was breastfeeding at the time of the visit she was eligible to provide a blood sample for the analysis of 25(OH)D.

A venous blood sample was taken from the mothers by a trained phlebotomist (SOP; Appendix 18). The participant was prepared by seating them in an upright position; a venous occlusion tourniquet was placed on the mothers arm just above the elbow and fastened. An appropriate vein was selected and venipuncture was performed, 10ml of blood was collected into a gold capped pre-labelled collection tube. This was stored upright in a cool (approximately 4-8°C) polystyrene box for safe transporting along with infant samples.

3.10.2.4 Blood Sample Processing

Following home visits infant and maternal blood samples for 25(OH)D analysis were delivered promptly to the Massey University Human Nutrition laboratory for processing (SOP Appendix 19).

Maternal and infant samples were processed separately due to different collection volumes; however the same method was used. The 'Labofuge 400' centrifuge machine was used to separate samples. Samples were spun at 3000rpm for 10 minutes at room temperature. Serum aliquots were then pipetted into low binding eppendorf tubes, pre-labelled with the participants identification code. Low binding eppendorf tubes were used to maximise sample recovery as these significantly reduce sample to surface binding.

Maternal and infant samples were separated and organised by date and ID into labelled polystyrene containers and placed in a -80°C freezer in the human nutrition laboratory at Massey University. At the end of the data collection process in August 2013 all samples were checked to ensure they had an appropriate ID number, and to ensure they were in date order to match the inventory sheet. Once checked all eppendorf tubes were placed into polystyrene containers containing dry ice to ensure they remained frozen. Inventory and sample delivery forms were attached and samples were then transported (frozen) promptly to Waitemata District Health Boards (WDHB) laboratory services located at North Shore Hospital in Takapuna, Auckland. The SOP for preparation to send samples for batch analysis can be found in Appendix 20 and the blood sample delivery form can be found in Appendix 21.

3.10.2.5 Analysis of 25(OH)D

Batch analysis of maternal and infant blood samples were carried out by a trained laboratory technician at WDHB. WDHB laboratory is IANZ accredited; this recognises the technical competence of the laboratory.

The ADVIA Centaur Vitamin D Total assay was used in the quantitative determination of total 25(OH)D (25(OH)D₂ and 25(OH)D₃), using the ADIVA Centaur XP systems (SOP Appendix 22). The ADIVA Centaur Vitamin D Total assay is a one pass, eighteen minute competitive immunoassay that uses an anti-fluorescein monoclonal mouse antibody covalently bound to paramagnetic particles, an anti-25(OH)D monoclonal mouse antibody labelled acridinium ester and a vitamin D analogue labelled with fluorescein.

Bloods were thawed and mixed thoroughly prior to analysis. Prior to the assay procedure the samples were checked to ensure they were free of fibrin, other matter and air bubbles, if present a second centrifugation was carried out.

For the analysis of 25(OH)D a minimum 150µL serum sample was necessary, this required at least 600µL of blood to be obtained. Upon batch analysis if there was significantly less than 150µL of serum the laboratory technician diluted such samples in accordance with the recommendations of the ADVIA Centaur Vitamin D Total assay procedure (SOP Appendix 22) and analysed these if possible.

The assay measures 25(OH)D concentrations from 10.5-375 nmol/L. Any 25(OH)D results greater than 375 nmol/L are checked and retested. Coefficients of variation of the assay range with the differing 25(OH)D concentrations; 25(OH)D concentration of 29 nmol/L has a total CV% of 11.1, a 25(OH)D concentration of 139 nmol/L has a total CV% of 7.8. The functional sensitivity of the vitamin D assay is 8.33 nmol/L. The vitamin D assay is linear from 10.5-375 nmol/L and has a high specificity for 25(OH)D₂ (cross reactivity % (CR%) 104.5) and 25(OH)D₃ (CR% 100.7).

One limitation of this assay method was any samples that were grossly haemolysed could not be analysed, as a false reading would be produced. Therefore 25(OH)D results were not reported by the laboratory for any samples that were grossly haemolysed. For a complete description of the assay method and procedure refer to Appendix 22.

3.10.2.6 Definition of Vitamin D Status

To determine the number of infants and mothers classified as vitamin D deficient or insufficient National Health and Medical Research Council (NHMRC) recommended values were used. According to these values vitamin D deficiency was defined as 25(OH)D \leq 27.5 nmol/L, insufficiency was defined as 25(OH)D \leq 50 nmol/L and sufficiency was defined as $>$ 50- \leq 125 nmol/L. The upper level included any value above 125 nmol/L (NHMRC, 2006).

Further classification was also done based on values recommended by Holick (2011) according to these values vitamin D deficiency was defined as 25(OH)D \leq 75 nmol/L and vitamin D sufficiency was defined as \geq 75 nmol/L.

3.10.3 Anthropometric Measurements

All anthropometric measurements were completed by a paediatric trained phlebotomist and trained researcher at the home visits. Standardised operating procedures were followed for all measurements to ensure accuracy (Appendix 14, 15 and 16).

3.10.3.1 Infant Weight

Infant weight was obtained by the researchers at home visits using ATRONICS SBB-003 Low Profile Digital Baby Scale (SOP; Appendix 14).

The mother was asked to undress the baby removing all clothes, accessories and nappy. However if the nappy was dry and was left on this was adjusted for by weighing it, and subtracting this from the final measure. The scales were zeroed and the infant was placed in the middle of these. To ensure accuracy 3 readings were taken and averaged to the nearest 0.1g. Once the measurement was complete the infant was redressed by the mother. In the case of twins or triplets the same process was repeated.

3.10.3.2 Infant Length

Infant length was obtained by the phlebotomist and trained researcher at home visits using an age appropriate length board (SOP; Appendix 16).

The infant was placed gently on its back onto the base of the length board. Any accessories that may have interfered with an accurate measurement were removed. The base of the infants heels were placed so that they touched the bottom edge of the length board. The infant was adjusted to ensure correct positioning, slight pressure was put onto the infants knees to assist with extending the legs. The head piece of the length board was then moved down to firmly touch the infants head ensuring that the vertical line from the infants' ear canal to the lower border of the eye socket was perpendicular to the horizontal board, this is known as the Frankfort Vertical Plane. To keep the infant's head in the correct position, the assisting measurer gently cupped his or her hands over the infant's ears. During measurements the mother was encouraged to stand close on the side to reassure the infant which helped ensure the infant lay still thus allowing a more accurate measurement. The average of 3 readings was recorded to the nearest 1mm.

3.10.3.3 Infant Head Circumference

Infant head circumference was obtained by the researchers at home visits using an age appropriate flexible plastic measuring tape (SOP; Appendix 15).

The infant was held by the mother in a comfortable position whilst the researcher proceeded with the measurement. The tape was wrapped around the widest part of the infants head; just above the infants eyebrows, ensuring it was positioned at the fullest protuberance of the skull at the back of the head. Once in the correct position the tape was gently fastened, and the reading was taken to the nearest 1mm. The average of 3 readings was recorded.

3.10.4 Fitzpatrick Skin Type Scale

The Fitzpatrick Skin Type Scale was used on all infants and all mothers at home visits. The scale allowed us to determine participant's skin colouring (The Skin Cancer Foundation, 2013). The pictorial scale was held to the inner part of the forearm of the participant, this allowed the comparison of colours. The definition of each skin type was also taken into consideration when determining skin type.

The scale defines 6 different skin types; determined by colour and certain characteristics including time the skin takes to burn when exposed to sunlight. Skin types 1-3 refer to very fair to moderately fair skin. Skin types 4-6 refer to moderately brown to very brown skin. Skin types 1-3 burn quickly when exposed to sunlight, whereas skin types 4-6 rarely burn and usually tan (The Skin Cancer Foundation, 2013). The complete description of skin types 1-6 along with the pictorial scale used can be found in section 2.3.4 and Appendix 23.

3.10.5 Data Collection from Medical Notes

Medical information of infants and mothers were obtained from the Auckland City Hospital database, where medical charts, biochemistry data and inpatient notes were accessed. The data collection sheet for medical notes can be found in Appendix 24. Informed consent was provided for this by all mothers who participated.

Medical information collected included a total of 25 questions. The first 8 questions were in relation to the infant's birth, including DOB, gestational age, weight, length and head circumference at birth, type of delivery, reason for preterm birth and whether the baby was a single, twin or other. The next

3 questions were regarding inpatient medical data including whether the infant was admitted to the NICU and if so duration of their stay, whether the infant had any medical complications after birth and if so what these were and the date of discharge from hospital. Twelve questions were in relation to the infants feeding and supplement use whilst in hospital and at discharge. Questions included whether the infant received parenteral nutrition and if so for how long, whether enteral nutrition was received and if so how long for and what this was as well as if there was any fortification. Whether the infant received any supplements during hospital stay including Vitadol C or 'other' and if so the date the supplements were started and the dose that they were provided at. Other questions included feeding type, method (parenteral/enteral/oral) and supplement use and dose at hospital discharge. The remaining 2 questions were in relation to the mother, and included whether she had any complications during pregnancy and if so what these were, and whether the mother was a smoker or not.

3.11 Statistical Analysis

Statistical analyses were performed using SPSS package version (SPSS Inc., Chicago, IL, USA). All variables were tested to determine if they were normally distributed using Kolmogorov-Smirnov and Shapiro-Wilk tests together with examining Normal Q-Q box and stem and leaf plots. Infant vitamin D concentrations were log transformed to determine geometric mean and 95% confidence intervals.

Feeding groups were classified as: exclusively breastfed with supplements, exclusively breastfed without supplements, formula fed with supplements and formula fed without supplements. Exclusively breastfed refers to the provision of breast milk only, however, if infants had been given water they were still included within this group. The infants who were formula fed with and without supplements contained some infants who were being given breast milk occasionally.

Change in anthropometric measurements of infants from birth to appointment was determined by calculating Z scores. United Kingdom – World Health Organisation (UK-WHO) data were used to calculate these. Four groups were defined; infants who received Vitadol C supplements versus those who did not and infants who were vitamin D sufficient versus those who were vitamin D insufficient. The difference in Z scores between groups were determined via Independent Student t-tests and reported as *P* values.

Factors affecting 25(OH)D concentrations in infants were determined using Wilcoxon ranked-sum tests and Mann-Whitney tests for non-parametric data. A Bonferroni correction was applied so all effects are reported at a 0.008 level of significance. Spearman's correlation coefficients were calculated for non-parametric data to assess the correlation between maternal and infant 25(OH)D concentrations. Chi square and Independent student t tests were used to determine significance between variables in supplemented versus non supplemented groups. All non-normally distributed variables are presented as median, 25th and 75th percentiles and range. Normally distributed variables are presented as mean, standard deviation and range. Log transformed data are presented as mean and 95% confidence intervals. Categorical variables are presented as frequencies and percentages. A *P* value of <0.05 was used to determine significance when comparing groups.

3.12 Dissemination of Results

A letter with the summary of results including 25(OH)D concentrations for the infant and mother (if available) and anthropometric measurements of infants taken at appointment were sent to the mother. If 25(OH)D concentrations were outside of normal ranges mothers were informed in the letter and a letter was also sent to their GP. Letter templates for maternal and infant results as well as the letter to GP can be found in Appendix 25, 26 and 27. A summary of the overall findings of the study will also be sent out to all participants. In addition the results will be presented at the 2013 Vitamin D symposium at Massey University, Albany and will be published in a peer reviewed journal.

Chapter 4.0 Results

4.1 Description of Participants

The recruitment of preterm babies to this study and the final number of participants for whom a blood sample was available for 25(OH)D analysis is shown in Figure 4.1. Infant characteristics are summarised in Table 4.1. The mean \pm SD gestational age of the infants was 33 weeks and 5 days (236 days \pm 20.6).

At four months after hospital discharge the mean \pm SD corrected age of infants was 99 \pm 16.07 days. All infants, except 2 were seen in winter.

The geometric mean (95% CI) serum 25(OH)D concentration of the infants at four months after discharge was 73.8 (60.2, 90.4) nmol/L. The 25(OH)D range was 16 nmol/L to 314 nmol/L.

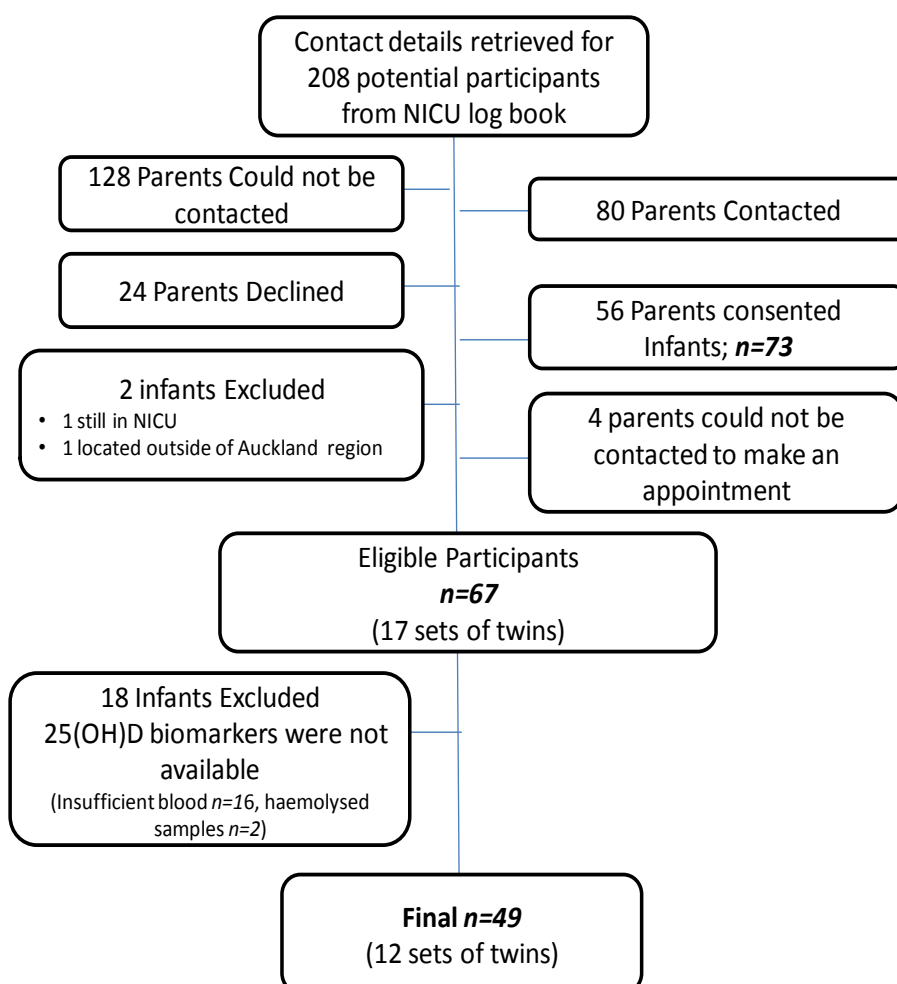


Figure 4.1: Flow Diagram of Recruitment and Final Number of Participants

Table 4.1: Characteristics of Participants (n=49)

Characteristics		
Gender, n (%)		
Male		27 (55.1)
Female		22 (44.9)
Single Birth, n (%)		
Twin Birth, n (%)		
Ethnicity, n (%)		
European		24 (49)
Maori		3 (6.1)
Pacific		6 (12.2)
Asian		6 (12.2)
Indian/Fiji Indian		6 (12.2)
Other		4 (8.3)
Gestational Age (days)		
Mean ± SD (range)		236 ± 20.58 (170-258)
≤32 weeks, n (%)		11 (22.5)
>32 weeks, n (%)		38 (77.5)
Birth Weight (kg)		
Mean ± SD (range)		1.9 ± 0.66 (0.7-3.7)
≤1800g, n (%)		21 (42.9)
>1800g, n (%)		28 (57.1)
Birth Length (cm)		
Mean ± SD (range)		44.0 ± 4.40 (31-54)
Birth Head Circumference (cm)		
Mean ± SD (range)		31.0 ± 3.08 (22.0-37.0)
Time Since Hospital Discharge (days) Mean ± SD (range)		
Corrected Age at Appointment (days) Mean ± SD (range)		
Age in days at Appointment (days) Mean ± SD (range)		
Mode of Feeding at 4 months after Discharge ^c		
Breastfed, n (%)		27 (56.2)
Formula Fed, n (%)		21 (43.8)
25(OH)D (nmol/L)		
Mean, Confidence Interval, (range) ^ϕ		73.8, 60.2-90.4, (16-314)

^cMode of feeding missing for one infant, ^ϕLog transformed data

4.2 Vitamin D Status of Infants

The Vitamin D status of the infants based on cut off values routinely used in New Zealand and Australia to define deficiency, insufficiency, sufficiency and upper levels are presented in Table 4.2. Based on these classifications 28.6% of babies have insufficient vitamin D status.

Table 4.2: Classification of Vitamin D Status in Infants^Y (n=49)

Classification	25(OH)D (nmol/L)	n	%
Severely Deficient	<12.5	-	-
Mild –Moderate Deficiency	>12.5 - ≤25	4	8.2
Insufficient	>25 - ≤50	10	20.4
	>50 - ≤75	6	12.2
Sufficient	≥75 - ≤125	20	40.8
Upper Level	>125	9	18.4

^Y25(OH)D cut off values are based on values routinely used in New Zealand and Australia (NHMRC, 2006)

4.3 Characteristics of Participants According to Supplement Use

Of the 49 infants included in this study, 25 infants received Vitadol C supplements when discharged home. Infant characteristics according to supplementation group are shown in Table 4.3. All of the infants who received Vitadol C supplements met the age and weight criteria for supplementation (<32 weeks gestation and/or <1800g at birth). Additionally 6 infants who did not meet the criteria received Vitadol C supplements. Two infants who were <1,800 g at birth and who therefore met the criteria for Vitadol C supplementation did not receive Vitadol C supplements; all other infants who did not receive supplements did not meet the criteria for supplementation.

Table 4.3: Description of Participants according to Supplement Use (n=48[†])

	Received Vitadol C Supplements n=25	Did not Receive Vitadol C supplements n=23	P value*
Gender, n (%)			
Male	8 (32)	18 (78.3)	0.0001
Female	17 (68)	5 (21.7)	0.0001
Single Birth, n (%)	13 (52)	11 (47.8)	0.772
Twin Birth, n (%)	12 (48)	12 (52.2)	0.772
Ethnicity, n (%)			
European	11 (44)	13 (56.5)	0.384
Maori	2 (8)	1 (4.3)	0.603
Pacific	2 (8)	4 (17.5)	0.327
Asian	4 (16)	1 (4.3)	0.187
Indian/Fiji Indian	4 (16)	2 (8.7)	0.447
Other	2 (8)	2 (8.7)	0.928
Birth Weight (kg)	1.5 ± 0.44 (0.70-2.3)	2.5 ± 0.51 (1.5-3.7)	0.0001
Mean ± SD (range)			
≤1800g, n (%)	19 (76)	2 (9.5)	0.0001
>1800g, n (%)	6 (24)	21 (90.5)	0.0001
Gestational Age (days)	224 ± 21.89 (170-254)	249 ± 8.0 (231-258)	0.0001
Mean ± SD (range)			
≤32 weeks, n (%)	11 (44)	0	
>32 weeks, n (%)	14 (56)	23 (100)	0.0001
Birth Length (cm)	41.3 ± 3.90 (31.0-47.0)	46.9 ± 2.80 (43.0-54.0)	0.0001
Mean ± SD (range)			
Birth Head Circumference (cm)	29.3 ± 2.80 (22.0-33.0)	33 ± 1.99 (30.0-37.0)	0.0001
Mean ± SD (range)			
Time Since Discharge	116.7 ± 13.30 (93.0-163.0)	119 ± 15.60 (102.0-163.0)	0.978
Mean ± SD (range)			
Mode of feeding, n (%)			
Breastfed	13 (52)	14 (60.9)	0.535
Formula Fed	12 (48)	9 (39.1)	0.535
25(OH)D (nmol/L)	110 (93-142)	37 (27-71)	0.0001
Median (25 th -75 th percentiles)			

[†]Data missing for one infant, *Significant differences between supplemented and non-supplemented (P<0.05) (Independent Student t-test, Mann-Whitney Test and Pearson's chi-square)

4.4 25(OH)D Concentration of Infants According to Birth Weight and Gestational age Categories by Supplement Use

The median (25th-75th percentile) serum 25(OH)D concentration of the infants at four months after hospital discharge was significantly higher in supplemented infants 110 (93-142) nmol/L compared to non-supplemented infants 37 (27-71) nmol/L ($P<0.001$). The serum 25(OH)D concentrations of the infants by supplement group according to gestational age and birth weight categories are shown in Table 4.4. Infants over 32 weeks gestation who did not receive supplements had a significantly lower serum 25(OH)D concentration compared to those infants who were over 32 weeks gestation and supplemented ($P<0.0001$).

Table 4.4: 25(OH)D of Infants According to Birth Weight and Gestational age Categories by Supplement Use*

	Received Vitadol C Supplements $n=25$	Did not Receive Vitadol C supplements $n=23$	P value*
Birth Weight Category			
$\leq 1800g$	110 (90-159) [†] $n=19$	37 (29-105) [†] $n=2$	<0.0001
$>1800g$	107 (94-116) [†] $n=6$	37 (27-71) [†] $n=21$	<0.0001
Gestational Age Category			
≤ 32 weeks	110 (90-159) [†] $n=11$	- $n=0$	
>32 weeks	109 (98-136) [†] $n=14$	37 (27-71) [†] $n=23$	<0.0001

*Significant differences between supplemented and non-supplemented ($P<0.05$) (Mann-Whitney Test). Values are medians (25th, 75th percentiles) unless otherwise indicated, †Range

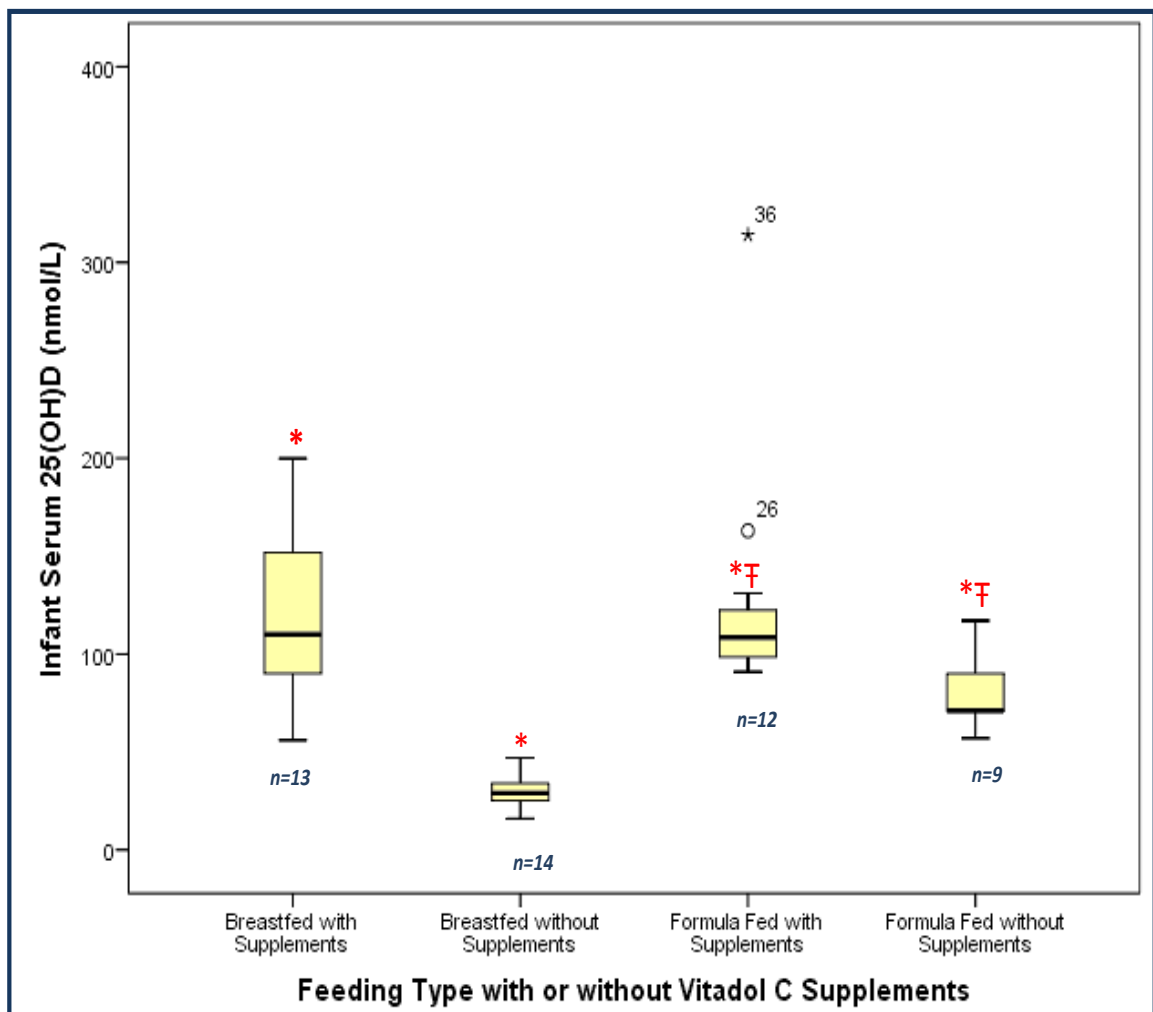
4.5 Serum 25(OH)D Concentrations of Infants by Mode of Feeding and Supplement Usage

Infants were grouped by mode of feeding and supplement usage (Figure 4.2). Infants who received Vitadol C supplements in addition to exclusive breastfeeding ($n=13$), or in addition to infant formula ($n=12$), all met the definition of vitamin D sufficiency (25(OH)D >50 nmol/L). All infants who were formula fed and did not receive supplements ($n=9$) also met the definition of vitamin D sufficiency.

25(OH)D concentrations were lowest in exclusively breast-fed infants who did not receive Vitadol C supplements. All exclusively breast-fed infants who did not receive Vitadol C supplements ($n=14$) met the definition of vitamin D deficiency (8.2%) or insufficiency (20.4%) (≤ 25 nmol/L and ≤ 50 nmol/L, respectively) (NHMRC, 2006). Sub-group analysis of the infants who were exclusively breast fed without supplements showed that 9 out of the 12 infants in this category were over 32 weeks.

25(OH)D concentrations were significantly affected by Vitadol C use $H(3)=33.85$, $P<0.05$. Vitadol C supplements in addition to exclusive breastfeeding was most significant ($P=0.0001$) ($U = 0.0001$, $r = -0.85$) (large effect).

There were 2 outliers within the group of infants who were formula fed and received Vitadol C supplements who had 25(OH)D concentrations of 314 nmol/L and 163 nmol/L. These infants were being given a Vitadol C dose of 0.3ml and 0.6ml, respectively (Figure 4.2).



<0.008 = level of significance, n=number

*P=0.0001; Exclusive breastfeeding without Vitadol C versus exclusive breastfeeding with Vitadol C, formula fed without Vitadol C and formula fed with Vitadol C

†P=0.002; Formula fed with Vitadol C versus formula fed without Vitadol C

Figure 4.2: Feeding Type With or Without Vitadol C supplements, by Serum 25(OH)D Concentrations in Infants

4.6 Relationship between Infant Serum 25(OH)D Concentrations and Maternal 25(OH)D Concentrations in Breastfeeding Mothers

Vitamin D concentrations in breastfeeding mothers were analysed to determine whether there was a correlation between maternal and infant vitamin D concentrations in exclusively breastfed infants. Median 25(OH)D concentrations in exclusively breastfeeding mothers (n=10) was 69.5 nmol/L (range 25 nmol/L – 107 nmol/L). Spearman’s correlation coefficient between maternal and infant vitamin D status was not significant (P=.738), therefore no further analysis was conducted.

4.7 Change in Anthropometric Measurements in Infants According to Supplement group, and Vitamin D Sufficiency versus Vitamin D Insufficiency

There were no significant differences in Z scores for anthropometric measurements from birth to appointment in infants who received Vitadol C supplements versus those who did not (Table 4.5). Additionally there was no significant difference between Z scores for anthropometric measurements from birth to appointment in infants who were vitamin D insufficient (25(OH)D ≤50 nmol/L) versus those that were vitamin D sufficient (25(OH)D >50 nmol/L) (Table 4.6).

Table 4.5: Change in Z Scores for Weight, Length and Head Circumference between Birth and Appointment in Infants who Received Vitadol C supplements versus those who did not

	Supplemented <i>n</i> =25	Not Supplemented <i>n</i> =23	<i>P</i> Value*
Length	0.53 ± 1.29 [†]	-0.07 ± 1.24 [†]	0.601
Weight	0.06 ± 1.76	0.17 ± 1.17	0.821
Head Circumference	0.46 ± 1.09	0.27 ± 1.30	0.982

[†]Z scores for length missing for one baby <25 weeks gestation. Values presented as mean ± SD, *Differences between supplemented and non-supplemented (*P*<0.05) (Independent Student t-test)

Table 4.6: Change in Z Scores for Weight, Length and Head Circumference between Birth and Appointment in Infants who were Vitamin Sufficient versus those who were Vitamin D Insufficient

	Vitamin D Sufficient <i>n</i> =35	Vitamin D Insufficient <i>n</i> =14	<i>P</i> Value*
Length	0.36 ±1.24 [†]	-0.08 ± 1.49 [†]	0.744
Weight	0.12 ± 1.52	0.11 ± 1.24	0.854
Head Circumference	0.52 ± 1.16	0.04 ± 1.21	0.778

[†]Z scores for length missing for one baby <25 weeks gestation. Values presented as mean ± SD, *Differences between vitamin D sufficient and insufficient (*P*<0.05) (Independent Student t-test)

4.8 Supplement Use and Compliance in Infants

Overall, 25 infants were prescribed Vitadol C after hospital discharge (Table 4.3). At 4 months post hospital discharge, 23 of the 25 infants were still receiving Vitadol C (Table 4.7). For one of these infants Vitadol C supplements were stopped at approximately 3 months post hospital discharge as solids had been introduced. This infant was being formula fed and had a 25(OH)D concentration of 98 nmol/L at 4 months post hospital discharge. For the second infant, Vitadol C was also stopped at approximately 3 months post hospital discharge as the mother had ran out. This infant was being formula fed and had a 25(OH)D concentration of 105 nmol/L at 4 months post hospital discharge.

Of the 23 infants receiving Vitadol C supplements at 4 months post hospital discharge the majority were being given Vitadol C daily (*n*=11, 48%) or most days (*n*=10, 44%) (Table 4.7).

The main barrier for not giving Vitadol C daily was forgetting to do so. Other barriers included “worsens reflux” and “dislikes taste”.

Table 4.7: Vitadol C Compliance in Preterm Infants at 4 months post Hospital Discharge (n=23)

How Often Is Vitadol C Given	n	%
Everyday	11	48
Most Days	10	44
Some Days	2	8
Never	0	0

4.9 Vitadol C Dose in Infants

Of the 23 infants who received Vitadol C at 4 months post hospital discharge, 82.6% were receiving a 0.3ml dose. The doses ranged from 0.2ml to 0.6ml (Table 4.8).

Table 4.8: Vitadol C Dose in Infants, by Serum 25(OH)D Concentrations (n=23)

Dose of Vitadol C	n	%	Mean 25(OH)D (nmol/L)	Range 25(OH)D (nmol/L)
0.2ml	1	4.3	107	-
0.3ml	19	82.6	120	56-314
0.4ml	1	4.3	200	-
0.6ml	2	8.8	136	110-163

4.10 Sun Exposure Behaviours in Infants after Hospital Discharge

Exposure to sunlight was variable in the infants. When the babies were outside in sunlight the majority were placed in protective clothing; 62.5%, most were placed in the shade; 52.1% and very few had sunscreen applied. Wearing a hat in the sun was also variable in all infants (Table 4.9). A full description of sun exposure behaviours in preterm infants can be seen in Table 4.9.

Table 4.9: Sun Exposure Behaviours in Infants after Hospital Discharge (n=48)[†]

Description	Always	Usually	Sometimes	Rarely	Never
How Often Is the Infant Exposed to Sunlight	13 (27)	12 (25)	14 (29.2)	7 (14.6)	2 (4.2)
How Often does the Infant Wear Protective Clothing in the Sun	30 (62.5)	10 (20.8)	4 (8.3)	1 (2.1)	3 (6.3)
How Often Is the Infant in the Shade	25 (52.1)	16 (33.3)	5 (10.4)	2 (4.2)	0
How Often does the Infant Wear a Hat In the Sun	18 (37.5)	7 (14.6)	5 (10.4)	1 (2.1)	17 (35.4)
How Often does the Infant Wear Sunscreen	4 (8.33)	0	5 (10.4)	2 (4.2)	37 (77.1)

[†]Values presented as n (%)

Chapter 5.0 Discussion

This is the first study to describe the vitamin D status of preterm infants (less than 37 weeks gestation) after hospital discharge in New Zealand. In this sample of 49 preterm infants, the mean 25(OH)D concentration was 73.8 nmol/L, the range was notably variable; 16-314 nmol/L. According to National Health and Medical Research Council (NHMRC) guidelines 28.6% ($n=14$) of infants are classified as being vitamin D insufficient (25(OH)D ≤ 50 nmol/L), of which 8.2% ($n=4$) were further classified as having mild to moderate vitamin D deficiency (25(OH)D ≤ 25 nmol/L) (NHMRC, 2006). However, it should be mentioned that these NHMRC cut off values are those recommended for term infants, adolescents and adults in New Zealand and Australia. Optimal vitamin D concentrations for preterm infants are yet to be determined.

Vitadol C supplements had the most significant effect on 25(OH)D concentrations in infants ($P=0.0001$). All infants who received Vitadol C supplements or infant formula after hospital discharge had vitamin D concentrations above 50 nmol/L. All exclusively breastfed infants who did not receive Vitadol C supplements ($n=14$) had vitamin D concentrations below 50 nmol/L.

In addition 18.4% ($n=9$) of infants who received Vitadol C supplements were found to have levels above the recommended upper limit (25(OH)D >125 nmol/L) (NHMRC, 2006).

5.1 Purpose of the Study

The main purpose of this study was to present a situational analysis of the vitamin D concentrations of preterm infants at 4 months post hospital discharge, and to describe the factors affecting these.

We decided to assess infant 25(OH)D concentrations at 4 months post hospital discharge for several reasons. Shorter gestational lengths predispose preterm infants to reduced vitamin D accretion in *utero*, therefore increasing requirements in infancy (Tsang et al., 2005). This group of infants are therefore highly dependent on feed and supplemental sources for vitamin D (Holick, 2006; Tsang et al, 2005). Tsang et al. (2005) suggests that normalisation of vitamin D concentrations in a previously vitamin D insufficient (25(OH) <50 nmol/L) preterm infant however may take upwards of 3 months.

After hospital discharge while sun exposure may be a possible route of obtaining vitamin D in preterm infants it requires them to be exposed to sunlight in the UV β range. However, preterm infants are often subject to lengthy hospital stays and may spend their first few days to months of life in a neonatal intensive care unit (NICU), while in such an environment the cutaneous synthesis of vitamin D is impossible (Blencowe et al., 2012). In addition the Ministry of Health (MOH) recommends that all infants (less than 6 months of age) are not placed in direct sunlight, and that if they are protective clothing, shady areas and sunscreen should be utilised (Ministry of Health, 2013). Following such recommendations would effectively reduce the cutaneous synthesis of vitamin D. Therefore, ruling out sun exposure as a viable route for obtaining adequate vitamin D in this group. It was therefore important to assess sun exposure practices in these infants after hospital discharge, determine whether MOH guidelines for sun exposure were being followed in this group and whether these behaviours influenced infant vitamin D concentrations after hospital discharge.

Feeding type and supplement use also contribute to infant vitamin D concentrations after birth. The World Health Organisation (WHO) recommends exclusive breastfeeding for all infants, including those born preterm for the first 6 months of life (World Health Organisation, 2007). However, breast milk is a poor source of vitamin D, even in mothers who are vitamin D sufficient (25(OH)D >50

nmol/L) (Thiele et al., 2013; Wagner et al., 2006). If such recommendations are followed the likelihood of vitamin D deficiency is high; unless an additional source of vitamin D is provided.

In Auckland City Hospital's NICU Vitadol C supplements are available to provide an additional source of vitamin D. The standard dose of Vitadol C recommended at Auckland City Hospital's NICU after hospital discharge is 0.3ml per day (Cormack, 2013). This dose provides 11.7µg (467 IU) of vitamin D, 667µg of vitamin A and 33mg of vitamin C (PHARMAC; Pharmaceutical Management Agency New Zealand, 2013). This amount of vitamin D meets the values recommended for preterm and term infants set by the American Academy of Paediatrics (AAP) (10µg/400IU per day), and also meets the NHMRC recommended intake for term infants (5µg/200IU per day) (NHMRC, 2006; Wagner & Greer, 2008). At Auckland City Hospital's NICU preterm infants who are less than 32 weeks gestation and/or less than 1,800g at birth meet the criteria for Vitadol C supplementation. Infants who fall outside of these criteria are not routinely provided with Vitadol C supplements, unless individually prescribed (Cormack, 2013). Considering that the vitamin D content provided in a standard 0.3ml dose of Vitadol C meets both AAP and NHMRC recommendations it is highly likely that infants who receive vitamin D supplements will be vitamin D sufficient. However, those who do not receive supplements and are exclusively breastfed are at risk of vitamin D deficiency.

Considering it may take up to 3 months to normalise vitamin D concentrations in the previously vitamin D insufficient preterm infant, it was important to ensure this time had lapsed before vitamin D concentrations were measured to assess supplement effectiveness. However, we also wanted to assess the effect of breastfeeding and sun exposure recommendations in these infants. We therefore decided to assess vitamin D concentrations in all infants at 4 months post hospital discharge. This meant that while infants would be different ages at their time of appointment (depending on their gestational length) all would have been at home the same amount of time and routine feeding practices would likely be developed. Furthermore at this time breast milk or infant formula would still be the infant's primary nutrient source and under current recommendations they would still be recommended to avoid sunlight exposure and be exclusively breastfed (Ministry of Health, 2013).

The vitamin D status of preterm infants at 4 months post hospital discharge and the effect of feeding and supplementation practices on vitamin D concentrations will be discussed here.

5.2 Characteristics of Participants

The participants included in the current study were preterm infants (less than 37 weeks gestation) who were as close to 4 months post hospital discharge as possible. The mean gestational age of infants was 33 weeks and 5 days; defined as moderate to late prematurity. However, there was a notable range; the youngest infant had a gestational age of 24 weeks and 1 day whilst the oldest was 36 weeks and 6 days. Thus there was also substantial variation between other characteristics of infants including anthropometric measurements at birth, for example the lightest infant was 700g and the heaviest infant was 3,700g at birth.

The mean time at which infants were seen after hospital discharge was 4 months. However, not all infants were seen at the specified time point; the earliest appointment after hospital discharge was 3 months and 2 days and the latest was 5 months and 5 days. The appointments were booked according to the mother's convenience. Some appointments were rescheduled and this contributed to the range.

Infant characteristics were further grouped according to supplementation usage; those who received Vitadol C supplements after hospital discharge, and those that did not. In total 25 infants received Vitadol C supplements and the remaining 23 did not (supplementation data was missing for 1 infant). Of the 25 infants who received Vitadol C after hospital discharge, all met the criteria except 6 who were more than 1,800g and more than 32 weeks gestation at birth. Additionally, of the infants who did not receive Vitadol C supplements, all except 2 fell outside of the criteria; These infants were both more than 32 weeks gestation and had birth weights of 1,700g and 1,500g and thus should have qualified to receive Vitadol C supplements. As expected infants who were grouped within the supplementation group were significantly smaller ($P=0.0001$) and younger ($P=0.0001$) at birth in comparison to infants who were in the non supplemented group.

In addition infants were characterised according to feed and supplementation type; including exclusively breastfed with supplements ($n=13$), exclusively breastfed without supplements ($n=14$), formula fed with supplements ($n=12$) and formula fed without supplements ($n=9$).

A total of 49 preterm infants were included in the current study. Whilst we initially had a higher recruitment number of 67 infants there was some difficulty in obtaining sufficient blood from all infants via the heel prick method for the analysis of 25(OH)D. Whilst the best efforts were exerted, sufficient blood could not be obtained for 16 infants and 2 samples were haemolysed upon analysis. Thus providing us with a total of 49 infants with 25(OH)D concentrations available.

5.3 Vitamin D Status of Preterm Infants at 4 Months Post Hospital Discharge

The mean vitamin D status of preterm infants at 4 months post hospital discharge was 73.8 nmol/L; a value which is considered as vitamin D sufficient by the NHMRC (NHMRC, 2006). There was substantial variation between the range of vitamin D concentrations; the minimum and maximum values were 16 nmol/L and 314 nmol/L, respectively. Concentrations which are considered as mild to moderate vitamin D deficiency and vitamin D toxicity, respectively (NHMRC, 2006). The range of concentrations reflects different feeding and supplement practices within infants. The lowest value was obtained from an infant who was exclusively breastfed without Vitadol C supplements, whilst the highest value was obtained from a formula fed infant who received Vitadol C supplements.

From this cohort of 49 preterm infants, 28.6% ($n=14$) were classified as being vitamin D insufficient, of which 8.2% ($n=4$) were further classified as having mild to moderate vitamin D deficiency (NHMRC, 2006). All of these infants were exclusively breastfed and did not receive Vitadol C supplements.

In addition to this according to NHMRC guidelines 18.4% ($n=9$) of infants are classified as having vitamin D values above the recommended upper level (25(OH)D >125 nmol/L) (NHMRC, 2006).

It is important to recognise that classification of vitamin deficiency, sufficiency, and upper levels are based on population wide guidelines set in New Zealand and Australia by the NHMRC. In New Zealand and Australia there are no specific cut off values used to define deficiency, sufficiency and upper levels in preterm infants (NHMRC, 2006). Other countries also lack specific cut off values for preterm infants and instead provide population wide recommendations (AAP, 2008; Braegger, 2013; IOM, 2011). The AAP and the Institute of Medicine (IOM) recommend similar values for vitamin D deficiency and sufficiency to New Zealand and Australia (AAP, 2008; IOM, 2011; NHMRC, 2006). It still remains unclear whether utilising such values is appropriate in this unique population group.

Some vitamin D experts and health institutions regard population wide vitamin D deficiency and sufficiency levels utilised by the NHMRC, IOM and AAP as too low (Freeman; 2009; Heaney, 2008; Holick et al., 2011; Rollins, 2009; Vitamin D Council, 2013). Holick et al. (2011) suggests that any vitamin D concentration below 75 nmol/L should be classed as vitamin D deficient. Consensus on appropriate cut off values to define vitamin D deficiency and sufficiency states are yet to be determined for all population groups let alone in preterm infants, thus adding confusion to what values should be utilised to provide a true representation of vitamin D status in this group.

If such values recommended by Holick et al. (2011) are utilised an additional 12.2% ($n=6$) of infants would be classed as vitamin D deficient, bringing the group total to 39% ($n=20$). Five of these infants were formula fed without supplements and one was breastfed with supplements.

5.4 Major Determinants of Vitamin D status in Preterm Infants at 4 Months Post Hospital Discharge

Vitamin D concentrations were significantly affected by Vitadol C supplement use in infants at 4 months after hospital discharge ($P<0.05$). Vitadol C supplements in addition to exclusive breastfeeding had the most significant effect on infant 25(OH)D concentrations ($P=0.0001$). Vitadol C supplements in addition to formula feeding also had a significant effect on infant 25(OH)D concentrations ($P=0.002$).

All breastfed infants who received Vitadol C supplements had 25(OH)D concentrations above 50 nmol/L. In addition five of these infants had levels above the recommended upper limit (25(OH)D >125 nmol/L). The contrary was observed in breastfed infants who did not receive Vitadol C; all fourteen of these infants had 25(OH)D concentrations below 50 nmol/L, of these 4 had levels below 25 nmol/L. Values which are regarded as vitamin D insufficiency and mild to moderate vitamin D deficiency, respectively.

All infants who received Vitadol C in addition to formula feeding had 25(OH)D concentrations above 50 nmol/L, however, there was also 3 infants who had 25(OH)D concentrations above 125 nmol/L; considered above the recommended upper level (NHMRC, 2006). Whereas all infants who were formula fed without Vitadol C supplements were classified as having vitamin D concentrations within the safe recommended range; 25(OH)D more than 50 nmol/L and less than 125 nmol/L.

Other factors were explored to determine if they had a significant effect on infant 25(OH)D concentrations at 4 months post hospital discharge. However, only factors predictive of receiving Vitadol C supplementation were significant; including birth weight and gestational age. Ethnicity, season, sun exposure behaviours, Fitzpatrick score, and maternal vitamin D status were not significantly associated with infant 25(OH)D concentrations.

This study demonstrates that Vitadol C supplementation and formula feeding are effective at preventing vitamin D deficiency and insufficiency in preterm infants at 4 months post hospital discharge. In addition we can conclude that exclusive breastfeeding without concurrent vitamin D supplementation is not adequate to achieve and maintain sufficient vitamin D status in this group.

5.5 Vitamin D status in Exclusively Breastfed Infants without Vitadol C Supplements

As already described 100% ($n=14$) of preterm infants who were exclusively breastfed and did not receive Vitadol C supplements were classified as vitamin D insufficient at 4 months post hospital discharge.

While it is clear that human breast milk is the best nutrition for preterm and term infants in the first year of life, there is substantiated concern regarding vitamin D concentrations in maternal milk (Amukele et al., 2012; Hollis & Wagner, 2004; Thiele et al., 2013; World Health Organization, 2007). Even in a vitamin D sufficient mother (25(OH)D >50 nmol/L) breast milk vitamin D concentrations are low; approximately 0.4µg (16IU) per litre (Thiele et al., 2013). However, reported levels do differ within the literature. Accurate analysis of breast milk vitamin D concentrations require the measurement of 25(OH)D₂ and 25(OH)D₃ concentrations directly from maternal milk. Estimating maternal milk concentrations through the analysis of maternal vitamin D status can be performed however does not provide as accurate representation of these levels. As with vitamin D status, differing concentrations in maternal milk are expected. Nonetheless, research does demonstrate a high percentage of vitamin D deficiency and insufficiency in exclusively breastfed infants (Gesner et al., 1997; Wall et al., 2013; Ziegler et al., 2006). Based on a low vitamin D concentration of 0.4µg (16IU) per litre 25 litres of breast milk would be required to be ingested daily to meet the AAP recommendations of 10µg (400IU) of vitamin D per day. Such levels put an exclusively breastfed preterm infant at substantial risk of vitamin D deficiency. In comparison to term infants, risk of deficiency in exclusively breastfed preterm infants is further exacerbated. Preterm infants have shorter gestational lengths and therefore miss out on optimising vitamin D accretion whilst *in utero*. Consequently, they are more likely to be born with suboptimal vitamin D stores (Tsang et al., 2005).

Vitamin D supplementation is necessary in exclusively breastfed preterm infants to prevent vitamin D deficiency. Criteria for Vitadol C supplements currently utilised in Auckland City Hospital's NICU (less than 32 weeks gestation and/or less than 1,800g at birth) is based on gestational age and birth weight of the infant and does not currently take into consideration feeding type (Cormack, 2013). A change in criteria at Auckland City Hospitals NICU based on best available evidence however would ensure that all babies who are breastfed also receive vitamin D supplements.

Other than Vitadol C supplementation the addition of breast milk fortifier (BMF) into expressed breast milk (EBM) is an effective way of increasing breast milk vitamin D concentrations. The vitamin D content in a standard 2.2g sachet of BMF ranges from 2.5µg (100IU) – 4µg (160IU) of vitamin D (Cormack, 2013). The regular use of fortifier would be sufficient to achieve and maintain adequate vitamin D status in exclusively breastfed preterm infants. However there are some concerns with utilising BMF; including the necessity to express breast milk. Expressing breast milk requires the mother to have the correct equipment and can be a lengthy and uncomfortable procedure for some. In addition expressing breast milk increases the risk of exogenous contamination and requires strict hygiene as well as appropriate storage and temperature control (Cossey et al., 2011). Additionally expressing breast milk, removes the benefits of directly feeding from the breast (Recidoro, 2010). In addition the World Health Organisation recommends avoiding bottle use in infants who are being breastfed as it causes 'nipple confusion' - "a phenomenon that refers to an infant's difficulty in achieving the correct configuration, latching technique and sucking pattern necessary for successful breastfeeding after bottle-feeding or exposure to an artificial nipple" (Recidoro, 2010, p.1). When some of these issues are taken into consideration compliance with expressing breast milk and adding BMF must be questioned (Cossey et al., 2011).

Furthermore, the criteria to receive BMF are identical for receiving Vitadol C supplements (infants less than 32 weeks gestation and/or less than 1,800g at birth). Thus any exclusively breastfed preterm infant who does not meet this criterion is still at extreme risk of vitamin D deficiency. This is of great concern considering that it is these moderate to late preterm infants who are more likely to be successful in breastfeeding. Moderate to late preterm infants are those who are more likely to

have developed the necessary reflexes to suckle, swallow and breathe in a coordinated manner (Berseeth, 1993; Ingham, 2008; Neu, 2007). However it is these infants who are missing out on additional sources of vitamin D (Cormack, 2013).

Other than providing an additional source of vitamin D to the breastfeeding infant, maternal vitamin D supplementation whilst lactating has been suggested as a method for increasing maternal breast milk vitamin D concentrations, therefore resulting in improved vitamin D status in the breastfed infant (Thiele et al., 2013; Wagner, Hulseley, Fanning, Ebeling & Hollis, 2006). A supplement of 10µg (400IU) per day has been shown to increase vitamin D content in maternal milk to approximately 2µg (80IU) per litre. A higher dose supplement is shown to increase levels further; women supplemented with 160µg (6400IU) of vitamin D per day over a 6 month period saw an increase in breast milk vitamin D concentrations from 2 to 22µg (80 to 880IU) per litre (Wagner et al., 2006). This dramatic increase in vitamin D content would meet the vitamin D requirements of the preterm infant, however the safety of such doses are not currently well understood (NHMRC, 2006). However, this is an area of rapidly growing research.

Routine vitamin D supplementation for all lactating women in New Zealand is not part of current health policy. The NHMRC currently recommends that lactating women consume an adequate intake (AI) of 5µg (200IU) of vitamin D per day which is consistent with recommendations for infants, children and adults (NHMRC, 2006). However for women at risk of vitamin D deficiency, for example those with dark skin, or with limited access to sunlight a 10µg (400IU) supplement of vitamin D per day is recommended (NHMRC, 2006; The Royal Australian and New Zealand College of Obstetricians and Gynaecologists RANZCOG, 2009). However, as already discussed even a supplement of 10µg (400IU) per day has only been shown to increase vitamin D content in maternal milk to approximately 2µg (80IU) per litre, and thus would not be effective in improving vitamin D concentrations in the breastfed infant (Thiele et al., 2013). Ambiguity regarding dose and timing of maternal supplementation combined with differing definitions of vitamin D deficiency, sufficiency and upper levels are a barrier toward implementing vitamin D supplementation guidelines for lactating women (NHMRC, 2006).

It is crucial that guidelines are put in place to prevent vitamin D deficiency and insufficiency in exclusively breastfed preterm infants. Currently it seems the most appropriate way for preterm infants to receive adequate vitamin D is via a direct supplemental source. However, as already mentioned maternal vitamin D supplementation during lactation is a growing area of research and findings may later indicate this is also an effective method of improving the vitamin D status of exclusively breastfed preterm infants.

5.6 Vitamin D status in Exclusively Breastfed Infants with Vitadol C supplements

There was a total of 13 infants who were exclusively breastfed and received Vitadol C supplements, all infants had vitamin D concentrations above 50 nmol/L. The minimum and maximum vitamin D concentrations in this group were 56 nmol/L and 200 nmol/L, respectively. Whilst the minimum value is considered as being within the safe recommended range (>50 - ≤125 nmol/L) the maximum value is above the recommended upper level (NHMRC, 2006).

The infant with a 25(OH)D concentration of 200 nmol/L was receiving a 0.4ml dose of Vitadol C daily, providing 15.6µg (624IU) of vitamin D per day, which is above the intake recommended by the AAP of 10µg (400IU) daily and NHMRC of 5µg (200IU) daily for preterm and term infants, respectively.

In addition 4 other infants had values above 125 nmol/L, these values were 131 nmol/L, 152 nmol/L, 159 nmol/L and 194 nmol/L. All of these infants received a standard 0.3ml Vitadol C dose in addition to breast milk.

As there were 5 infants who had 25(OH)D values above 125 nmol/L with Vitadol C supplements in addition to receiving breast milk suggests that a lower dose of Vitadol C may be required to ensure vitamin D levels remain within the safe recommended range (25(OH)D >50 nmol/L ≤125 nmol/L).

It is evident that Vitadol C in addition to breastfeeding is necessary to prevent vitamin D deficiency. However, as there were some infants who had vitamin D concentrations above the recommended safe level (25(OH)D >125 nmol/L) the dose of Vitadol C provided after hospital discharge should be revised.

5.7 Vitamin D Status in Formula fed Infants without Vitadol C Supplements

There was a total of 9 infants who received infant formula without vitadol C supplements, all infants were classified as vitamin D sufficient (25(OH)D >50 nmol/L). In addition all of these infants had vitamin D concentrations within the safe recommended range (25(OH)D >50 - ≤125 nmol/L), the minimum and maximum values in this group was 57 nmol/L and 117 nmol/L, respectively.

All infant formula in New Zealand is supplemented with vitamin D₃; concentration varies according to brand, however ranges from 0.76-1.2µg (30-48IU) per 100ml (Cormack, 2013). A term infant formula with a vitamin D concentration of 1µg (40IU) per 100ml would require the ingestion of 1000ml daily to meet the AAP recommendations of 10µg (400IU) per day for term and preterm infants and 500ml daily to meet NHMRC recommendations of 5µg (200IU) per day for term infants (Abrams & the Committee on Nutrition, 2013).

As suggested from the results of this study vitamin D deficiency is unlikely in formula fed preterm infants. The provision of Vitadol C supplements to formula fed infants is not necessary to achieve vitamin D sufficiency at 4 months post hospital discharge. However, formula fed infants who were born preterm may benefit from short term supplementation to attain adequate vitamin D status during early life when only small volumes of infant formula are ingested. However, additional research is required to confirm this.

We can conclude from these results that infant formula alone is sufficient to achieve and maintain vitamin D levels recommended by the NHMRC at 4 months post hospital discharge. However, that low dose supplementation may be beneficial in some to reach higher levels of sufficiency.

5.8 Vitamin D status in Formula Fed Infants with Vitadol C Supplements

Twelve infants received Vitadol C supplements in addition to infant formula. All infants had vitamin D concentrations above 50 nmol/L on analysis. The minimum and maximum vitamin D concentrations were 91 nmol/L and 314 nmol/L, respectively. Whilst the minimum value is considered as being within the safe recommended range (>50 - ≤125 nmol/L) the maximum value is more than double the upper level (NHMRC, 2006).

The infant who had a vitamin D concentration of 314 nmol/L received a standard 0.3ml dose of Vitadol C after hospital discharge. There were no other indicators of why this infant had such a high concentration of 25(OH)D on analysis. This result may have been spurious. This infant was followed up after the completion of the study, the general practitioner who had been provided with the

infant's 25(OH)D blood results was asked to identify what further action was taken. The results had been referred to a paediatrician and the general practitioner reported back that the mildly elevated vitamin D concentration was likely due to the use of Vitadol C, since the mother was no longer giving the infant this, and considering the infant showed no signs of vitamin D toxicity no further testing was carried out (personal communication, November 19, 2013). It should also be mentioned that others do not regard this level as vitamin D toxicity (Holick et al., 2011).

In addition 2 other infants had vitamin D concentrations above 125 nmol/L. Concentrations were 131 nmol/L and 163 nmol/L. The infant with a vitamin D concentration of 131 nmol/L was also receiving a standard 0.3ml dose of Vitadol C daily, whereas the infant with the 25(OH)D concentration of 163 nmol/L was receiving a 0.6ml dose daily, providing 23.4µg (934IU) of vitamin D per day. Interestingly however, this infant had a twin who received the exact same dose of Vitadol C after hospital discharge, and had a vitamin D concentration within the normal safe range; 110 nmol/L. Furthermore, these infants had no differences in feeding or supplement usage from birth. In addition these infants had very similar weights at birth (1,800g and 1,900g (representing the infants with the higher and lower vitamin D concentration, respectively).

Considering that 3 infants who received Vitadol C in addition to infant formula had vitamin D values above the recommended safe upper level, suggests that caution with the vitamin D supplementation dose in addition to formula feeding is necessary. Furthermore, as all infants who received infant formula without Vitadol C were vitamin D sufficient, questions whether additional supplementation would confer additional benefit. High quality longitudinal research is required to determine if there are any benefits of higher vitamin D concentrations at this stage of life on longer term health.

We can conclude that whilst Vitadol C supplementation may be beneficial in formula fed infants to reach higher levels of vitamin D sufficiency, more research is required before a conclusion can be made. Currently if a Vitadol C is provided to formula fed preterm infants a lower dose than that currently prescribed is recommended.

5.9 Vitamin D Toxicity

Some infants (18.4%, $n=9$) had 25(OH)D concentrations above the recommended upper limit (25(OH)D >125 nmol/L). All infants who had 25(OH)D levels above 125 nmol/L received Vitadol C supplements in addition to infant formula ($n=3$) or in addition to breast milk ($n=5$) (supplementation data was missing for 1 infant).

However, as with deficiency values there is confusion with what values should be considered toxic. The AAP and IOM also define the upper limit as 25(OH)D concentrations more than 125 nmol/L, and suggest that levels above this may be associated with adverse effects (NHMRC, 2006; Ross, 2011; Wagner, 2008). However, levels at which symptoms of toxicity occur are highly variable. Holick et al. (2008) suggests that vitamin D toxicity does not occur until 25(OH)D levels reach 375 nmol/L and above. Symptoms associated with toxicity include hypercalcemia and hypercalciuria, clinical symptoms of which can include faltering growth, polyuria and ectopic calcification (Tsang et al., 2005). Literature suggests that vitamin D intakes must be consistently high in order to reach a toxicity state, and toxic levels have not been sited with current prescribed levels of Vitadol C in Auckland City Hospital's NICU (0.3ml providing 11.7µg (467IU) vitamin D). Even levels up to 25µg (1000IU) of vitamin D per day; which is recommended for preterm infants by ESPGHAN have not been associated with toxicity (Agostoni, 2010; Markestad, 1987). Pharmacological doses with levels greater than 250µg (10,000IU) per kilogram per month have been associated with toxicity

(Markestad, 1987). One study provided term infants with different supplementation doses for the treatment of vitamin D deficiency induced rickets; whilst a one off dose of 3750µg (150,000IU) was associated with improved symptoms and no toxicity, doses of 7500µg (300,000IU) and 15000µg (600,000IU) resulted in hypercalcemia (Cesur, Caksen, Gundem, Kirimi & Odabas, 2003).

In the current cohort of infants the highest vitamin D concentration as already noted was 314 nmol/L. This infant was formula fed and received a standard 0.3ml dose of Vitadol C which provides 11.7µg (467 IU) of vitamin D. An average one litre intake of infant formula daily in addition to 0.3ml of Vitadol C would have provided 23.7µg (948IU) of vitamin D daily, however intake would have varied with differing intakes of infant formula. Such values of vitamin D as already suggested have not previously been associated with toxic levels of vitamin D, and furthermore are in line with intakes recommended by ESPGHAN (Agostoni, 2010; Markestad, 1987). Therefore whether a different dose was provided or if there are other factors that may have contributed to this level is largely unknown. Interestingly the highest dose of Vitadol C; 0.6ml providing 23.4µg (934IU) of vitamin D was prescribed to two infants (twins), who were also being formula fed, these infants had vitamin D concentrations of 110 nmol/L and 163 nmol/L. Discrepancies between Vitadol C dose and 25(OH)D concentrations may suggest that accurate doses are not being provided to infants. There has been one case acknowledged outside of this study where a mother was confused by dosage information and was providing her infant with a 3ml dose instead of a 0.3ml dose of Vitadol C daily. On analysis this infant had a vitamin D concentration of 175 nmol/L. However, it was unclear how long this dose was being provided to the infant (J. Crawford, personal communication, November 21, 2013). In addition Vitadol C dose is routinely provided drop by drop directly into the infant's mouth. This has been suggested as the incorrect method of providing any liquid medications as the first drop is always going to be larger than subsequent drops, therefore, providing an accurate dose via this method is highly unlikely, and has the potential to provide a higher dose than what is prescribed (J. Crawford, personal communication, November 21, 2013).

Frequency of taking Vitadol C may have been an additional factor that contributed to differences in vitamin D concentrations between infants. However the majority of mothers stated that they gave their infants Vitadol C everyday (48%) or most days (44%). The main reason for mothers not giving Vitadol C daily was forgetting to do so.

The reason for such large discrepancies between 25(OH)D concentrations in preterm infants being prescribed similar doses of Vitadol C is largely unknown and further investigation in subsequent populations is recommended.

5.10 Optimal Vitamin D Dose in Preterm Infants after Hospital Discharge

Optimal vitamin D supplementation dose in preterm infants is debatable (Agostoni, 2010; Ministry of Health, 2013; Ross, 2011; Wagner & Greer, 2008). Markestad et al. (1983) demonstrated that a vitamin D supplement of 12.5µg (500IU) per day in addition to a combination of breast and standard term formula was sufficient to normalise vitamin D levels (25(OH)D \geq 50 nmol/L) in all preterm infants by one month chronological age, even in those who were previously vitamin D deficient (25(OH)D <25 nmol/L). Backstrom et al. (1999) looked at vitamin D supplementation dose in preterm infants from birth to 3 months chronological age and the effects of different dose on bone density; the authors concluded that a vitamin D dose (from feed and supplemental sources) of 5µg (200IU) per kilogram per day up to a maximum of 10µg (400IU) per kilogram per day had similar effects on vitamin D status and bone mineral accretion compared to a dose of 24µg (960IU) per day. McCarthy

et al. (2013) showed that a vitamin D intake of 10µg (400IU) per day (5µg/200IU from both feed and supplements) from birth for a median of 104 days was adequate to achieve sufficient vitamin D status (25(OH)D >50 nmol/L) in most (87%) VLBW preterm infants. In addition McCarthy et al. (2013) also demonstrated 8% ($n=12$) of cases where 25(OH)D status was above 125 nmol/L.

Our study has shown that a 0.3ml dose of Vitadol C providing 11.7µg (467IU) of vitamin D per day is adequate in all infants to reach vitamin D sufficiency at 4 months post hospital discharge, regardless of feed type. Furthermore, a 0.2ml dose providing 7.7µg (311IU) when provided with infant formula was also adequate to reach vitamin D sufficiency in one infant. However, that a 0.3ml dose and above was also associated with toxic levels of vitamin D in some infants.

From the results of this study, we can conclude that Vitadol C use is necessary in preterm infants after hospital discharge to reach and maintain vitamin D sufficiency, however that the optimal dose necessary still needs to be determined.

5.11 Vitadol C Supplements and Vitamin A Content

There is further concern with the current standard 0.3ml dose of Vitadol C; as already described this dose also provides 667µg of vitamin A, and 33mg of vitamin C. Whilst there are no current concerns with this level of vitamin C, the amount of vitamin A provided in this dose is above the safe upper limit set (600µg) for infants, children, adolescents and adults (NHMRC; 2006; PHARMAC; Pharmaceutical Management Agency New Zealand, 2013).

In the Ministry of Health's latest companion statement on Vitamin D and Sun Exposure during Pregnancy and Infancy they acknowledge that Vitadol C should not be routinely provided to all breastfed infants due to the high concentration of Vitamin A (Ministry of Health, 2013). Recently the MOH provided a submission to the Pharmacology and Therapeutics Advisory Committee (PTAC) detailing the importance of providing a vitamin D only preparation for treatment of infants with rickets; response to which was acknowledged, however considered low priority. The basis of these recommendations by PTAC was that there was no evidence available for vitamin A toxicity with current prescribed levels of Vitadol C, and further, that a lower dose could be provided to be within the safe ranges for vitamin A (Pharmacology and Therapeutics Advisory Committee, 2013).

Such barriers make providing routine vitamin D supplementation to all preterm and term infants extremely difficult. Whilst it is clear that risk of vitamin D deficiency and insufficiency is high in exclusively breastfed preterm and term infants, it may not be safe to routinely prescribe a standard 0.3ml dose of Vitadol C. Until a vitamin D only preparation for infants is made available with subsidy it is important to review the current prescribed dose of Vitadol C in preterm infants after hospital discharge.

The current study did not analyse vitamin A levels in this group, however high levels may have been present. As mentioned earlier there has been one acknowledged case outside of this study where an infant was being given a 3ml dose of Vitadol C instead of the 0.3ml dose prescribed. This dose would have provided 6,670µg of vitamin A; which is more than ten times the upper limit (NHMRC, 2006). This infant had a vitamin A concentration of 1.3µmol/L; which is considered within normal reference ranges (0.7 – 2.8µmol/L) (Cormack, 2013; J. Crawford, personal communication, November 21, 2013).

5.12 Sun Exposure Practices in Preterm Infants after Hospital Discharge

Sun exposure practices in preterm infants after hospital discharge were previously unknown. It was assumed that in view of the current recommendations for all infants to avoid sun exposure that this would not have a significant effect on vitamin D concentrations in preterm infants after hospital discharge. It has however been suggested that incidental sun exposure may be sufficient to maintain adequate vitamin D concentrations in infants (Ministry of Health, 2013). However, there are several knowledge gaps regarding this suggestion, for example how much incidental sun exposure is required and how much skin must be shown to produce an effect. Whilst there is research to indicate how much incidental sun exposure is required in adults to produce adequate circulating vitamin D, this knowledge is lacking in infants.

In the current study sun exposure was not significantly associated with infant 25(OH)D concentrations at 4 months after hospital discharge. All infants except two were seen in winter. Sun exposure practices in these infants after hospital discharge were variable. However, what was apparent was that when infants were exposed to sunlight the majority were placed in protective clothing or alternatively were placed in the shade. Both of these factors are effective in preventing the cutaneous synthesis of vitamin D (Holick et al., 2006). Sunscreen was rarely used in all the infants. However, considering these infants were either placed in protective clothing or in the shade when outside sunscreen use would not have necessarily conferred any additional protection from the sun's rays. Because the majority of infants were seen in winter, it is unclear from this study whether sun exposure practices would change with season, and then in turn if summer months would have a significant effect on 25(OH)D concentrations in infants.

It is also suggested that preterm infants who have lengthy hospital stays are more likely to be vitamin D deficient as the cutaneous synthesis of vitamin D is not possible whilst indoors. However, research indicates that it is extremely and moderately preterm infants who are more likely to suffer prematurity related health consequences and subsequently have longer hospital stays (Blencowe et al., 2012). However, extremely and very preterm infants are those who meet the criteria for vitamin D supplementation, and further are less likely to be successfully breastfed, thus are more likely to be provided with preterm infant formula and thus have sufficient vitamin D intake (Berseth, 1993; Cormack, 2013; Ingham, 2008; Neu, 2007). Risk of vitamin D deficiency in these preterm infants is low. However, the contrary would be observed if an infant has a prolonged hospital stay and is not provided with any additional source of vitamin D.

Recommending the avoidance of sun exposure in infants is based on sound evidence. The epidermal barrier is known to remain immature for the first two years of life. Thus ultraviolet (UV) light may have a more damaging and possibly accumulative effect in infants (American Academy of Pediatrics, 1999). Furthermore, due to the immobility of infants at this age they are unable to remove themselves from uncomfortable heat. Sweating capacity may also be reduced in this group, therefore significantly increasing the risk of heatstroke (American Academy of Pediatrics, 1999). Considering these factors it is deemed unsafe for infants to be exposed unprotected to direct sunlight for at least the first 6 months of life (American Academy of Pediatrics, 1999). Furthermore, epidemiological evidence indicates that the age at which sun exposure is begun has a more significant effect on skin cancer risk compared to total sun exposure over ones lifetime (Autier & Dore, 1998; Marks, Jolley, Leclercq & Foley, 1990).

From this study we can conclude that sun exposure practices in preterm infants after hospital discharge are not conducive to obtaining sufficient vitamin D, and considering sun exposure recommendations for this group it is important that sun exposure is not considered as a viable source for attaining adequate vitamin D in all preterm infants. These findings exacerbate the importance of establishing routine vitamin D supplementation recommendations for all preterm exclusively breastfed infants after hospital discharge.

5.13 Conclusions

We can conclude that preterm infants who are exclusively breastfed and do not receive Vitadol C supplements are at risk of vitamin D deficiency after hospital discharge. All of the infants in this cohort who were exclusively breastfed and did not receive Vitadol C ($n=14$) were vitamin D insufficient ($25(\text{OH})\text{D} \leq 50 \text{ nmol/L}$) at 4 months after hospital discharge. All infants who received either infant formula or Vitadol C supplements in addition to infant formula or breast milk were vitamin D sufficient ($25(\text{OH})\text{D} > 50 \text{ nmol/L}$). From this study we conclude that a vitamin D supplement may be essential for all exclusively breastfed preterm infants after hospital discharge to prevent deficiency. Furthermore, that post discharge vitamin D supplements may be important for formula fed infants to reach higher levels of sufficiency. However, due to the number of infants (18.4%, $n=9$) who had $25(\text{OH})\text{D}$ levels above the upper level deemed safe, caution with Vitadol C supplement use and dose is warranted.

Furthermore, we conclude that sun exposure is minimal in preterm infants after hospital discharge and therefore nutritional vitamin D intake takes precedence over sun exposure in this group. Sun exposure should not be relied on as a viable source of vitamin D for preterm infants after hospital discharge.

5.14 Strengths and Limitations

The strengths of the current study include having low dropout rates and including an extended range of feeding variables. In addition the majority of infants were seen at the specified time point; 4 months post hospital discharge. Furthermore, there were a higher percentage of infants in this study who were being breastfed. In addition these infants were fairly evenly distributed between receiving Vitadol C supplements ($n=13$) and not receiving Vitadol C supplements ($n=14$), this allowed us to compare the effects of breastfeeding with and without Vitadol C supplements after hospital discharge.

Another strength of this study was conducting all appointments at the participants homes, this meant participants could be seen at their convenience and may have been a factor contributing to low dropout rates. However, a disadvantage of this scenario was that participants sometimes cancelled and rescheduled appointments, which meant not all participants, were seen at the specified time point. The effect of seeing infants at different time periods on 25(OH)D concentrations was not assessed, however, due to the different ages of these infants it may have potentially affected the amount of breast milk or infant formula they were being given, in addition to having a shorter or longer period to reach vitamin D sufficiency.

There were also several potential limitations in the current study. The sample size was small; however, despite this, the finding that 100% of preterm infants who were exclusively breastfed and did not receive Vitadol C were vitamin D insufficient 4 months after hospital discharge is striking.

Additionally there was an uneven spread between ethnicities and Fitzpatrick skin tone of infants, which may have potentially influenced the non statistical findings of these factors in comparison to 25(OH)D concentrations in infants. Furthermore, the majority of infants were seen in winter, thus sun exposure behaviours of this group during summer still remains largely unknown. In future having a longer recruitment process to ensure that a larger number of infants were recruited in addition to conducting the study over both winter and summer periods is recommended.

Recruiting infants from hospitals other than Auckland City Hospital's NICU would have been beneficial. As well as increasing the number of infants recruited, there may have been a more even distribution between ethnicities and therefore Fitzpatrick skin tone. In addition this would have allowed us to determine the effectiveness of different feeding and supplementation practices used across a number of different hospitals in New Zealand. Furthermore, recruiting infants from both the North and South Island may have allowed us to analyse differences in 25(OH)D concentrations of the infants based on their geographical location.

Another acknowledged limitation is that there is currently no specific reference range for vitamin D deficiency, sufficiency and upper levels in preterm infants. The outcome of this research was therefore defined by using existing reference ranges set for term infants, adolescents and adults in New Zealand. Whilst this concurs with the majority of paediatric literature it is still considered as a potential limitation, as the relevance of these values in preterm infants is yet to be determined. Having specific vitamin D cut off values for preterm infants would have substantially improved this study and further research in this area. Currently it is largely unknown whether utilising population wide cut off values in preterm infants is appropriate.

Analysing vitamin A concentrations of infants in addition to vitamin D would have further strengthened this study. Vitadol C as already described also contains vitamins A and C. At a 0.3ml

dose, Vitadol C provides 667µg of vitamin A which is above the upper limit (600µg) recommended by the NHMRC for all ages. Considering some infants had vitamin D levels above the recommended upper limit, there may also have been a number of infants who had toxic levels of vitamin A. However upon analysing 25(OH)D concentrations there was insufficient serum left for the analysis of vitamin A. Whilst this was not an objective of the current study, the analysis of Vitamin A concentrations would have contributed to the findings of this study and assisted us with assessment of the safety of this dose of Vitadol C.

To further strengthen this study and improve knowledge on Vitadol C use in infants, we would have asked the mother to give the infants their daily dose of Vitadol C when we were present. This would allow us to determine whether the correct dose was being provided and would have allowed us to assess the safety and effectiveness of the mode through which it was being given. As previously mentioned it has been suggested that all liquid medications should not be provided drop by drop as the first drop is always larger than subsequent drops and that this can therefore result in an inaccurate dose being provided. However, 0.3ml is also a very small dose and could be easily confused with 3ml on a medicine dispenser. Therefore observing such practices in future would allow specific recommendations to be made to ensure that Vitadol C is being given as safely as possible.

Obtaining infant and maternal 25(OH)D concentrations at birth would have also added to the current study. This would have allowed us to determine the effect of gestational length and maternal 25(OH)D concentrations on infant 25(OH)D concentrations at birth, and would have allowed us to determine if there was any correlation present at four months after hospital discharge.

In addition whilst it was outside the scope of this study, it would have been advantageous to directly assess the vitamin D content of maternal milk by retrieving a breast milk sample and assessing the correlation between breast milk vitamin D concentration and maternal vitamin D status.

5.15 Recommendations

On the basis of the current findings we recommend a vitamin D nutritional intake of 10µg (400IU) per day combined from feed and supplemental sources for all preterm infants from birth. Once vitamin D sufficiency is achieved and sun exposure is increased a lower vitamin D intake as suggested by the NHMRC of 5µg (200IU) per day, may be appropriate to maintain sufficiency. This would equate to a 0.25ml dose of Vitadol C per day for all exclusively breastfed preterm infants which would provide 9.6µg (387IU) of vitamin D, and a 0.1ml dose for all infants being formula fed which would provide 3.8µg (155IU) of vitamin D per day in addition to infant formula which provides from 0.74-1.3µg (29.6-52IU) per 100ml. The newer doses proposed of 0.1ml and 0.25ml would provide 222µg and 555µg of vitamin A, respectively. Both of these concentrations are below the recommended upper limit (NHMRC, 2006).

Caution with higher intakes of vitamin D from both feed and supplementation is warranted. As already discussed in this group of preterm infants 18.4% ($n=9$) of those who received Vitadol C had 25(OH)D values considered toxic (>125 nmol/L) (AAP, 2008; IOM, 2011; NHMRC, 2006). Thus indicating that the current standard dose of Vitadol C (of 0.3ml per day providing 11.7µg (467IU) of vitamin D) may be excessive, especially when provided in combination with infant formula. However, as previously discussed consensus on what vitamin D concentration should be used to determine vitamin D toxicity is yet to be determined. Furthermore, some infants had much lower values with a standard dose of Vitadol C and furthermore, one infant had a normal vitamin D concentration (110 nmol/L) with a 0.6ml dose of Vitadol C, thus indicating that the dose is being given incorrectly or infants are responding differently. In addition it needs to be considered that errors can occur when biomarkers are being measured. Therefore, to further add to this research and provide sound recommendations a larger more robust study should be conducted to determine optimal vitamin D dose in this group.

It is also important that future research determines optimal vitamin D concentrations in preterm infants. Currently it is largely unknown whether utilising population wide cut of values is appropriate for preterm infants. Additionally the effects of such concentrations on short and longer term health need to be determined.

In addition it is recommended that in future a supplement preparation which contains vitamin D only is made available for preterm infants.

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APPENDICES

APPENDIX ONE: Information Sheet



MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

INFORMATION SHEET

You have been sent or given the information sheet about this research study because your baby was born preterm (before 37 weeks' gestation) and we would like to invite you to take part in a study looking at the nutrition of preterm babies after hospital discharge. Thank you for taking the time to think about enrolling your baby in this study.

ABOUT THE RESEARCHERS

We are a group of researchers from Massey University in Albany and the Neonatal Intensive Care Unit (NICU) at Auckland Hospital. Our research team includes Cath Conlon (PhD) and Pamela von Hurst (PhD) (Massey University), Professor Frank Bloomfield (Professor of Neonatology and Specialist neonatologist, National Women's Health, Auckland City Hospital), Barbara Cormack (Neonatal Dietitian, Auckland City Hospital), Briar Emmett and Charlotte Moor who are both doing their MSc in Nutrition and Dietetics at Massey University.

PROJECT DESCRIPTION AND INVITATION

Feeding preterm babies is often hard. We are recruiting **all** babies born before 37 weeks' gestation in the Auckland area, including extremely preterm babies, moderately preterm babies and late preterm babies (who may not have experienced any problems due to being born preterm) in order to look at their nutrition after they have been discharged from hospital. Because preterm babies are born too soon, they often have not had enough time to develop sufficient nutrient stores. Iron and vitamins D, A and E are some of these nutrients which are often low in preterm babies. These nutrients all have important roles within in the body. They are needed for the normal growth, brain development and the health and well-being of your baby. Therefore, we would like to find out whether the current feeding and supplementation practices are enough to prevent deficiencies in these babies. Results of this study will hopefully guide future feeding and supplementation practices of preterm babies.

We are asking you to consider including your baby in this study as she/he was born preterm (before 37 weeks' gestation). Preterm babies are unlike any other babies and it is therefore not possible to conduct this research in any other group.

PROJECT PROCEDURES

This study will involve 2 home visits over your baby's first year of life to find out about feeding, nutrition and how she/he has grown. We would like to visit you in your home or you can attend our research facilities at Massey University in Albany, Auckland at a time which is convenient to you. Our first visit would be 4 months after your baby was discharged home from hospital and our second visit would be when your baby is 1 year old (corrected age).

At each visit we would like to take a small blood sample from your baby so that we can look at his/her iron and vitamin status. This may cause your baby some discomfort. If you are breastfeeding, we would also like to take a blood sample from you. This will be to determine whether you are deficient in iron or vitamin D which could affect your baby's nutritional status.

If you or your baby are found to be deficient in iron or vitamin D we will send your results directly to you GP so that they can advise you.

Obtaining information on how preterm babies grow after they have been discharged is one of the key outcomes of the research, so we will measure your baby's weight, length and head circumference at each visit.

We would also like to collect some additional information using 4 simple questionnaires. These questionnaires will be used to collect information about you and your baby, on feeding practices, nutritional supplementation and sun exposure. At the second visit when your baby is 1 year corrected age we will also ask about starting solid foods.

Taking part in the study will take about an hour on each visit. The questionnaires will take between 20 to 30 minutes. Other measures which include weight, length and head circumference and a blood test will take between 10 to 15 minutes. We also allow time for you to ask questions. All blood samples will be taken by a trained phlebotomist or neonatal research nurse who has experience with taking bloods from babies.

We will also collect some health information about your baby from baby's medical notes. This is to record how your baby was fed after birth, his/her birth weight, head circumference and length, and whether any assessment of nutritional status was made during the hospital admission or after birth.

BENEFITS

By taking part in this study you will receive your baby's individual blood results on their iron and vitamin D status. Both of these nutrients are essential for optimal development and growth of your baby. If we find that your baby is low in these nutrients then we will refer you to your GP, often the solution is as easy as providing a supplement but it's important that this is decided by your medical practitioner. You will also find out your baby's length, weight and head circumference, these will be taken by trained researchers. If you are breastfeeding and consent to giving a blood sample, you will also find out whether you are sufficient in iron and vitamin D. If these values are outside of the normal ranges you will be notified and these will be forwarded on to your GP. Your GP will be able to provide you with best advice and if any treatment is needed.

By taking part in this study you and your baby are helping us find out whether feeding and supplementation practices currently followed are appropriate to prevent iron and vitamin D deficiencies in babies born preterm. With your help we can find out whether feeding and supplementation practices are currently sufficient or whether these need changing or if babies need to be routinely monitored.

DATA MANAGEMENT

Any information collected from you and your baby will be used only for the purposes of this study. This information will be stored in a secure manner at Massey University. Once collected, data will be entered into a database and analysed in a way that does not identify you or your baby.

We will not be sharing information about you or your baby outside of the research team. The information that we collect from this research project will be kept confidential. All questionnaires with information about you and your baby will be given a number and will not display any names.

A summary of your baby's results will be given to you. In addition, the overall findings from the study will be shared with all parents; however, individual results will be kept private. We will also publish the results of this study so that we can make sure that future feeding and supplementation practices are in the best interests of preterm babies.

If we find that any of the results from the blood sampling are outside of a normal range we will, with your permission, contact your baby's General Practitioner directly and give them a copy of the results.

PARTICIPANT'S RIGHTS

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- *decline to answer any particular question;*
- *decline to have the blood sample taken from your baby or yourself (if breast feeding)*
- *withdraw from the study within the timeframe of data collection;*
- *ask any questions about the study at any time during participation;*
- *provide information on the understanding that your name or your baby's name will not be used unless you give permission to the researcher;*
- *be given access to a summary of the project findings when it is concluded.*

PROJECT CONTACTS

If you have any questions, you may ask them now or later. If you wish to ask questions later, you may contact any of the following

Owen Mugridge
O.Mugridge@Massey.ac.nz
09 414 0800 extension 41174
TXT 021 160 5949

Massey University Oteha Rohe
Albany Highway, Albany 0632
New Zealand

Cath Conlon (PhD)
09 414 0800 extension 41206

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 13/06. If you have any concerns about the conduct of this research, please contact Dr Brian Finch, Chair, Massey University Human Ethics Committee: Southern A, telephone 06 350 5799 x 84459, email humanethicsoutha@massey.ac.nz.

APPENDIX TWO: Contact Letter to Parents



MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

CONTACT LETTER TO PARENTS

Dear Parent or Caregiver,

We are writing to you because your baby was born preterm (before 37 completed weeks of gestation).

Preterm babies are at risk of having iron and fat soluble vitamin deficiencies. Preterm babies are born too soon, which can mean they have not had enough time to develop adequate nutrient stores. These nutrients all have important roles within the body and are needed for the normal growth, health and well being of your baby. Preterm babies are particularly at risk of having iron and vitamin D deficiencies. We would therefore like to find out whether the current feeding and supplementation practices are enough to stop deficiencies developing in these babies born preterm. From this study you will find out if your baby is deficient in any of these nutrients.

We are interested in investigating nutrient stores in any baby born preterm, including those with varying degrees of immaturity (from those babies born extremely preterm to those who are only just preterm). The study is being run through Massey University in collaboration with Auckland District Health Board and specialist health professionals from the Neonatal Intensive Care Unit. If your baby was born before 37 weeks gestation and you live in the Auckland area you may qualify to take part in this study. The aim of the study is to look at the nutrient status and feeding practices of preterm babies after they have been discharged from hospital.

If you think you might be interested in this study, please complete the sheet below and leave at reception OR contact Owen Mugridge on:

O.Mugridge@massey.ac.nz
OR 09 414 0800 extension 41174
OR TXT 021 160 5949

APPENDIX THREE: Contact Details Sheet



Post Discharge Nutrition of Preterm Babies: micronutrient status and feeding practices of preterm babies after hospital discharge

CONTACT DETAILS SHEET

CONTACT DETAILS

Your Name: _____

Phone Number: _____

Mobile Number: _____

Email Address: _____

APPENDIX FOUR: Participant Consent Form - Infant



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UNIVERSITY OF NEW ZEALAND

Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

PARTICIPANT CONSENT FORM

INFANT

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

We would also like to ask your permission to have access to your baby's medical records, these will only be used for the purpose of this study, and all data obtained will be kept confidential.

As the parent/legal caregiver to my baby

Baby's Name

Please print

.....

I agree to have myself and my baby participate in this study under the conditions set out in the Information Sheet.

Signature:

Date:

.....

Full Name of Parent/legal caregiver

Please print

.....

APPENDIX FIVE: Participant Consent Form – Mother



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UNIVERSITY OF NEW ZEALAND

Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

PARTICIPANT CONSENT FORM

MATERNAL BLOOD TESTING

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I am currently breast feeding my baby and I agree to have a blood test taken, under the conditions set out in the Information Sheet.

Signature:

Date:

.....

Full Name – printed

.....



Preterm Infants – Post Discharge Nutrition Study - Standard Operating Procedure – Recruitment

Recruitment

1. Collect NHI number from Auckland City Hospital NICU log book for all live preterm infants born before 37 weeks gestation – Contact: Maureen Cassin, email: MCassin@adhb.govt.nz
2. Provide NHI numbers to Ward Clerk and have her post the patient labels to Massey University, IFNHH
3. Record batch labels in spread sheet – Record batch labels in R://PiFAN/PRETERM/Recruitment/
4. Send letters to each patient. Letters include:
 - a. Information Sheet
 - b. Contact letter
 - c. Contact detail sheet
 - d. Free post, self addressed envelope
5. Phone parents who return contact detail sheet and complete the Demographic sheet and assign a study ID i.e. PT0001B for infant and PT0001M for mother. Book participant in at a date as close as possible to 4 months after discharge. Collate data on the Volunteer Log Excel document – R://PiFAN/PRETERM/Recruitment/Volunteer Log
6. If contact detail sheet not returned to Massey University, call parents one week after sending letters to determine whether they would like to take part in the study. If yes, complete the Demographic sheet and assign a study ID i.e. PT0001B for infant and PT0001M for mother. Book participant in at a date as close as possible to 4 months after discharge. Collate data on the Volunteer Log Excel document.
7. File all completed demographic questionnaires and contact detail sheets in ring binder. Ring binder 1 has participants 1-35. Folder 2 participants 36 onwards.

NB: When booking appointments endeavour to book participants who live in the same area on the same day.

Preparation

1 week prior to appointment, send parent email with link to Survey Monkey questionnaires

Dear _____,

Thank you for enrolling yourself and your preterm baby in the Post-discharge Nutrition of Preterm Babies study. In preparation for your appointment on _____ at _____ am/pm could you please take some time to complete a quick questionnaire about yourself and your baby. There are no "right" or "wrong" answers. Accurate and thoughtful responses will allow us to pinpoint current practices.

If you have more than one baby enrolled in this study, please fill out a separate questionnaire for each child.

All of the data collected is anonymous and your answers will be held in strict confidence.

Below is the link to the questionnaire. Your baby's individual study ID is PT----B.

http://www.surveymonkey.com/s/preterm_baby_study

If you have any questions, please do not hesitate to contact me via telephone or email. I will be joined by my colleague Charlotte/Briar/ Jenny and we look forward to meeting you at our appointment at _____ address. In preparation for our arrival please keep _____ hydrated and their feet nice and warm as it will make things a little easier!

Kind regards,

Owen Mugridge

Telephone number

APPENDIX SEVEN: Demographics Questionnaire



MASSEY UNIVERSITY
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UNIVERSITY OF NEW ZEALAND

Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

MOTHER AND BABY DEMOGRAPHICS QUESTIONNAIRE

Date: _____
Participant ID: _____

Mother Name: _____

Mother Age: _____

Mother DOB: _____

Address: _____

Telephone: _____ Mobile: _____

Email: _____

Number of Infants: _____

Baby gender: _____

Baby name: _____

Baby DOB: _____

Hospital Discharge date: _____

Venue: _____

Section 1

What we need to know about the mother

1. Which ethnic group do you/the baby's mother belong to? (Please circle the one that most applies to you)

- New Zealand/European
- Other European
- New Zealand Maori
- Cook Island Maori
- Fijian
- Niuean
- Samoan
- Tongan
- Tokelauan
- Other Pacific Island
- Chinese
- Other Asian
- Indian
- South East Asian
- Other

3. Was this your first baby? (Please circle) Yes No

If no, how many other children do you (the mother) have and what are their ages? (Please state)

Section 2 - What we need to know about your baby

4. Which ethnic group does your baby belong to? (Please circle the one that most applies to your baby)

- New Zealand/European
- Other European
- New Zealand Maori
- Cook Island Maori
- Fijian
- Niuean
- Samoan
- Tongan
- Tokelauan
- Other Pacific Island
- Chinese
- Other Asian
- Indian
- South East Asian
- Other

5. What gestational age was your baby born at?

_____ **Weeks** _____ **Days**

Appointment date/time: _____

Appointment location: _____

Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

Interview Schedule

Prior to conducting the interview, the interviewer must ensure that an appointment has been made and that consent has been obtained from the mother/family. Any culturally specific or additional support requirements should also have been discussed with the parent prior to interviews.

Each interviewer should follow this schedule when conducting the data collection to ensure consistency in data collection.

1) Meet and Greet

a. Introduce yourself by name.

b. Explain to the parent(s) that you are there to collect data for the “Post discharge nutrition of preterm babies” study.

c. Ask the parent(s) if this is good time for you to complete your data collection. If not, organise another appointment date.

2) Explain to the parent(s) what you are going to do during this interview

a. Explain that you will be weighing their baby and measuring their length and head circumference.

b. Explain that you will be obtaining blood samples from the baby and from the mother if she is still breast feeding and consents to the blood tests.

c. Explain that you will also be administering questionnaires: a feeding questionnaire, a supplement questionnaire and a sun behaviour questionnaire if mother has not already completed the online questionnaires.

3) Ask the parent(s) where is the most convenient place for you to conduct your measuring, blood sample taking and interviews. Ensure that this is a safe place to measure the baby.

4) Explain that if they have any questions that they should feel free to ask you to clarify anything.

5) If mother has not completed online questionnaires, administer the feeding questionnaire following the questions in order.

a. Administer a separate questionnaire for each infant.

6) If mother answers yes to currently breast feeding advice her that:

“Since you are currently breastfeeding it would be really valuable for this study to know whether your iron and vitamin D status is affecting your infant’s status. Would you be willing to have your vitamin D and iron status tested as well?” (Or words to that affect).

- a. If she answers yes, ask her to complete the maternal blood collection consent form.
- 7) Administer the supplement questionnaire following the questions in order.
 - a. Administer a separate questionnaire for each infant.
- 8) Administer the sun behaviour questionnaire following the questions in order.
 - b. Administer a separate questionnaire for each infant.
 - c. Assess skin colour using the Fitzpatrick skin colour tool – record on data collection sheet
- 9) Weigh the baby - refer to the SOP
- 10) Measure the baby's head circumference – refer to the SOP
- 11) Measure the length of the baby using a length board with a fixed foot piece and movable headpiece - refer to the SOP
- 12) Collect maternal blood samples - refer to the SOP
- 16) Collect infant's blood samples - refer to the SOP for Infant blood collection.
- 17. Advise the parents that they will receive a summary of the blood results (if applicable) along with a summary of the anthropometric measurements taken, when 25(OH)D results are available.
- 18. Ensure that you have completed the below checklist – which should be checked off on the data collection sheet.

Thank the mother and family for their time and that they should feel free to contact Massey University if they have any questions or concerns.

Checklist

Task	√ or X
Completed demographic form for each infant	
At least two recorded weight measurements per infant	
At least two recorded head circumference measurements per infant	
At least two recorded length measurements per infant	
Infants blood samples obtained	
Completed interviewer administered medical questionnaire per infant	
Completed interviewer administered feeding questionnaire per infant	
Completed interviewer administered sun behaviour questionnaire per infant	
Completed Maternal blood sample consent form (if currently breastfeeding)	
Mothers blood samples (optional for breast feeding mother)	
Administer the three day food record for each infant	



Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

Data Collection Sheet

Date: _____

Baby ID: _____

Baby DOB: _____

Age in days: _____

Health Screening Questionnaire completed	<input type="checkbox"/>
Criteria for inclusion in the study met	<input type="checkbox"/>
Informed Consent completed	<input type="checkbox"/>
Demographic Questionnaire completed	<input type="checkbox"/>
Anthropometric Measurements taken	<input type="checkbox"/>
Fitzpatrick Skin Assessment score	<input type="checkbox"/>

Baby's weight at 4 months after discharge: _____ kg

Baby's length at 4 months after discharge: _____ cm
_____ cm
_____ cm
Mean : _____ cm

Baby's head circumference at 4 months after discharge: _____ cm
_____ cm
_____ cm
Mean : _____ cm

Infant's Blood Tests:

Completed:

Detail of any issues with blood collection

Mother's Blood Tests:

Currently breast feeding or expressing breast milk YES/NO

If no, blood sample from mother not required. If yes:

Informed consent for blood sample	<input type="checkbox"/>
Consent for blood sample declined	<input type="checkbox"/>
Fitzpatrick Skin Assessment score	<input type="checkbox"/>

Completed:
Arranged: Date: _____

Feeding and Supplement Questionnaire completed

Sun exposure questionnaire completed

Second Visit

Date arranged _____

To be arranged _____

Declined _____

Explained to parent how they will receive their results

Thank parent for their time and participation in the study



Sun Exposure Questionnaire

Date: _____

Participant ID: _____

Participant DOB: _____

1. Since your baby was discharged from hospital have you received any advice about sun exposure for your baby?

Yes/No/Not sure/Can't remember (delete as appropriate)

If mother said yes please ask her what the advice was and who gave the advice

General practitioner

Midwife

Plunket Nurse

Other (please state)

Is the data for this questionnaire being collected in summer (September to April) or winter (May to August).

Summer

Winter (delete as appropriate)

2. For the following questions please answer

Always, Usually, Sometimes, Rarely or Never. (Please circle)

How often do you expose your baby to sunlight? Always Usually Sometimes Rarely Never

Do you usually keep your baby in the shade? Always Usually Sometimes Rarely Never

Do you usually apply sunscreen to your baby before going into the sun? Always Usually Sometimes Rarely Never

Do you put a hat on your baby? Always Usually Sometimes Rarely Never

Do you put protective clothing on him/her? Always Usually Sometimes Rarely Never

(protective clothing, e.g. long shirts, long pants, rash vests)

We would like to assess your baby's skin tone using this skin colour guide.

Place the skin tone guide next to the skin and identify which skin type most accurately reflects the baby's skin tone. Use under the arm for this measurement as this skin is not usually exposed to sun.

Mother's Score: _____

Baby's score: _____

If you (the mother) are currently breastfeeding please fill out question 3 below

3. For the following questions please answer

Always, Usually, Sometimes, Rarely or Never. (Please circle)

How often do you expose your skin to sunlight? Always Usually Sometimes Rarely Never

Do you usually keep in the shade? Always Usually Sometimes Rarely Never

Do you usually apply sunscreen whilst outside in the sun? Always Usually Sometimes Rarely Never

Do you wear a hat whilst outside? Always Usually Sometimes Rarely Never

Do you wear protective clothing whilst outside in the sun? Always Usually Sometimes Rarely Never

4. Do you wear covering for cultural or religious reasons? (*question for the mother*)

Yes

No

Please provide details (for example veil)

Thank you



Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

Supplement Questionnaire

Date_____

Participant ID:_____

Participant DOB_____

1. Was your baby discharged from Auckland Hospital on any supplements? *(please tick)*
Yes
No *(Finish questionnaire)*
Not sure *(List supplements: Vitadol C, Ferro-Liquid (or Ferrous Sulphate) to jog the mother's memory)*
2. Was your baby discharged from your local hospital on any supplements? *(please tick)*
Yes
No *(Finish questionnaire)*
Not sure *(List supplements: Vitadol C, Ferro-Liquid (or Ferrous Sulphate) to jog the mother's memory)*
N/A
3. Which supplements were your baby discharged home on? *(please tick)*
Vitadol C
Ferro-Liquid/ Ferrous Sulphate
Other:_____
4. What dose was your baby discharged from hospital on?
(Write N/A if vitamin/mineral not prescribed or don't know if mother doesn't know)
Vitadol C: _____
Ferro-Liquid/Ferrous Sulphate: _____
Other: _____
5. Has the dose of this/these supplements ever been changed since your baby was discharged? *(please circle)*
Yes

No
Don't Know

6. What was the dose changed to?
(Write N/A if vitamin/mineral not prescribed or don't know if mother doesn't know)

Vitadol C: _____

Ferrod-Liquid/Ferrous Sulphate: _____

Other: _____

7. After discharge, did you give these to your baby daily? (please circle)

Yes, every day (continue to question 8)

Yes, most days

Yes, some times

No, never

8. If not every day, how often did you give them to your baby?

9. What were some barriers/ issues you experienced with giving your baby supplements every day?

10. Are you still giving your baby these supplements? (please circle)

Yes

No

11. If no, when did you stop giving your baby these supplements?

12. If no, why did you stop giving your baby these supplements?

Thank you



Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

Feeding Questionnaire

Date ___/___/___

Participant ID: _____

Participant DOB ___/___/___

When your baby was discharged

These questions relate back to when your baby was discharged from the hospital.

1. On the day you were discharged from hospital how was your baby fed? (please circle the one which most applies)

Breast fed

Bottle fed

Tube fed (*tube through their nose or mouth*)

Combination of breast/bottle /tube

Please specify for example breast fed and topped up with a tube feed

2. What was your baby fed when first discharged? (You can chose more than one option if relevant)

Breastmilk (Go to question 3)

Breast milk and formula (Go to question 3)

Formula (Go to question 4)

Cows milk (go to question 4)

Other: _____

3. If you were breastfeeding or giving expressed breast milk, how long did you continue this after discharge? (or document if still breast feeding/providing EBM)

- 3b. Are you currently:

Exclusively breast feeding

Breast feeding and solids

Partially breastfeeding/ formula feeding

Formula feeding

3a. If you are currently breast feeding have you/the mother been diagnosed with iron deficiency?
(Please circle)

- Yes
- No
- Don't know

3b. Are you currently vitamin D deficient? (Please circle)

- Yes
- No
- Don't know

4. After you were discharged from hospital were you adding anything to your baby's feeds or giving any supplements after feeds?

- Yes _____(specify)
- No

5. . If formula fed, what formula was your baby fed after discharge? (Please circle)

- Post discharge preterm formula (S-26 Gold Pregro)
- De- Lact
- Heparon Junior
- Karicare Gold Plus 1 from Birth
- Karicare Gold Plus 2 from 6 months
- Karicare AR All Ages
- Karicare Follow On 2 From 6 months
- Karicare Goat 1 From Birth
- Karicare Goat 2 From 6 months
- Karicare Gold 1 From Birth
- Karicare Gold 2 From 6 months
- Karicare HA AR All ages
- Karicare HA Gold Plus All ages
- Karicare Infant 1 From Birth
- Karicare Soy All ages
- Kindergen
- Locasol
- MCT Peptide
- Monogen
- NAN HA 3
- NAN HA GOLD 1
- NAN HA GOLD 2
- Neocate Advance (unflavoured)
- Neocate Advance (vanilla)
- Neocate LCP
- Novalac AC

- Novalac AR
- Novalac IT
- Novalac SD
- Novalac Stage 1 and 2
- Nurture Follow-on Formula 2
- Nurture Gold Follow-on Formula 2
- Nurture Gold Infant Formula 1
- Nurture Plus Gold Infant Formula All Ages
- Nurture Infant Formula 1
- Pepti-Junior Gold
- S-26 Gold AR
- S-26 Gold Lactose Free
- S-26 Gold Newborn
- S-26 Gold Progress
- S-26 Original Newborn
- S-26 Original Progress
- S26 Soy
- SMA
- Other _____(specify)
- Don't remember

If mother is currently breast feeding or expressing breast milk go to question 6 otherwise skip to section on starting solids

6. If you are currently breast feeding or expressing breast milk (fully or partially) are you willing to have a blood test to check your iron and vitamin D status? *(please tick)*
- Not applicable (Go to section on starting solids)
- Yes *(arrange for mother to sign consent form and have a blood sample taken)*
- No

7. Are you taking any supplements during this time? If yes, which ones *(collect brand name of any supplements the mother is taking) (please tick)*

- Elevit
 - Blackmores Pregnancy and Breastfeeding Gold
 - Other multivitamin *state*
- which* _____

- Iron
 - Calcium
 - Vitamin D
 - Iodine
 - Other *state*
- which* _____

8. Are you a vegetarian/vegan? *(please tick)*
- Yes
- No

Baby's First Foods

1. Is your baby currently eating any solids? *(please circle)*
- Yes
- No (Go to question 10)
2. Who suggested that you started solids? *(please tick)*

- Plunket nurse
- General practitioner
- Neonatologist/paediatrician
- Family member
- Myself
- Other (please state) _____

Explore the reason for starting solids (Find out if it was due to advice or because the baby seemed hungry)

3. When did your baby start eating solids? _____ (Date)
or if not known – age

Weeks _____

Months _____

(ask whether this is their chronological age (age since their birth) or their corrected age).

Don't remember

4. What was your baby's first food? *(please circle)*

Ready-made baby food

Homemade foods

Rusk

Baby rice

Fruit

Vegetables

Yoghurt

Breakfast cereal

Meat

Other _____

5. When did you introduce red meat?

Date: _____

Or if you are unsure of date, how long after introducing the first food did your baby eat red meat?

Days: _____

Weeks: _____

Haven't introduced red meat yet *(please circle if this applies)*

6. When did you introduce other meat, for example chicken, pork or fish?

Date: _____

Or if you are unsure of date, how long after introducing the first food did your baby eat other meat?

Days: _____

Weeks: _____

Haven't introduced other meat yet *(please circle if this applies)*

7. How many times a day does your baby eat solid foods? _____

8. Approximately how much does your baby eat at each time? *(hint: teaspoons, tablespoons etc)*

9. How often do you usually give your baby these types of solid foods?

	More than once per day	Once per day	3 or more times per week	Once or twice per week	Less than once per week	Never
Fresh fruits						
Fresh Vegetables						
Ready made foods (such as jars of baby food)						
Breakfast cereals						
Rice or Pasta						
Breads						
Potatoes						
Potato products e.g. chips, crisps						
Butter or margarine						
Beef						
Lamb						
Pork including ham						
Chicken & other poultry						
Fish						
Eggs						
Beans, lentils, chickpeas						
Tofu						
Nuts						
Cheese or yoghurt						
Puddings or desserts						
Biscuits, sweets or cake						

10. Has your baby ever had any liquids other than breast milk or infant formula? *(please circle)*

Yes

No (Finish interview)

11. How old was your new baby the first time he or she drank liquids other than breast milk or formula?

Weeks _____

Months _____

Don't remember

12. What was your baby's first liquid other than breast milk or formula? *(please circle)*

Cow's Milk

Soya Milk

Goats Milk

Juice

Tea

Water

Other: _____

Thank you



Preterm infants – Post Discharge Nutrition Study – Blood collection Protocol – Study code: 26

Barcode labelling before collection day:

1. Complete participant details – name and DOB on Waitemata District Health Board laboratory services request form. Make one photocopy of this form and keep as record.

2. Each family has a set of unique identification barcode labels. Each member has 5 barcode strips labels. Suffix **M** for mother and suffix **B** for baby.

Naming convention for sample: (There are four components to barcode label)

- a) PT – Preterm study code
- b) Visit time – 0 for baseline and 2 for second visit
- c) Patient consecutive number: 001, 002 to 999
- d) Suffix M for mother and B for baby

For example:



Indicate - Preterm/baseline visit/subject#001/mother
Her child would be PT0001B

3. Stick one of each barcode labels on sample collection record.

4. Stick one of each ‘mother’ labels onto 10ml BD vacutainer tubes (1x gold and 1x purple). And stick remaining ‘mother’ label onto maximum recovery 2ml microcentrifuge tubes, put barcode labels length wise on the microcentrifuge tubes.

5. Stick one of each ‘baby’ labels onto small BD microtainer tubes (1x gold and 1x lilac). And stick remaining label onto maximum recovery 2ml microcentrifuge tubes, put barcode labels length wise on the microcentrifuge tubes.

6. Upon completion of blood collection run, the phlebotomist will deliver the purple top tubes directly to North Shore hospital for analysis. Meanwhile, the remaining gold top tubes which have been kept in cold storage will return to B27 laboratory for processing.

Blood collection:

The phlebotomist will take the original completed Waitemata District Health Board laboratory services request form to verify participant identity at their home.

The vacutainer must be filled to full capacity to ensure sufficient amounts of serum and plasma and more importantly, to ensure the proper blood-to-additive ratio.

Invert gold top tubes (serum collection) 5 times at blood collection. (Manufacturer recommendation)

After the blood samples have been taken the blood tubes should be placed into cold storage box and keep in an upright position in a suitable container. The phlebotomist should then take the blood tubes to the respective laboratory.

Prepared By;
O.Mugridge
Date: July, 2013

Reviewed By:
B.Emmett, C.Moor, P.C.Tong
Date: July, 2013

Approved By
Dr. C. Conlon
Date: July, 2013



Standard Operating Procedure Infant Weight

BACKGROUND

The purpose of this SOP is to standardise weighing procedures to ensure that all measurements are accurate and precise.

SCOPE

Applies to all researchers involved in the Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge study.

PROCEDURE

Equipment

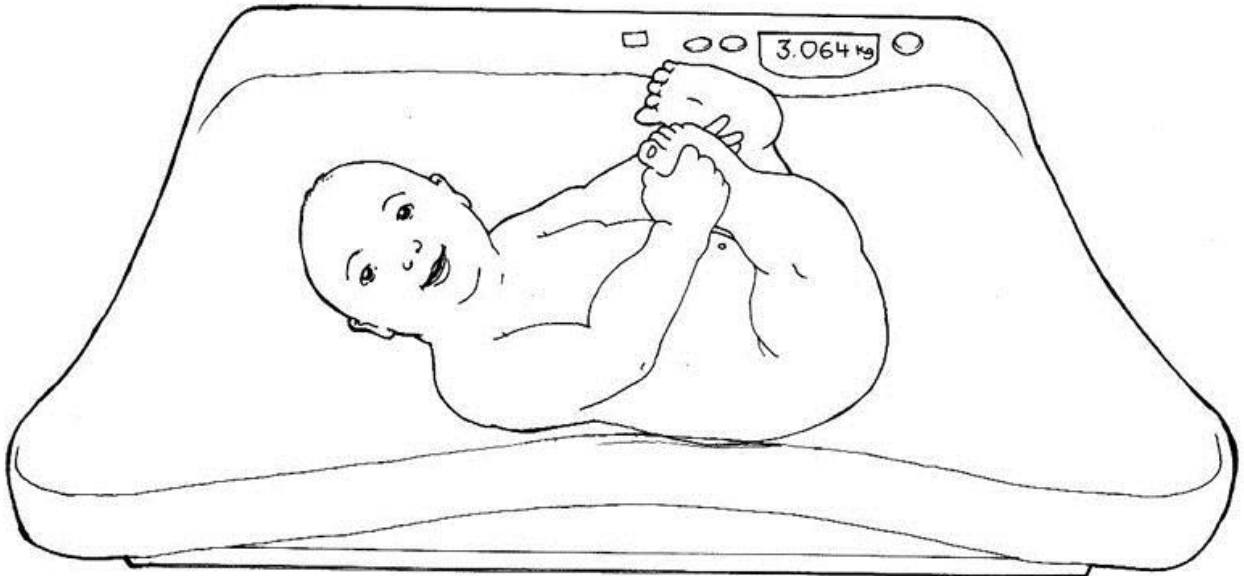
- Infant weighing scales

Calibration of Scales

- Calibration of the baby scale is done twice a week at a minimum.
- Ensure that the scale is placed on an even, flat surface. Check whether the scale is level using the bubble on the far right-hand leg of the scale. If you find that the scale is not level, the legs of the scale may be individually adjusted until the bubble lies in the centre of the window. There must be enough light to read the display.
- Turn on the scale
- Beginning with the 0.5kg weight, place the weight in the middle of the scale. The display should now read 0.500. Mark the value on the calibration form.
- Repeat this process with the 1kg, 2kg, 3kg 5kg and 8kg weights. This will allow checking across the full range of weights required for this study. Record the obtained values on the calibration form.
- Also calibrate the scale using the tare function. Place the 0.5kg on the scale, press the tare button. Wait until the display shows 0.000. Then place the 2kg weight on the scale and check that the value is 2kg.
- If the reading deviates from the expected value, remove the weight, ensure that the scale is on an even surface and that nothing is interfering with the weighing platform. Repeat the measurement again.
- If the reading still deviates from the expected value, inform the lead anthropometrist.

Weighing Procedure

1. Ensure that the digital baby scales are clean and calibrated.
2. Place digital baby scales on a hard surface.
3. Explain to the parent(s) that you will need to weigh the baby while they are naked to ensure that you get an accurate weight. Ask for their consent and help to remove their baby's clothes and diaper. If the parent(s) refuse, weigh the baby with their diaper on and record that consent was not obtained to weigh their baby naked.
4. Ask parent whether infant has recently been fed. Record how long since last feed.
5. Tare/zero the scales.
6. Place baby in the middle of the scales.



7. Record weight to nearest gram.
8. Take baby off scales and tare/zero scales again.
9. Reposition the baby in the middle of the scales and weigh again. Record weight.
10. If the two measurements differ by more than 50g, take a third measurement
11. Place a nappy on the baby. Ask the parent(s) if they would like the same nappy to be put back on or if they would prefer a clean nappy.
12. Repeat steps 3 to 11 for the next infant if more than one baby is being assessed.
13. If the infant becomes very agitated during measurements, the measurers should wait for him or her to calm down before continuing. This is important for both mothers and babies.
14. Before ending the anthropometry session check the forms for completeness.

Prepared By;
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Date: January, 2013

Reviewed By:
O. Mugridge
Date: January 2013

Approved By:
Dr. C. Conlon
Date: January 2013



Standard Operating Procedure ***Head Circumference Measurement***

BACKGROUND

The purpose of this SOP is to standardise head circumference measurement procedures to ensure that all measurements are accurate and precise.

SCOPE

Applies to all researchers involved in the Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge study.

PROCEDURE

Equipment

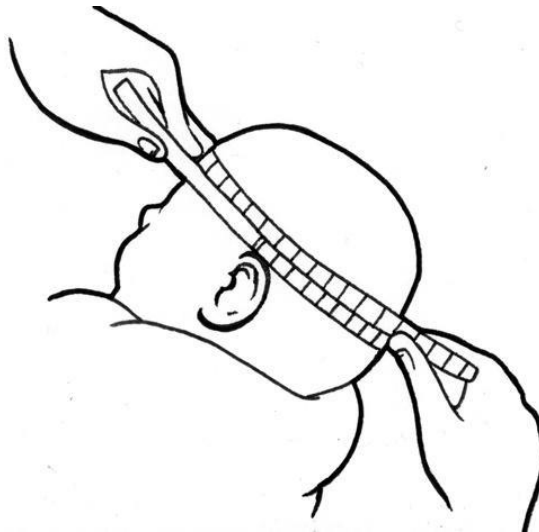
- Metal tape measure

Measuring Procedure

1. Hair pins or head bands should be removed as they interfere with the positioning of the tape around the head.
2. The infant is held on the assistant measurer or mother's lap. It is not always easy for the measurer to manipulate and secure the tape correctly around the head because many infants, especially the older infants, find this measurement uncomfortable.



3. The lead measurer sits by the side of the mother or of the observer, who is holding the infant.
4. Take care that the side of the tape marked in centimetres is on the outside for the reading, with the zero end in the inferior position.
5. Loop the tape before slipping it over the head.
6. The measurer anchors the tape just above the eyebrows, with the zero point on the side closest to him or her. *In some manuals, it is recommended to wrap the tape around the fullest head circumference. However, the forehead anchor point is important for standardized measurement within and across sites.*
7. At the back of the head, the tape is positioned over the fullest protuberance of the skull.
8. The other measurer helps by positioning the tape correctly, i.e. level, on the other side of the head.
9. Once the tape is positioned correctly, pull tight to compress the hair and skin. Be careful not to pull the tape too tight and cause injury to newborns. Keep hands and fingers out of the way for the reading.



10. Take the reading to the last completed 1mm and remove the tape from the infant's head.
11. Write the value obtained in the corresponding section of the form.
12. Repeat the measurement ensuring that the measurements are within 5mm. Do a third measurement if necessary.

Prepared By;
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Date: January, 2013

Reviewed By:
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Date: January 2013

Approved By:
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Date: January 2013



Standard Operating Procedure ***Infant Length***

BACKGROUND

The purpose of this SOP is to standardise length measurement procedures to ensure that all measurements are accurate and precise.

SCOPE

Applies to all researchers involved in the Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge study.

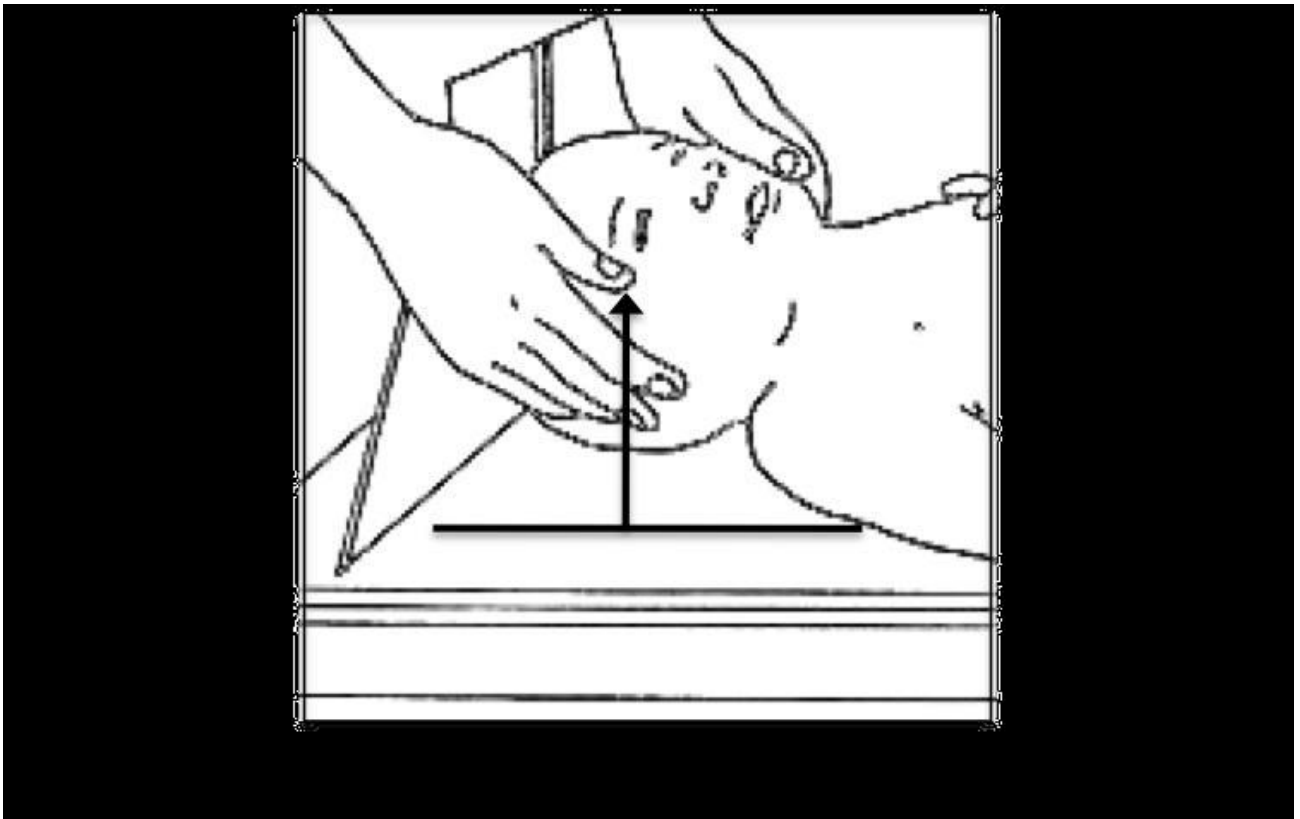
PROCEDURE

Equipment

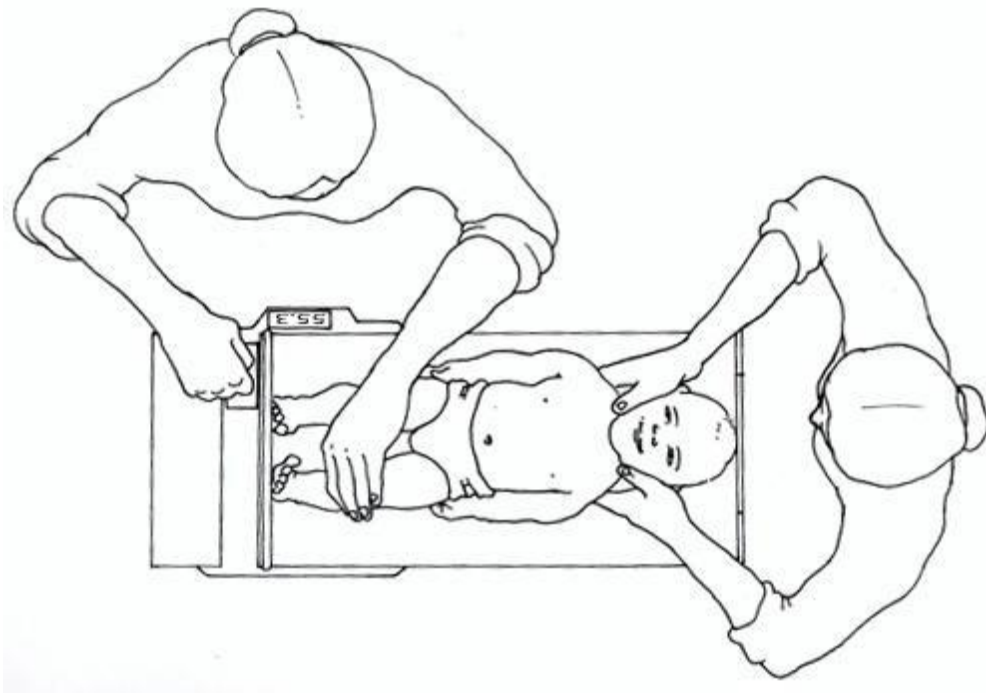
- Length Board

Measuring Procedure

7. The infantometer is placed on a raised flat surface like a large table so that it is level and stable.
8. Ask the mother to remove the infant's clothes if this has not already been done for the weight measurement. Measuring length can provoke anxiety and crying in infants. The mother should be asked to calm the baby. To avoid causing discomfort, cover the horizontal board with a thin cloth or soft paper.
9. Any hair ornaments should be removed if they interfere with positioning the head. Diapers increase the difficulty of holding the infant's legs together and straightening them out, so they should be removed for this measurement.
4. The lead measurer stands on the side to hold down the baby's legs with one hand and move the foot board with the other hand. The assisting measurer stands at the head board and positions the infant's head.
5. The head should be positioned correctly and legs and feet held firmly to allow an accurate measurement. The assisting measurer holds the infant's head so that the top of the head touches the fixed headboard. Position the infant's head such that a vertical line from the ear canal to the lower border of the eye socket is perpendicular to the horizontal board. This head position is known as the Frankfort Vertical Plane.



To keep the infant's head in the correct position, the assisting measurer gently cups his or her hands over the infant's ears. The mother can stand close on the side to reassure the infant. The lead measurer positions the infant so that shoulders and hips are aligned at right angles to the long axis of the body. Gentle pressure is applied on the knees to straighten the legs.



6. To take the measurement, the foot board is positioned gently against the infant's feet. The soles of the feet should be flat on the board, toes pointing upwards. If the infant bends the toes and prevents the foot board touching the soles of his or her feet, scratch the soles slightly and draw in the foot board when he or she draws the toes up. Take care that the knees are straightened only as far as they can go without causing harm to the infant. Be aware that for newborns and very premature infants, it is impossible to straighten the knees to the same degree as in older infants as they can be very fragile and could easily be injured if too much pressure is applied to their knees. Therefore, the measurer should apply only very minimum pressure on their knees. The assisting measurer should check that the infant is not arching the spine when the reading is taken, and should alert the lead measurer should the infant shift out of position. The footboard is pressed against the feet gently so that there is small compression of the tissue on the feet. The measurement is recorded to the last completed 1mm. For example, if the length is between 61.3cm and 61.4cm, write 61.3cm.
7. As a general principle, if the measurer cannot hold both legs because the infant is restless, obtain a one-leg measurement.
8. Read the measurement as soon as possible after the footboard has been positioned and make a note of this.
9. Re-measure the infant. Ensure that measurements are within 7mm.
10. Hand the infant back to the mother.
11. Write the value obtained in the corresponding section of the form without delay.

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Date: January, 2013

Reviewed By:
O. Mugridge
Date: January 2013

Approved By:
Dr. C. Conlon
Date: January 2013



Standard Operating Procedure
Infant Heel Prick

- 1) Ensure the mother has provided written and verbal consent for a blood sample to be taken from the infant
- 2) Ask parent to place infant on their knee, supporting their back and letting the infants foot hang off their leg so that gravity can help with blood collection
- 3) Place a paper towel below the infants leg and on the ground below the infants foot
- 4) Remove any socks/shoes
- 5) Ensure hands are clean and sterilised and wear fitted non sterile gloves
- 6) Gently warm and massage the infants foot to increase blood flow
- 7) Using an alcohol wipe clean the puncture site and allow to air dry
- 8) Using the lancet, puncture the skin on the outer side of the infants heel using a quick deliberate stroke
- 9) Wipe away the first drop of blood to avoid contamination with tissue fluid or debris
- 10) Hold the infants heel firmly, but avoid squeezing it too tightly as this may cause dilution of the specimen with tissue fluid and increases risk of haemolysis
- 11) Collect blood in the gold capped pre-labelled collection tube. Collect no less than 600µL
- 12) If the infant's blood clots preventing sample removal, ask the parent if you can make another heel prick and follow steps 1-7.
- 13) When blood collection is complete apply pressure with cotton wool to stop bleeding.
- 14) Ask parent if their infant is allergic to plasters, and if not ensure it is ok to place plaster over puncture site.
- 15) Thank the mother and infant
- 16) Discard all waste into the sharps bin
- 17) Place blood collection tube into the ice filled polystyrene box to keep cool, ensure that it is kept out of direct light.
- 18) Repeat steps 16 and 17 if more than one infant

Prepared By;
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Date: February, 2013

Reviewed By:
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Date: February 2013

Approved By
Dr. C. Conlon
Date: February 2013



Standard Operating Procedure Maternal Blood Sample

- 1) Ensure the mother has provided verbal and written consent for the blood sample
- 2) Ask the mother to sit upright in a comfortable position
- 3) If the mother is anxious or afraid reassure her and ask what would make her more comfortable
- 4) Ensure hands are cleaned and sterilised and put on fitted non sterile gloves
- 5) Place a clean paper towel under the mothers arm
- 6) Extend the mothers arm locate a vein that is clearly visible and of good size
- 7) Apply the tourniquet about 4-5 finger widths above the venipuncture site.
- 8) Re-examine the vein
- 9) Clean the site to be punctured with an alcohol swab and allow to air dry
- 10) Anchor the vein by holding the patients arm and placing a thumb below the venipuncture site
- 11) Ask the mother to form a fist to allow the veins to be more visible
- 12) Place the needle into the vein at a 30 degree angle and remove blood sample required.
- 13) Once sample is retrieved release the tourniquet before removing the needle
- 14) Remove the needle gently, apply pressure to the puncture site with a clean piece of cotton wool to stop blood flow
- 15) Ask the mother to hold the piece of cotton wool in place whilst her arm is extended and slightly raised.
- 16) Ask the mother if she is allergic to plasters, if she is not place one over the puncture site.
- 17) Thank the mother and reassure she is ok.
- 18) Place the labelled collection tube into the ice filled polystyrene box to keep cool
- 19) Discard all waste into the sharps bin
- 20) Remove gloves and sanitise hands.
- 21) Ensure blood samples are kept cool and out of direct light until sample processing can be carried out.

Prepared By;
O.Mugridge
Date: February, 2013

Reviewed By:
B.Emmett, C.Moor, P.C.Tong
Date: February 2013

Approved By
Dr. C. Conlon
Date: February 2013



Preterm infants – post discharge nutrition study – Blood Processing – Study code: 26

Blood processing:

Ensure ALL laboratory personnel must have their Hepatitis antibody checked (and be immunised, if necessary) BEFORE handling human blood. While risk associated with exposure to blood and tissues (potentially) contaminated with Hepatitis B can be eliminated by vaccination, the potential risk of infection from other infectious agents such as Hepatitis C, HIV and CJD can only be REDUCED by following careful safety measures when handling specimens.

NOTE:

A. Serum and plasma should be separated from cells (centrifugation) within 2 hours after sample has been taken.

B. Gold top tube (serum collection) should stand for at least 30 min at room temperature until clotted before centrifuging. Manufacture recommendation is 60 minutes.

C. Heracus Labofuge 400R internal chamber must be pre-cooled to 4°C before use.

D. By holding the vacutainer tube away from your face and over the top of the biohazard waste container, then wrap the lip of vacutainer tube with large tissue paper and gently twist to remove blood tube lid. This will avoid exposure to any aerosols created by opening the vacutainer tubes. Place tissue paper in biohazard waste container and return the vacutainer tube to the rack.

Big vacutainer tube:

Using Heracus Labofuge 400R swing bucket rotor **#8179** for this step.
Centrifuge the vacutainer tubes @ 3500 rpm (1547rcf) for 10 minutes at room temperature.

Small vacutainer tube:

Using Heracus Labofuge 400R microcentrifuge rotor **#3325** for this step.
Proceed to centrifuge the vacutainer tubes @ 4000 rpm (1520rcf) for 10 minutes at room temperature.

Sample Aliquoting

Use the labelled eppendorf tubes to aliquot the serum. Make sure that the barcode on the vacutainer matches the barcode on the eppendorf tube. Aliquot a minimum of 250µL from the big vacutainer and everything from the small microtainer.

Storing

Store the labelled sample by Date and ID in the -80°C Freezer.

Prepared By;
O.Mugridge
Date: July, 2013

Reviewed By:
B.Emmett, C.Moor, P.C.Tong
Date: July, 2013

Approved By
Dr. C. Conlon
Date: July, 2013

APPENDIX TWENTY: Standard Operating Procedure – Preparation to Send Samples for Batch Analysis



- 1) Ensure WDHB are aware that samples are being dropped off at the previously arranged time
- 2) Ensure all participant ID codes are in date order and placed onto an A4 sheet of paper so that these are easily accessible
- 3) Ensure all barcodes are readable and all freezer boxes are labelled with Study ID and Study Centre
- 4) Have a polystyrene box filled with dry ice
- 5) Remove samples from -80 degree freezer and double check all samples to ensure there is enough serum for sample analysis
- 6) Once checked place all of the containers with the eppendorf tubes into the polystyrene box and place lid on so these remain frozen. Seal lid loosely to allow CO2 to escape.
- 7) Label all packaging boxes with address of recipient and sender
- 8) Tape inventory and all sample delivery documents to the top of the box
- 9) Transport these promptly to North Shore Hospital using Sub60 sample courier services.
- 10) Leave them with the arranged staff member for analysis – recipient to sign both copies of sample delivery form
- 11) Discard of the dry ice appropriately

Prepared By;
O.Mugridge
Date: July, 2013

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Date: July, 2013

Approved By
Dr. C. Conlon
Date: July, 2013

APPENDIX TWENTY ONE: Blood Sample Delivery Form



MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Sample Delivery Form

I can confirm that I have received the following samples and documentation:

Study ID _____

Sample Type _____

Sample Number _____

Institution _____

Sender _____

A Copy of the Sample Delivery Form

Sample Inventory

Recipient:

Signature _____

Printed _____

Sender:

Signature _____

Printed _____

Prepared By;
O.Mugridge
Date: July, 2013

Reviewed By:
B.Emmett, C.Moor, P.C.Tong
Date: July, 2013

Approved By
Dr. C. Conlon
Date: July, 2013

Assay Procedure

Principles of the Procedure

The ADVIA Centaur VitD assay is a one-pass, 18-minute antibody competitive immunoassay that uses an anti-fluorescein monoclonal mouse antibody covalently bound to paramagnetic particles (PMP), an anti-25(OH) vitamin D monoclonal mouse antibody labelled with acridinium ester (AE), and a vitamin D analog labelled with fluorescein.

An inverse relationship exists between the amount of vitamin D present in the patient sample and the amount of relative light units (RLU) detected by the system.

For detailed instructions on performing the procedure, refer to the system operating instructions or to the online help system.

Before placing samples on the system, ensure that samples have the following characteristics:

- Samples are free of fibrin or other particulate matter. Remove particulates by centrifugation at 1000 x g for 10 to 15 minutes.
- Samples are free of bubbles.

This assay requires 20 µL of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. For detailed information about determining the minimum required volume, refer to *Sample Volume Requirements* in the system operating instructions or to the online help system.

Vitamin D

The ADVIA Centaur and ADVIA Centaur XP systems automatically perform the following steps:

1. Dispenses 20 µL of sample into a cuvette, and incubates for 15 seconds.
2. Dispenses 200 µL of Ancillary Pack Reagent, and incubates for 4.5 minutes at 37°C.
3. Dispenses 50 µL of Lite Reagent, and incubates for 5.5 minutes at 37°C.
4. Dispenses 100 µL of Solid Phase reagent, and 50 µL of ancillary well reagent, and incubates for 2.75 minutes at 37°C.
5. Separates the Solid Phase from the mixture, and aspirates the unbound reagent.
6. Washes the cuvette with Wash 1.
7. Dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction.

The ADVIA Centaur systems report results according to the selected option, as described in the system operating instructions or in the online help system.

Calibrating the Assay

The ADVIA Centaur VitD assay requires a Master Curve calibration when using a new reagent lot number. For each new lot number of Lite Reagent and Solid Phase, use the barcode reader or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values. For detailed information about entering Master Curve values, refer to the system operating instructions or to the online help system.

Calibrate the assay at the end of the 28-day calibration interval. Additionally, this assay requires a two-point calibration when:

- Changing lot numbers of primary reagent packs.

- Replacing system components.
- Quality control results are repeatedly out of range.

For detailed information about entering calibration values, refer to the system operating instructions or to the online help system.

Using Bar-Code Labels

Calibrator bar-code labels are lot-number specific. Do not use bar-code labels from one lot of calibrators with any other lot of calibrators.

Use the ADVIA Centaur VitD Calibrator bar-code labels to identify the Low and High Calibrator sample cups when performing the ADVIA Centaur VitD assays. Place the bar-code label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing a Calibration

Each lot of calibrators contains a Calibrator Assigned Value card to facilitate entering the calibration values on the system. Enter the values using the bar-code scanner or the keyboard.

Perform the calibration procedure using the following steps:

Note *This procedure uses calibrator volumes sufficient to measure each calibrator in duplicate.*

1. Schedule the calibrators to the work list.
2. Label two sample cups with calibrator bar-code labels: one for the low and another for the high.
3. Gently mix the Low and High Calibrators and dispense at least 0.5 mL into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay and ancillary reagents are loaded.
7. Start the entry queue, if required.

Note *Dispose of any calibrator remaining in the sample cups after 10 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh calibrators.*

Performing Quality Control

Follow government regulations or accreditation requirements for quality control frequency. To monitor system performance and chart trends, as a minimum requirement, 2 levels of quality control material should be assayed on each day that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

For quality control of the ADVIA Centaur VitD assay, use ADVIA Centaur VitD quality control material. Refer to the Expected Value card for the suggested expected values specific for the lot number of the controls.

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

Using Bar-Code Labels

Control bar-code labels are lot-number specific. Do not use bar-code labels from one lot of controls with any other lot of controls.

Use the ADVIA Centaur VitD quality control bar-code labels to identify the positive and negative sample cups when performing the ADVIA Centaur VitD assay. Place the bar-code label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup. Perform the quality control procedure using the following steps:

Note *This procedure uses control volumes sufficient to measure each control in duplicate.*

1. Schedule the quality control samples to the work list.
2. Label two sample cups with quality control bar-code labels: one for the positive and another for the negative.
3. Gently mix the quality control materials and dispense at least 250 μ L into the appropriate sample cups.
4. Load the sample cups in a rack.
5. Place the rack in the sample entry queue.
6. Ensure that the assay reagents are loaded.
7. Start the entry queue, if required.

Note *Dispose of any quality control materials remaining in the sample cups after 10 hours.*

Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials

Vitamin D

Taking Corrective Action

If the quality controls results do not fall within the Expected Values or within the laboratory's established values, do not report results.

Take the following actions:

1. Determine and correct the cause of the unacceptable control results:

- a. Verify that the materials are not expired.
- b. Verify that required maintenance was performed.
- c. Verify that the assay was performed according to the instructions for use.
- d. Rerun the assay with fresh quality control samples, and confirm that quality control results are within acceptable limits before running patient samples.
- e. If the quality control results are not within acceptable limits, recalibrate the assay, and repeat step d.
- f. If necessary, contact your local technical support provider or distributor for assistance.

2. Repeat testing of patient samples before reporting results.

Perform corrective actions in accordance with your established laboratory protocol.

Results

Results should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.

The system reports serum and plasma VitD results in ng/mL (common units) or nmol/L (SI units), depending on the units defined when setting up the assay. The conversion formula is $1 \text{ ng/mL} = 2.5 \text{ nmol/L}$.

For detailed information about how the system calculates results, refer to the system operating instructions or to the online help system.

Dilutions

Dilute and retest serum samples with vitamin D levels greater than 150 ng/mL (375 nmol/L) to obtain accurate results. Manually dilute the patient samples with ADVIA Centaur Vitamin D Diluent, and then load the diluted sample in the sample rack, replacing the undiluted sample. The recommended dilution is 1:2.

Ensure that results are mathematically corrected for dilution. If a dilution factor is entered when scheduling the test, the system automatically calculates the result.

Limitations

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.¹⁵ Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

Do not use hemolyzed samples.

Expected Values

Vitamin D Status Range

Deficiency < 20 ng/mL (50 nmol/L)

Insufficiency 20–30 ng/mL (50–75 nmol/L)

Sufficiency 30–100 ng/mL (75–250 nmol/L)

Toxicity > 100 ng/mL (250 nmol/L)

Performance Characteristics

Assay Range

The ADVIA Centaur VitD assay measures 25(OH)D from concentrations of 4.2 to 150 ng/mL (10.5 to 375 nmol/L). The low end of the assay range is defined by the limit of quantitation (LoQ).

Specificity

The ADVIA Centaur VitD Total assay shows high specificity for 25(OH)D₂ and 25(OH)D₃. The following compounds were tested with total 25(OH)D concentrations of 35 and 115 ng/mL. Percent change is calculated as: Percent cross-reactivity = (corrected assay value / amount of compound spiked) x 100

Sensitivity

The limit of blank (LoB), limit of detection (LoD), and the limit of quantitation (LoQ) were determined as described in CLSI Document EP17-A.20 The ADVIA Centaur VitD assay had an LoB of 1.7 ng/mL (4.3 nmol/L), an LoD of 3.20 ng/mL (8.0 nmol/L), and an LoQ of 4.2 ng/mL (10.5 nmol/L). The LoD is defined as the lowest concentration of 25(OH)D that can be detected with 95% probability.

The functional sensitivity of the ADVIA Centaur VitD assay is 3.33 ng/mL (8.33 nmol/L). The functional sensitivity was determined using multiple samples in the range of 2 to 10 ng/mL (5 to 25 nmol/L). All samples were assayed twice a day in replicates of 4 over 10 days using 2 lots (n = 320 for each sample) of ADVIA Centaur VitD reagents.

Linearity

Linearity was evaluated according to the CLSI protocol EP6-A.21. A sample containing high levels of total 25(OH)D was mixed in various proportions with a sample containing low levels of total 25(OH)D. The resulting sample mixtures were assayed for total vitamin D. On the ADVIA Centaur system, the VitD assay is linear from 4.2 to 150 ng/mL.

Standardization

The ADVIA Centaur VitD assay is standardized using internal standards which are traceable to LC/MS/MS. The relationship between the ADVIA Centaur VitD assay (y) and liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) (x) is described using linear regression as: $\text{ADVIA Centaur VitD} = 1.01 (\text{LC/MS/MS}) + 8.9 \text{ ng/mL}$, $r = 0.99$

APPENDIX TWENTY THREE: Fitzpatrick Scale of Skin Types

Table 11: Fitzpatrick scale of skin types

Type	Description
1	Always burning, never tan; sensitive to exposure; redheaded, freckles, Celtic background
2	Burns easily, tans minimally; fair-skinned, blue, green or grey eyes, Caucasians
3	Burns moderately, tans gradually to light brown; average Caucasian skin
4	Burns minimally, always tans well to moderately brown; olive skin
5	Rarely burns, tans profusely to dark; brown skin
6	Rarely burns, least sensitive; deeply pigmented skin

Source: SunSmart Partnership (2005).

Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge

Data Collection from Medical Notes

Date ___/___/___

Participant ID: _____

Date of discharge from hospital ___/___/___

About the Birth

1. Patient's DOB: _____

2. Patient's Gestational Age at birth: _____

3. Birth weight: _____

4. Birth length: _____

5. Head circumference at birth: _____

6. Is the baby a Single baby
 Twins
 Triplets
 Other (Please state) _____

7. Type of delivery:

Caesarean
Vaginal birth

8. What was the reason for the premature birth?

Spontaneous preterm labour
Severe infant growth restriction
Pre-eclampsia
Foetal distress
Placental abruption
Gestational diabetes
Infection
Other: _____

Inpatient relevant data

1. After the baby was born, was he or she put in an intensive care unit? (Please circle)

Yes
No

2. Did the baby have any medical complications after birth?

Respiratory distress syndrome

Pneumonia

Jaundice

Sepsis

Necrotizing enterocolitis

Anaemia

PDA

ASD

VSD

Other: _____

3. How old was the baby when he/she was discharged from hospital?

Date of discharge: _____

Corrected Age: _____

Chronological Age: _____

Feeding and Supplement History

1. Did the baby receive parenteral nutrition?

Yes, how long? (Days) _____

No

2. Whilst in hospital did the baby receive enteral nutrition (EXPRESSED BREAST MILK and/or infant formula)?

Yes

No

3. If baby was fed formula, which formula were they fed?

4. Whilst in hospital was the baby breast fed?

Yes

No

5. How was the baby fed when discharged?

Nasogastric or orogastric

Breast feeding

Bottle feeding

6. What was the baby being fed upon discharge

7. If baby was fed formula, which formula were they fed?

8. What supplements did the baby receive whilst in hospital?

Vitadol C

Other: _____

None: _____

9. What date were the supplements started?

Vitadol C: _____

Other: _____

10. What dose were supplements initially prescribed at?

Vitadol C: _____

Other: _____

11. What supplements were the baby discharged on?

Vitadol C

Other: _____

None: _____

12. What dose of supplements was the baby discharged on?

Vitadol C: _____

Other: _____

About the Mother

1. Did the mother have any medical complications during pregnancy?

Yes _____

Pre-eclampsia

Gestational Diabetes

Other : _____

2. Did the mother smoke during pregnancy?

Yes

No

APPENDIX TWENTY FIVE: Letter to Participants – Infant Blood Results



MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Address

Date

Dear

Thank you very much for enrolling into the Preterm baby research trial run by Massey University. You are one of the families recruited. The results collected show great promise to make a real difference in the feeding and supplementation practises of preterm infants.

Please find below a summary of the anthropometric and blood measurements taken at our appointment. “Normal” ranges are also included for your reference. If your child’s vitamin D value is outside of the normal range, a copy of this letter has been sent to your general practitioner. They will contact you if further assessment, clinical advice and intervention is necessary.

Whilst every effort was made to collect enough blood from our volunteering babies, on occasions it was not always possible. If a result reads “Insufficient sample size” unfortunately the blood sample collected was not large enough to analyse this measure.

Childs Name _____		
Measure	Result	Range
Length (cm)		
Weight (kg)		
Head circumference (cm)		
25(OH)D (vitamin D) (nmol/L)		<25nmol/L – Deficient <50nmol/L – Insufficient >200nmol/L – Above normal range

If you have any questions, please do not hesitate to contact me on the number below, or by email.

Kind regards,
 Owen Mugridge

Research Trials Manager
 o.mugridge@massey.ac.nz
 09 414 0800 extension 41174
 Massey University Oteha Rohe
 Albany Highway Albany 0632 New Zealand

APPENDIX TWENTY SIX: Letter to Participants – Maternal Blood Results



MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Address

Date

Dear

As a breastfeeding mother, you opted into the measurement of a blood sample for the Preterm baby research trial run by Massey University. Thank you for your participation, we will find the results extremely important when comparing your results with your baby's.

Please find below a summary of the blood measurements taken at our appointment. "Normal" ranges have also been included for your reference.

Mothers Name _____		
Measure	Result	Range
25(OH)D (vitamin D) (nmol/L)		<25nmol/L – Deficient <50nmol/L – Insufficient >200nmol/L – Above normal range

If you have any questions, please do not hesitate to contact me on the number below, or by email.

Kind regards,
Owen Mugridge

Research Trials Manager
o.mugridge@massey.ac.nz
09 414 0800 extension 41174
Massey University Oteha Rohe
Albany Highway Albany 0632 New Zealand

APPENDIX TWENTY SEVEN: Letter to GP – Participant Blood Results



(Insert date)

(Insert GP name and Practice address)

Dear Doctor,

_____ (name) has been enrolled in the research study '**Post discharge nutrition of preterm babies: micronutrient status and feeding practices of preterm babies after hospital discharge**' study run through Massey University Albany.

Recent blood tests taken as part of this research study to assess vitamin D status have indicated that results _____ are outside of normal ranges. These results have been attached.

The parents have been informed of these results but have not been given any clinical advice. The parents have consented to these results being sent to their GP.

Please contact Cath Conlon (PhD) with any further questions.

Kind regards,

Cath Conlon (PHD)

Cath Conlon (PhD)
C.Conlon@massey.ac.nz
09 414 0800 extension 41206
Massey University Oteha Rohe
Albany Highway
Albany
0632
New Zealand

APPENDIX TWENTY EIGHT: ADHB Standard Parenteral Nutrition Solution

	Starter Nutrition Formulae (Used from birth to 2 days)	P 100 (Used from Day 2 and beyond)
Nutrient	Concentration per 1Litre	
Protein	67.9g	42g
Nitrogen	10.9mmol	6.7mmol
Amino Acid	70	43
Glucose	150	100
Sodium	3.5mmol	55mmol
Potassium	3.5mmol	55mmol
Chloride	0 mmol	28mmol
Gluconate	32.3mmol	34mmol
Acetate	67.9mmol	56.71mmol
Calcium	16.10mmol	17.02mmol
Phosphate	0mmol	19mmol
Magnesium	0	2.8mmol
Trace Elements	0	11ml
Zinc	5mg	Included in trace elements
Heparin	500U	500U
Energy	782kcal	497kcal