# Preventing Vitamin B12 Deficiency in South Asian Women of Childbearing Age - the VitB12 Study

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# Ethical Approval

Ethical approval for both the qualitative and quantitative phases of this project was been granted by AUTEC: Ethics Application Number 08/02. Approved on 23rd December, 2008. (Ethics approval letter attached as Appendix 1).

# Confidential Material

No confidential material

# Abbreviations

| | |
|---|---|
| AdoCbl | Adenosylcobalamin |
| ANOVA | Analysis of variance |
| ANCOVA | Analysis of covariance |
| ARR | Absolute risk reduction |
| B12 | Vitamin B12 |
| B12FFQ | Vitamin B12 food frequency questionnaire |
| BCMRC | Body Composition and Metabolism Research Centre |
| BF% | Body fat percentage |
| BIA | Bioelectrical impedance analysis |
| CBPR | Community based participatory research |
| CCRG | Community collaborative research group |
| CI | Confidence interval |
| CMDHB | Counties Manukau District Health Board |
| CVD | Cardiovascular disease |
| FM | Fat mass |
| FFM | Fat free mass |
| FFQ | Food frequency questionnaire |
| FOS | Framingham Offspring Study |
| GDM | Gestational diabetes mellitus |
| GIT | Gastrointestinal |
| GM | Geometric mean |
| GP | General practitioner |
| Hb | Haemoglobin |
| Hcy | Homocysteine |
| HDL-C | High density lipoprotein cholesterol |
| HHM | Holdsworth Memorial Hospital Study |
| HoloTC | Holotranscobalamin |
| HOMA2β% | Pancreatic Beta cell function |
| HOMA2S% | Cellular insulin sensitivity |
| HOMA2 IR | Homeostatic model of insulin resistance version 2 |
| HOMA1 IR | Homeostatic model of insulin resistance version 1 |
| ITT | Intention to treat |
| LDL-C | Low density lipoprotein cholesterol |
| LOCF | Last Observation Carried Forward |
| MeCbl | Methylcobalamin |
| MCV | Mean corpuscular volume |
| MCHC | Mean corpuscular haemoglobin concentration |
| MD | Missing data |
| MMA | Methylmalonic acid |
| MUAC | Mid-upper arm circumference |
| NCD | Noncommunicable disease |

| | |
|---|---|
| NTD | Neural tube defect |
| NTT | Number needed to treat |
| NZFANS | New Zealand Food and Nutrition Survey |
| PMNS | Pune Maternal Nutrition Study |
| RCT | Randomised controlled trial |
| TC | Total cholesterol |
| TG | Triglycerides |
| TC/HDL-C | Total cholesterol/high density lipoprotein cholesterol ratio |
| TG/HDL-C | Triglyceride/high density lipoprotein cholesterol ratio |
| T2DM | Type 2 diabetes mellitus |
| Tukey's HSD | Tukey's honestly significant difference |
| WHO | World Health Organisation |
| WHR | Waist to hip ratio |
| WHtR | Waist to height ratio |

# Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously written or published by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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Gael Janine Mearns

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# Abstract

Maternal nutrition during pregnancy and breastfeeding has long-term effects on the growth and development of offspring. Longitudinal studies in India provide evidence that maternal vitamin B12 deficiency in pregnancy is associated with increased insulin resistance and relative adiposity in the offspring, potentially increasing the risk for developing type 2 diabetes mellitus in later life. Vitamin B12 is an exclusive vitamin because it is only available naturally in foods of animal origin. In New Zealand, vitamin B12 deficiency is considered rare because of a predominantly meat-eating diet and it is mainly older adults with malabsorption of B12, and those with extremely strict vegetarian or vegan dietary practices who are assumed to be at risk. Consequently, public funding for vitamin B12 supplements, decisions on folic acid supplementation and food fortification, plus screening and management of vitamin B12 deficiency, are all orientated towards addressing vitamin B12 deficiency in older adults with vitamin B12 malabsorption.

Worldwide, there are high prevalence rates for noncommunicable disease (cardiovascular disease and type 2 diabetes mellitus) in the South Asian population, and evidence supporting a high prevalence of vitamin B12 deficiency related to dietary patterns that contain little or no meat. Phenotypic changes in response to maternal vitamin B12 deficiency may exacerbate the already high noncommunicable disease risk for subsequent generations of offspring. The South Asian population in New Zealand is growing rapidly, but there is little published information on the prevalence of vitamin B12 deficiency among South Asian women living in New Zealand. Health professionals need a better understanding of risk factors for vitamin B12 deficiency, including how to work with the South Asian community to effectively prevent deficiency, particularly in women preconception.

The aim of this vitamin B12 (VitB12) study was to explore ways to reduce vitamin B12 deficiency in Auckland based, South Asian women of childbearing age. The mixed methods community-based participatory research design involved enlisting a group of South Asian community members to guide and participate in the planning, conduct, and implementation of the focus group discussions and the randomised controlled trial components of the research, as well as subsequent dissemination of findings. Six focus group interviews with community members (five groups) and health professionals (one group), were recorded and thematically analysed. Key themes identified from the focus group interviews were **dietary practices as integral to identity and belonging**, **managing B12 deficiency** and **study participation as a positive experience.** The double blind, randomised controlled trial recruited 62 women of childbearing age, stratified by meat/non meat-eating dietary patterns, then randomly allocated to one of three treatment groups: vitamin B12 dietary advice, 6µg vitamin B12 supplement, or placebo capsule. Measurements were undertaken at baseline, two, then six months and included fasting blood for biomarkers of vitamin B12 status (serum B12 and holotranscobalamin), serum folate, glucose, insulin, lipids and a full blood count. Measures also included weight, height, body fat by single frequency hand to foot bioimpedance, and blood pressure. A 30-item, researcher-administered, food frequency questionnaire developed for this VitB12 study was used to assess reported dietary vitamin B12 intake at each time point.

Women in the study presented with metabolic risk factors for noncommunicable disease and gestational diabetes. Nearly 75% of women had total cholesterol and 50% low-density lipoprotein-cholesterol results that exceeded New Zealand National Heart Foundation recommended guidelines, and although body mass index was within recommended guidelines, all of the women were within the obesity range based on body

fat percentage estimated using bioelectrical impedance (range 41to 61%). Waist to hip ratio and waist to height ratio, two indices of central obesity, were borderline high.

At baseline, 48% of women were low in serum vitamin B12 (< 222 pmol/L), and 51% were low in holotranscobalamin (< 45 pmol/L). All had sufficient folate status. The food frequency questionnaire developed for the study was a valid estimate of dietary B12 intake, supported by a moderate positive correlation with serum B12 ($r$=0.50, $p$ <0.001, 95 % CI [0.28, 0.67]) and holotranscobalamin ($r$=0.55, $p$ <0.001, 95 % CI [0.34, 0.71]). The B12 FFQ demonstrated greater than 62% sensitivity and specificity for detecting low B12 biomarkers. Compared with women who ate meat (45%), those with non-meat-eating dietary practices (55%) were 2.2 (95% CI [1.4, 4.4]) times more likely to be low in serum B12 and 2.8 [1.4, 5.9] times more likely to be low in holoTC. Concurrently, 40% of women reported a dietary vitamin B12 intake less than the recommended daily intake of 2.4 µg/day. Women who ate no meat or white meat only were more likely to report an inadequate intake of dietary vitamin B12.

Over six months of the randomised controlled trial, the B12 supplement group treatment was associated with improvements that were substantial in both serum B12 (geometric mean increase of 30 %, 95 % CI [11, 48]) and holotranscobalamin (42 % [12, 72]). A drop in supplement adherence over the latter four months of the study for participants in the B12 supplement group was detected, and was associated with less improvement. For the placebo group and dietary advice groups, over 6 months there was a trivial and insignificant change in both serum B12 and holoTC concentrations, with a small increase in the percentage of women low in serum B12, and no change in the percentage low in holoTC.

The evidence presented in this thesis confirms that in common with international studies, South Asian women of childbearing age living in Auckland, New Zealand, and in particular, women with vegetarian or low meat eating dietary preferences, are at high

risk for B12 deficiency. Low dose oral vitamin B12 supplementation may be a beneficial strategy for preventing vitamin B12 deficiency in South Asian women prior to pregnancy. Future work could include investigating a prescription of a weekly B12 supplement dose rather than daily, and the effect of early screening before and during pregnancy for vitamin B12 insufficiency and deficiency. The vitamin B12 food frequency questionnaire developed for the VitB12 study has potential as a useful screening tool for inadequate dietary vitamin B12 intake.

This study has identified that South Asian women who eat little or no meat have a high prevalence of vitamin B12 deficiency, which could be ameliorated by oral supplementation. Promoting adherence to a low dose vitamin B12 supplementation or food fortification programme may be a beneficial strategy. In order to prevent or reduce life-course risks in offspring of women with vitamin B12 deficiency, the health literacy, policies, and practices of health professionals and communities must be informed by evidence.

*Keywords:* Vitamin B12, vitamin B12 deficiency, noncommunicable disease, South Asian women.

# Chapter 1: Introduction

Vitamins, nutrients essential for life, are organic compounds required in relatively small amounts and usually obtained from the diet. Vitamin B12 (B12), a water-soluble vitamin only found naturally in animal and microbial products (Watanabe, 2007), and folate, have vital and distinct roles in one carbon metabolism where they act as dietary methyl donors (Herrmann, Schorr, Obeid, & Geisel, 2003; Yajnik, 2009b). One carbon metabolism contributes to a network of interrelated metabolic pathways that are essential for normal growth, development, and physiological function across the life course (Herrmann et al., 2003). Some populations are more at risk of B12 deficiency because of their beliefs around eating meat and animal products, or because poverty limits intake of foods that contain B12. People of South Asian ethnic origin are at risk of B12 deficiency because of their low or non-meat eating dietary practices that have spanned across many generations (Jayanthi, 2001; Sharma, Soni, Murthy, & Malhotra, 2003; Yajnik et al., 2006).

In Maharashtra, India, half to two thirds of women have B12 deficiency (Krishnaveni et al., 2007; Yajnik et al., 2008).There is limited information on the B12 status of South-Asian women living in New Zealand. Two small AUT University based pilot studies found that when red meat was not included in the diet, two of six Indian preadolescent girls (Chhichhia, 2007; Rush, Chhichhia, Hinckson, & Nabiryo, 2009), and five of twelve young women (four of five Indian)(Xin, 2008), were B12 deficient.

In New Zealand, the number of people of South Asian origin almost doubled from 65,862 in 2001 to 112,893 in 2006, and represented 3% of the total New Zealand population of 4.2 million people (Statistics New Zealand, 2006). Furthermore, in 2006, the South Asian population was relatively young with 46% of the female population aged 15 to 39 years (Statistics New Zealand, 2006). The Asian population in New

Zealand, including those of South Asian origin, is predicted to more than double again by 2026 (Statistics New Zealand, 2008b), so prevalent health issues such as maternal B12 deficiency become even more crucial to address.

The nutritional environment during pregnancy and infancy may have profound effects on growth and development (Barker, 2004). Convincing evidence around the importance of periconceptual folate in the aetiology of neural tube defects (NTD) has led to folic acid food-fortification and advice for folic acid supplementation of women who plan to become pregnant. In Canada where folic acid fortification of all flour, and some corn and rice products was implemented in 1998, there were significant decreases in folate deficiency (Ray, Vermeulen, Boss, & Cole, 2002), with an associated reduction in NTD incidence (De Wals et al., 2007). Vitamin B12 deficiency in early pregnancy is also associated with an increased incidence of NTD (Kirke et al., 1993; Molloy et al., 2009; Ray & Blom, 2003), but when compared with folate, there is not the same emphasis on preventing periconceptual B12 deficiency. In an Ontario, study of 10,622 women aged 15 to 46 years exposed to folic acid food fortification, 5.2% of the 1244 pregnant women who were less than 28 weeks gestation, had biochemical B12 deficiency (Ray, Cole, & Boss, 2000). In Canada, the reduction in NTD has plateaued following folic acid food fortification, and a systematic review of studies linking B12 deficiency to increased NTD risk indicates that preventing maternal B12 deficiency prior to pregnancy may further reduce the plateaued incidence of NTD (Molloy et al., 2009; Ray, Goodman, O'Mahoney, Mamdani, & Jiang, 2008).

The longitudinal Pune Maternal Nutrition Study (PMNS) in India (Yajnik et al., 2008), found that low maternal B12 concentrations in pregnancy were associated with metabolic changes, including abdominal adiposity and insulin resistance in offspring at six years of age, despite some of these children being of ‘normal’ weight (Yajnik et al., 2008). The risk was increased when maternal B12 deficiency was accompanied by high

concentrations of folate (Yajnik et al., 2008). Phenotypic changes in children secondary to maternal B12 deficiency may increase the life-course risk for noncommunicable disease (NCD) such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) in adult life (Barker, 1993; Barker, Osmond, Forsen, Kajantie, & Eriksson, 2005; Sadeghian et al., 2006; Yajnik, 2009b). These phenotypic changes are in addition to the recognised association of neurological impairments and macrocytic anaemia with overt B12 deficiency (Lindenbaum et al., 1995; Oosterhuis, 2000; Reynolds, 2006). The proposed mechanism for these changes is altered expression of genes (epigenetic). A suboptimal availability of B12 as a methyl donor interrupts one carbon metabolic pathways, trapping folate and increasing the production of metabolites such as homocysteine (Hcy) that in turn may alter gene expression, fat deposition, and protein synthesis (Depeint, Bruce, Shangari, Mehta, & O'Brien, 2006a, 2006b; Fenech, 2001; Herrmann et al., 2003; Yajnik, 2009b; Yajnik et al., 2008). The effects of these epigenetic changes, and alterations in phenotypic plasticity and resilience may be passed on to subsequent generations, exacerbating the NCD risk profile of populations (Barker, 2004; Devaskar & Thamotharan, 2007; Gardiner, 2008; Kuh & Ben-Shlomo, 1997; Smith & Ozanne, 2006).

The risks to the child from maternal B12 deficiency may present in early infancy. A baby whose mother has B12 deficiency also acquires low B12 stores, and these may drop even lower if the child is breast fed by a B12 depleted mother, with potential for severe and irreversible neurological consequences if not detected and treated early (Bak, Gökgöz, & Ünalp, 2009; Sklar, 1986; von Schenck, Bender-Götze, & Koletzko, 1997). For South Asian women, the low amount of B12 food consumed in their diet is the main contributor to B12 deficiency (Jayanthi, 2001; Refsum et al., 2001).

The recommended daily intake of dietary B12 for an adult is 2.4 µg per day, and this increases to 2.6 µg per day in pregnancy or if breastfeeding. (Commonwealth Department of Health and Ageing Australia, Ministry of Health, & National Health and Medical Research Council, 2005). Diets that include standard portions of meat usually contain sufficient B12 to meet daily requirements and to maintain existing B12 stores, but a diet low in animal-based foods may not. Meat is a plentiful source of vitamin B12 but, as with all foods, there is a variation in nutrient density within, and between food sources. In general, red meat (beef) has two to six times more vitamin B12 than the equivalent weight of chicken flesh. B12 sourced from milk, yoghurt, and cheese is relatively low in B12, but in lactovegetarian diets particularly, it is the main source of dietary B12 intake (Jayanthi, 2001; Watanabe, 2007).

Historically in Western cultures a non-vegetarian and largely red meat eating diet protected against insufficient B12 intake, so deficiency was, and still is, considered a rare problem and isolated to those (predominantly older adults) with B12 malabsorption.(Carmel, 1997; Hudson, 2010). Consequently B12 deficiency is not commonly tested for unless specifically indicated, such as suspected symptoms. Treatment options are traditionally prescribed on the assumption that deficiency is due to gastrointestinal B12 malabsorption, and therefore, large intramuscular doses of B12 are required (Bolaman et al., 2003; Hudson, 2010). Macrocytic changes only present in approximately 30% of people with low serum B12 (Oosterhuis, 2000), so diagnosis and management of B12 deficiency based on presentation of overt symptoms may not address the increased susceptibility for disease risk factors secondary to B12 tissue deficiency; effects that may take many years to physically present (Depeint et al., 2006a, 2006b; Fenech, 2001; Lindenbaum et al., 1995). These subtle changes may affect the life course health of an individual, and in terms of widespread population B12

deficiency, may significantly affect longevity for that population (Yajnik, 2009a, 2009b).

The drivers for development of chronic disease need closer examination and explication in order to curtail the current epidemic of noncommunicable disease, particularly for those in susceptible populations (Bristow, Rowan, & Rush, 2009; Yajnik, 2009a). Worldwide, people of South Asian origins have high rates of T2DM and CVD (Hossain, Kawar, & Meguid El, 2007; Wild, Roglic, Green, Sicree, & King, 2004), and these findings are mirrored in the New Zealand Indian population (Gala, 2008; Ministry of Health, 2006; Rasanathan, Ameratunga, & Tse, 2006). Identified risk factors in South Asian, and in particular Indian populations, include high rates of central adiposity, a high body fat percentage relative to an apparently normal body mass index, and a low percentage of muscle mass (Bhat et al., 2005; Rush, Freitas, & Plank, 2009; Yajnik, 2004). The prevalence of diagnosed gestational diabetes mellitus (GDM) is 16% in Indian women in Auckland, significantly higher than for other ethnic groups (10% for Other Asian, 6% for Pacific Island, and Māori, and 3 % for European women) (Auckland District Health Board, 2008, 2010). An action plan developed by both the Auckland District Health Board (ADHB) and Counties Manukau District Health Board (CMDHB) aligns with the New Zealand Government health strategy to reduce the burden of chronic diseases in South Asian communities (Gala, 2008; Ministry of Health, 2000, 2008). Priorities for action include (1) research at both a community and a national level to investigate and understand the risk factors for the South Asian population, (2) involvement of the community in the planning and implementation of interventions to address these risk factors, and (3) the inclusion of culturally appropriate health promotion activities to mitigate risks (Gala, 2008).

Congruent with the above priorities is the need for effective, acceptable and sustainable strategies to increase B12 status in women of South Asian origin at risk of

B12 deficiency, **before** they become pregnant. There are two interrelated problems; one problem is the high likelihood of B12 insufficiency and deficiency in young South Asian women and the other is the high prevalence of metabolic diseases such as GDM, T2DM and CVD in people of South Asian origin (Auckland District Health Board, 2010; Gala, 2008). The body of work in this thesis examined ways of improving B12 nutritional status in South Asian women living in New Zealand, with a long-term view of reducing the risks for metabolic disease in offspring of these women.

**Structure of this Thesis**

Chapter 2 of this thesis explores existing literature on the metabolism, physiology and consequences of B12 deficiency. Discussions include current knowledge around the prevention and management of B12 deficiency with the gaps in knowledge highlighted, particularly within the New Zealand context. Demographics of the South Asian population within Auckland and New Zealand are summarised, along with pertinent health issues and the need to address immediate and long-term health outcomes associated with suboptimal nutrition. Chapter 2 concludes with the aims and hypothesis of this VitB12 study, informed by the literature review, along with rationale supporting a community based participatory approach for conducting the research.

Chapter 3 describes the mixed methods design for this body of research, including the methodology underpinning the study and the paradigmatic influences on that methodology. The methods for community focus group interviews and analysis of data are included in this chapter. Chapter 4 presents the findings from the focus group interviews and their interpretation. Included in this is a discussion on how relevant themes from the focus group interviews informed the conduct of the randomised controlled trial. The methods used for the double blind, three-group treatment, randomised controlled trial are described in Chapter 5, with justification provided for the planned statistical analyses.

Chapter 6 describes the development of a thirty-question food-frequency recall questionnaire used to estimate dietary B12 intake (and dietary risk for B12 deficiency) in the research participants. Descriptions include validation of the B12 food frequency questionnaire as a dietary B12 measurement tool, plus the strengths and limitations of food-recall questionnaires for estimating nutrient intakes.

Findings from the three-group treatment (B12 supplement, placebo and dietary B12 advice) randomised controlled trial are presented in Chapter 7 along with interpretation of the statistical analyses. Chapter 8 discusses the findings from this course of investigation, the VitB12 study and the unique contribution that this body of works makes to knowledge about prevention of B12 deficiency. The emphasis is on how to translate this knowledge into prevention of B12 deficiency from inadequate dietary intake within the New Zealand context, but with suggestions as to the relevance of findings for the international context. Discussions include the strengths and limitations of the research, and recommendations for further research (Figure 1).

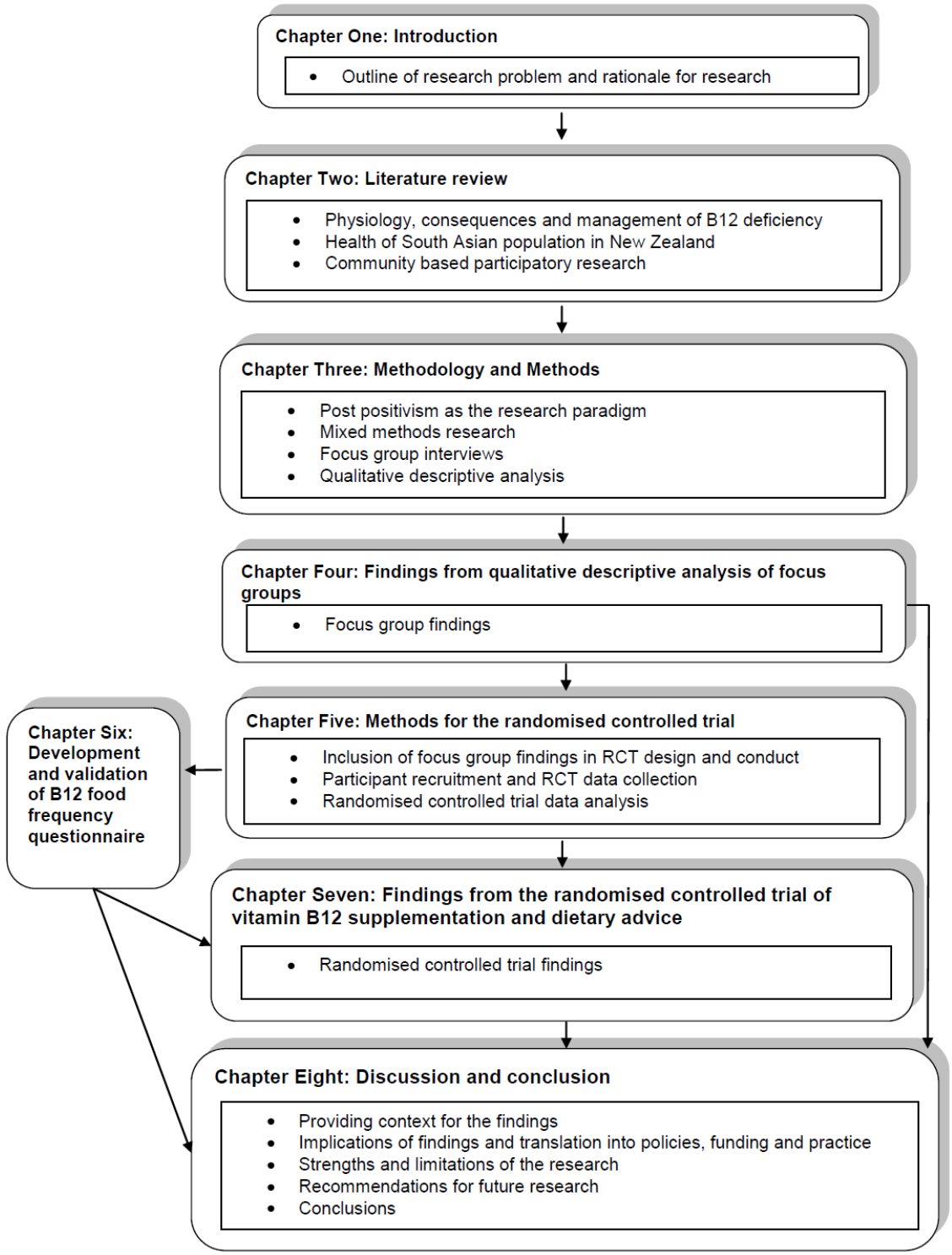



*Figure 1*. Structure and flow of the thesis.

**Chapter Summary**

Overseas research has shown that B12 deficiency is a problem in South Asian women of childbearing age, increasing the risks of poor pregnancy outcomes, neurological deficits, anaemia and noncommunicable disease for their future children. There is a lack of research on B12 deficiency in South Asian women living in New Zealand. Historically B12 deficiency has not been recognised as a problem in New Zealand, largely because the majority of the population consume a predominantly meat eating diet. Changing demographics and cultural composition of populations, and trends towards less meat-eating dietary preferences may change this, with an increase in the incidence of B12 deficiency among women of childbearing age. In order to promote an optimal pre-conceptual environment and to reduce the population risks associated with B12 deficiency, it is imperative to determine the extent of B12 deficiency and to develop strategies to effectively reduce B12 deficiency among women of childbearing age.

# Chapter 2: Literature Review

Vitamin B12 (B12) deficiency is prevalent in populations who do not eat meat. This is of particular relevance for women of South Asian origin and has implications for the future health of their offspring. This problem has not received the public health attention that it deserves. The body of research in the VitB12 study investigates the problem of B12 deficiency in South Asian women and investigates strategies to address this. Theoretical assumptions for the VitB12 study include the life course model of disease aetiology and the importance of community involvement in the study. The life course model frames the research project and is overviewed in **Section one** of this chapter, introducing why an adverse nutritional environment in utero reduces the potential for optimal development of the offspring (Kuh & Ben-Shlomo, 1997). **Section two** of the chapter covers sources for B12, the role of B12 in one carbon metabolism, then reasons for, and consequences of, B12 deficiency. Focus is on the relationship between maternal B12 status in pregnancy and the proposed epigenetic effects of metabolism of the child (Yajnik et al., 2008). Using the life course approach, the possible associations between B12 deficiency and increased population risk for noncommunicable disease (NCD) for people of South Asian origin are reviewed (Ben-Shlomo & Kuh, 2002; Yajnik, 2009a, 2009b). **Section three** explores concepts of cultural identity; describing who identifies as South Asian in New Zealand communities and the intersection of society, religion, culture and gender on dietary practices. Details include the demographics of the South Asian population in New Zealand, along with heath statistics for South Asian people.

Interventions trialled to improve B12 status are examined in **Section four** with details of the effect of these interventions on B12 biomarkers such as serum B12, holotranscobalamin (holoTC), methylmalonic acid (MMA) and homocysteine (Hcy).

**Section five** justifies the focus for the VitB12 study and outlines the study aim and research questions. Rationale is given for the embedded mixed methods approach for this study. Community collaboration in research is essential to successful translation of research findings into changes in health-related behaviours within communities. Chapter 2 concludes with a brief overview of the Community Based Participatory Research (CPBR) model (Minkler & Wallerstein, 2003) for community participation in the VitB12 study.

Literature was searched using Proquest Central, MEDLINE, Web of Science, Scopus, Science Direct, OVID and EBSCO databases. Search terms included 'vitamin B12', 'cobalamin' 'B12 deficiency', 'cobalamin deficiency', 'macrocytic anaemia', 'pernicious anaemia', 'vitamin B12 supplementation', 'cobalamin supplementation' 'homocysteine', 'methylmalonic acid', and 'holotranscobalamin'. Search terms were limited by adding 'South Asian', or 'Indian', 'women', 'pregnancy', 'noncommunicable disease', 'cardiovascular disease' and 'diabetes mellitus'. Secondary linking from references listed in research and review articles was also used to access information.

## Section One: Life Course Model as the Conceptual Framework for the Study

The conceptual framework for this study is the life course model of chronic disease aetiology where an adverse nutritional environment in-utero reduces the potential for optimal development of the offspring (Ben-Shlomo & Kuh, 2002; Devaskar & Thamotharan, 2007; Muthayya et al., 2006; Yajnik, 2009a, 2009b). Theoretical models propose that an adverse nutritional environment in-utero affects genetic expression in the offspring, programming metabolic susceptibility to risk factors such as over nutrition, increasing the offspring's risk for future chronic disease (Gluckman, Hanson, & Pinal, 2005; Yajnik, Godbole, Otiv, & Lubree, 2007). Risk accumulates throughout the lifespan, with the disease risk passing to subsequent generations (Ben-Shlomo & Kuh, 2002). Early in the life course, adaptive mechanisms

such as increased insulin release from pancreatic beta cells compensate for the risk factors, but when these adaptations fail, then disease symptoms can present (Ravelli et al., 1998). Risk management strategies for chronic disease have traditionally focused on addressing overt risk factors when adults present with early symptoms of disease. The estimated worldwide prevalence of type 2 diabetes mellitus (T2DM) was 2.8% in 2000 and is predicted to rise to 4.4% in 2030 (Wild et al., 2004). Urban populations in India, Sri Lanka, Pakistan and Bangladesh carry a high risk for diabetes mellitus, with marked differences between urban and rural prevalence rates. The urban prevalence rates for diabetes mellitus in 2005/2006 were 18.6%, 16.4%, 10.6% and 8.1% respectively for these countries, while rural prevalence rates were 9.2%, 8.7%, 7.7% and 2.3% respectively. (Ramachandran, Wan Ma, & Snehalatha, 2010). This burgeoning increase demands additional approaches to disease prevention in order to control T2DM and other NCD epidemics (Kuh & Ben-Shlomo, 1997; Yajnik, 2009a). The life course model advocates addressing risk for NCD throughout the lifespan, from pre-conception, in-utero, childhood and then throughout adult life (Ben-Shlomo & Kuh, 2002; Perry, 1997; Yajnik et al., 2008).

The VitB12 study is particularly concerned with addressing the foetal origins of disease and the altered growth and development associated with B12 deficiency in-utero that is postulated to affect offspring phenotype with increased lipogenesis, decreased muscle mass, increased abdominal adiposity and insulin resistance (Yajnik et al., 2008). Low vitamin B12 concentrations and the associated susceptibility to risk factors for NCD may be passing from one generation to the next, augmenting the already high population risk for NCD (Yajnik, 2009b).

## Section Two: Sources of Vitamin B12, Role in One Carbon Metabolism and Consequences of Vitamin B12 deficiency

### Food sources for B12.

Vitamin B12 (cobalamin) is an essential micronutrient sourced from microbial metabolism in animal products. In predominantly vegetarian populations, it can be a challenge to meet the recommended daily intake (RDI) of 2.4 µg /day (National Health and Medical Research Council, 2006). In some countries such as Australia, B12 fortification of cereals provides a useful source of B12 for vegetarians and vegans (Xin, 2008). In New Zealand however, cereals are not a B12 fortified food (Lesperance, 2009). Table 1 lists examples of foods that contain bioavailable B12 for humans. As humans cannot manufacture their own B12, they need to ingest it in food or take it in supplement form (Truswell, 2007; Watanabe, 2007). The bioavailability of B12 from different foods varies. For example, although the B12 content in milk is low (0.2-0.35 µg per 100ml) (Lesperance, 2009), it is readily absorbed, while the B12 from egg, particularly egg white, is less readily absorbed (Levine & Doscherholmen, 1983; R. M. Russell, Baik, & Kehayias, 2001; Tucker et al., 2000). The United States Framingham Offspring study of 2999 participants analysed the relationships between plasma B12 concentrations and dietary B12 intake from various food sources (Tucker et al., 2000). High and low consumption of dairy products was associated respectively with high and low plasma concentrations. This effect was more marked than for meat and poultry, suggesting that the B12 in dairy products is more readily absorbed than from meat and poultry. Cooking methods alter the bioavailability of B12, for example, scrambled eggs have a lower B12 bioavailability than fried or poached eggs, and boiling milk decreases its B12 bioavailability (Levine & Doscherholmen, 1983; Vogiatzoglou et al., 2009; Watanabe, 2007).

Table 1
*Dietary Sources of Cobalamin B12*

| **Food item** | **B12 content** | **Food item** | **B12 content** |
|---|---|---|---|
| Lamb liver | 81 µg/100g | Cheese | 0.7 – 1.4 µg/100g |
| Lamb kidney | 79 µg/100g | Eggs (large yolk) | 0.5-0.65 µg/100g |
| Sardines | 14 µg/100g | B12 fortified rice milk | 0.6 µg/100mL |
| Mussels | 25 µg/100g | B12 fortified soya milk | 0.4µg/100mL |
| Salmon | 3-4 µg/100g | Milk 0.1% fat calcium fortified | 0.33 µg/100mL |
| Tuna | 3-4 µg/100g | Homogenised milk 3% fat | 0.25 µg/100mL |
| Lamb (cooked) | 2.2 – 2.4 µg/100g | Reduced fat milk 1.5% fat | 0.03 µg/100mL |
| Prawns | 1.4 µg/100g | Trim milk 0.1% fat | 0.32 µg/100mL |
| White fish | 0.7 µg/100g | 'Calci-kid' enriched milk | 0.38 µg/100mL |
| Ham | 0.75 µg/100g | Marmite (fortified) | 0.7µg/teaspoon |
| Chicken | 0.5 – 1.3 µg/100g | Yoghurt | 0.3 µg/100g |
| Pork | 0.5 µg/100g | Ice cream | 0.5 µg/100g |

Sources for B12 cobalamin values: The Concise New Zealand Food Composition Tables 8th Edition (Lesperance, 2009)

According to the Australia New Zealand Food Standards Code (Standard 1.3.2), vitamin B12 is permissible as an additive to the following foods up to a maximum of the quantities specified (Food Standards Australia New Zealand, 2011):

- Meat, vegetable and yeast extracts (≤ 0.5µg B12 per 5gm of food).
- Beverages derived from legumes or rice (≤ 0.85µg B12 per 200mL of beverage).
- Analogues of yoghurt and dairy food derived from legumes (≤ 0.3µg B12 per 150gm of food).
- Analogues of meat derived from legumes (≤ 2.0µg B12 per 100gm of food).
- Analogues of ice cream derived from legumes (≤ 0.3µg B12 per 75gm of food).
- Analogues of cheese derived from legumes (≤ 0.2µg B12 per 25gm of food).
- Beverages containing protein derived from cereals (≤ 0.8µg B12 per 200mL of beverage).
- Formulated beverages (≤ 0.5µg B12 per 600mL of beverage).

**Absorption of B12**

Multiple factors influence the absorption and bioavailability of B12 from the gastrointestinal tract (GIT) (Herbert, 1994; Watanabe, 2007). Foods sources for B12 are bound to protein and the B12 is cleaved from the protein by gastric acid hydrolysis to initiate absorption. Once released, B12 then attaches to R-binder proteins for transport from the stomach to the duodenum. Disruption to gastric hydrolysis inhibits the absorption of food bound B12, however, cyanocobalamin preparations of B12 (found in fortified foods and oral supplements), are not protein bound and are readily absorbed regardless of gastric acidity (Andrès et al., 2003; Carmel, 1997). In the duodenum, pancreatic enzymes create a suitable alkaline environment to degrade the R-binder protein/B12 complex so that the B12 is released ready for attachment to intrinsic factor (Herbert, 1994).

Intrinsic factor, previously released from parietal cells of the gastric mucousa, transports B12 from the duodenum to the terminal ileum where, using calcium as a co-factor, the B12/intrinsic factor complex binds onto cubilin receptors and is internalized into enterocytes (Herbert, 1994). Inside the enterocyte, the B12/intrinsic factor complex is degraded and B12 then attaches to transcobalamin (TC) proteins for transport in plasma as holotranscobalamin (holoTC). These transport processes are saturable, and although approximately 98% of the daily requirement of B12 is absorbed via these processes, saturation of transport processes is reached at approximately 3µg of a B12 dose /dietary load, limiting how much vitamin B12 can be absorbed within a four to six hour period (Herbert, 1994; Herrmann, 2002). In vitamin B12 deficiency states, the cell surface receptors for transcobalamin up-regulate, increasing the absorption of B12 (Brady, Wilson, McGregor, Valente, & Orning, 2008; Clarke et al., 2007; Herbert, 1994). Approximately one to three percent of B12 intake bypasses transport proteins and is absorbed by passive diffusion across the wall of the GIT (Herbert, 1994).

HoloTC also binds inactive corrinoid or pseudo B12, permitting these to compete with cobalamin forms of B12 for transport. In some methods for B12 biomarker testing, this can falsely elevate both serum B12 and holoTC concentrations (Herbert, 1994; Herrmann, 2002). Corrinoid forms of B12 are contained in some in plant-based foods such as seaweed, but are not available for B12 metabolism in humans. Of the total cobalamin circulating in plasma, approximately six to twenty percent is transported as holoTC and it is this complex that is the useable form as it binds to specific cell surface receptors and has a rapid cellular uptake (Carmel, 2002; Herrmann & Obeid, 2011). B12 also attaches to the transport protein haptocorrin to form holohaptocorrin (holoHC). The larger percentage of total serum cobalamin is carried by holoHC, but as there are no cellular receptors for holoHC, this transported B12 is not immediately available for cellular functions (Carmel, 2002). Plasma holoHC appears to have an equilibrium role in maintaining B12 liver stores. (Carmel, 2006; Clarke et al., 2004; Herbert, 1994). HoloTC has a short half-life (approximately six minutes) because it distributes rapidly to cells (Herbert, 1994). In deficiency states holoTC concentrations fall more quickly than total serum vitamin B12 and because it represents the B12 available for cells, holoTC is a more sensitive indicator of cellular B12 deficiency than total serum vitamin B12 (Hvas & Nexo, 2005).

**The role of B12 in one carbon metabolism.**

Cobalamin B12 absorbed from animal-derived foods is converted into two forms that participate as co-enzymes in two different methylation or one carbon metabolism pathways; methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl) (Watanabe, 2007). In the first pathway, folate and B12 are co-enzymes in the methionine synthase mediated one carbon metabolism in the cytoplasm. In situations of normal B12 and folate concentrations, folic acid as 5-methyltetrahydrofolate, provides the methyl group needed for the remethylation of homocysteine to methionine, while the B12 (MeCbl)

acts as a cofactor in the methionine synthase mediated reaction (R. H. Allen, Stabler, & Lindenbaum, 1998; Stover, 2004). In the presence of low B12, folate becomes trapped as inactive 5-methyltetrahydrofolate (Herrmann, Schorr, Purschwitz, Rassoul, & Richter, 2001; Stover, 2004). Instead of being converted into methionine, increased concentrations of the metabolites homocysteine (Hcy) and cysteine accumulate (Herrmann et al., 2003). Methionine, through conversion to S-adenosylmethionine also has a critical role in biosynthesis of purines and thymidine, methylation of DNA (regulates gene expression), and proteins, lipids and neurotransmitter synthesis (R. H. Allen et al., 1998; Blom, Shaw, Martin den, & Finnell, 2006; Stover, 2004). Interruption to DNA methylation predominantly affects rapidly dividing cells such as erythrocytes, resulting in macrocytic anaemia, however, the presentation of macrocytic anaemia in B12 or folate deficiency is inconsistent and does not correlate well with Hcy, or serum B12 and folate concentrations (Oosterhuis, 2000). Inhibition of the methylation of homocysteine to methionine is also a suggested mechanism for increased risk of neural tube defects (NTD) in pregnancy if the mother is folate deficient (R. H. Allen et al., 1998; Blom et al., 2006; Refsum, 2001) or B12 deficient (Refsum, 2001).Some of the neurological deficits in B12 deficiency may also occur through inhibition of this pathway (R. H. Allen et al., 1998; Solomon, 2006).

In the second B12 dependent methylation pathway, B12 (AdoCbl) is a cofactor in the methylmalonyl CoA mutase conversion of methylmalonyl CoA to succinyl CoA. Insufficient AdoCbl inhibits this conversion and results in accumulation of the metabolite methylmalonic acid (MMA) (R. H. Allen et al., 1998). Folate is not cofactor in this pathway, and since folate deficiency does not produce any neurological deficits and B12 deficiency does, inhibition of this pathway is proposed to contribute to neurological deficits associated with B12 deficiency (Solomon, 2006; Stover, 2004). There is some uncertainty about this however, because metabolite studies comparing

metabolite concentrations (Hcy and MMA), neuropsychiatric abnormalities, serum B12, and folate concentrations, failed to find any significant associations (R. H. Allen et al., 1998). Symptoms of neuropsychiatric abnormalities range from decreased cognition, memory impairment, unsteady gait, incoordination, visual disturbances, mood and behaviour changes, and peripheral neuropathy (Lindenbaum et al., 1995). The term sub acute combined degeneration of the cord (SCD) describes posterior and lateral spinal cord lesions that develop secondary to B12 deficiency (Carmel, 2006; Lindenbaum et al., 1995; Molloy, Kirke, Brody, Scott, & Mills, 2008; Solomon, 2006).

**B12 deficiency and depletion.**

B12 depletion precedes deficiency and occurs at serum B12 concentrations previously classified as within the recommended reference range of normal (Herbert, 1994). Expected holoTC concentrations in persons with normal renal function is between 32 to 58 pmol/l (90 % confidence intervals) (Clarke et al., 2007). HoloTC concentrations < 45 pmol/L are indicative of B12 depletion (Herbert, 1994; William, Spencer, Elizabeth, Ann, & Victor, 2000). A serum B12 concentration of < 150 pmol/L has traditionally been defined as deficient, however these values are based on concentrations at which macrocytic anaemia presents (Oosterhuis, 2000). Serum B12 concentrations between 150 pmol/L and 222 pmol/L are indicative of depletion as the holoTC component of total serum B12 begin to fall at these concentrations. In stage 1 depletion, stores of holoTC deplete below 45 pmol/L, and this is the earliest indicator of inadequate cellular delivery of B12. At stage I depletion, serum B12 concentrations are frequently within normal range (> 258 pmol/L) Stage II occurs when serum B12 concentrations decrease to between 222 to 258 pmol/L. At these concentrations, serum holoHC has decreased and this indicates depletion of body B12 stores as HoloHC maintains in equilibrium to liver and body stores of B12. HoloHC has a half-life of 240 days so depletion takes longer to detect if using serum B12 as the only guide to B12

status (Herbert, 1994; Herrmann, 2002). People with vegetarian dietary preferences may remain in this state of B12 depletion for many years without progressing to overt deficiency because enterohepatic recycling of B12 is intact. In depleted states, when B12 absorption is intact, transcobalamin receptors are upregulated, facilitating increased reabsorption of B12 excreted via bile, keeping gastrointestinal losses of B12 to an absolute minimum (Herbert, 1994; Herrmann, 2002).

If B12 stores continue to deplete, there is insufficient B12 for one carbon metabolism, and excess accumulation of the metabolites Hcy and MMA. At this stage, subtle cellar changes such as mild cognitive impairment may occur, with symptoms so subtle that they go undetected. An elevated Hcy concentration or hyperhomocysteinaemia has been associated with increased risk of neurological deficits and cardiovascular disease, although there is still some debate over the nature of the latter association (Booth & Wang, 2000; Fenech, Aitken, & Rinaldi, 1998; Kumar et al., 2009; Refsum & Ueland, 1990; Sadeghian et al., 2006; van Oijen, Laheij, Jansen, & Verheugt, 2007; van Oijen, Vlemmix, et al., 2007).

B12 deficiency is often not diagnosed until symptoms such as macrocytic anaemia occur (Oosterhuis, 2000), however, the presentation of macrocytic anaemia occurs at late stage B12 deficiency and by this stage brain glial cells are depleted of B12 and the risk of irreversible neurological deficits is high (Herbert, 1994). Macrocytic anaemia is also an unreliable indictor of deficiency as not all B12 deficiency results in macrocytic changes and concurrent disorders such as iron deficiency anaemia or thallasemeia can obscure macrocytic changes (Herrmann et al., 2003; Oosterhuis, 2000).

Measurement of the serum B12 concentration has limitations for assessing B12 status because of the lag time in reflecting deficiency (Herbert, 1994). Measurements of serum Hcy and MMA concentrations are available as biochemical tests to diagnose B12 deficiency (Refsum et al., 2004), but these are only available at specialist laboratories in

New Zealand and they are expensive, preventing their routine use for diagnosing B12 deficiency. Furthermore, Hcy and MMA increase once changes in cellular biochemical functions occur in response to deficiency (Herrmann et al., 2001; Refsum et al., 2004). Measurement of the holoTC concentration is a preferable diagnostic test for B12 status as it can detect diminishing B12 stores before irreversible cell damage from deficiency occurs, and it has a high specificity for B12 deficiency (Brady et al., 2008; Čabarkapa, Stošić, Žeravica, Ilinčić, & Filipović, 2007; Herbert, 1994; Hvas & Nexo, 2005). MMA and Hcy are not specific for B12 deficiency; Hcy is raised in folate and B6 deficiency and both Hcy and MMA concentrations increase in renal impairment (Herrmann et al., 2001; Refsum et al., 2004).

There is wide variation in the literature cut-off points used to define B12 deficiency and insufficiency. For the purposes of the VitB12 study a cut–off point of 150 pmol/L is used to define B12 deficiency (De Benoist, 2008; Herbert, 1994; World Health Organization, 2008) and a cut off of 222 pmol/L used to define insufficiency (L. H. Allen, 2009; Molloy et al., 2009).

**B12 deficiency in pregnancy.**

There is plasticity in the expression of genes and although the phenotype or blueprint for genes is heritable, regulation can occur in DNA or RNA synthesis so that expression of certain genes are either silenced or activated (Lu et al., 2006). This can occur as an aberrant response in disease processes, as an adaptive response to adverse environmental conditions, or as physiological homeostatic mechanism (Fenech, 2001). The altered expression of genes in offspring, known as epigenetic adaptations or expression, is the mechanism by which maternal B12 deficiency in pregnancy is postulated to influence the lifelong NCD susceptibility for offspring (Devaskar & Thamotharan, 2007). Methylation pathways are vulnerable to plasticity, so folate and B12 play an important role in genomic stability (Fenech, 2001; Lu et al., 2006). In the

Pune Maternal Nutrition Study (PMNS) in Maharashtra, India (Yajnik et al., 2008), abdominal adiposity, insulin resistance and low muscle mass were most pronounced in the children born to mothers low in B12 but high in folate, suggesting that disruption of the methionine pathways with excess production of Hcy and impaired DNA methylation was involved in the nutritional reprogramming of offspring (Bavdekar et al., 1999; McKeigue, 1997; Yajnik, 2009b; Yajnik et al., 2008).

Animal studies of intrauterine nutritional deprivation resulting in foetal growth restriction have identified the nature of some of these changes(Devaskar & Thamotharan, 2007). These involve alterations in cellular insulin signalling mechanisms with restricted transport of glucose into cells, and disruption to the hypothalamic-pituitary adrenal axis with subsequent alterations in glucocorticoid release as contributors to the increased insulin resistance and increased susceptibility to central abdominal fat deposition (Devaskar & Thamotharan, 2007; Yajnik & Deshmukh, 2008). Epigenetic changes to metabolism are also induced through silencing or augmenting key genes involved in methylation reactions; these changes predisposing to expression of the insulin resistant phenotype (Devaskar & Thamotharan, 2007). In a population such as South Asian, where there is already a high prevalence of NCD, prevention of maternal vitamin B12 deficiency is one public health strategy that may reduce the epigenetic mediated susceptibility for NCD (Yajnik, 2009a).

In pregnancy, maternal B12 deficiency and insufficiency are also associated with an increased risk of NTD, early pregnancy loss, small for dates babies and poorer pregnancy outcomes (Molloy et al., 2008; Ronnenberg et al., 2002; Yajnik et al., 2005). In two case separate case control studies, increased risk of offspring NTD was identified at maternal serum B12 concentrations historically identified as normal or borderline low. In a large, three group, nested Irish case control study of European women, NTD risk increased two to three fold when maternal B12 decreased below 221 pmol/L

(Molloy et al., 2009). The increased risk of NTD at maternal B12 concentrations not previously considered as deficient was also supported in a Dutch case control study of 45 mothers and 85 matched case controls, plus their children. There was a 3.5 fold increase in risk of NTD if maternal B12 was below 186 pmol/L (Groenen et al., 2004).

Even when there is no indication of overt neurological impairments, low maternal B12 in pregnancy has been associated with subtle cognitive impairments in the offspring. In a follow up of the children from the PMNS, at nine years of age those children born to mothers low in B12 scored lower on cognitive function tests of short term memory (measured by the digit span test) and sustained attention (measured by the colour trails test) (Bhate et al., 2008). For the South Asian women in particular, this reinforces the need for more stringency with screening for B12 deficiency in women of childbearing age and consideration of the causes of B12 deficiency.

**Causes and consequences of B12 deficiency.**

Any factor that inhibits the unbinding of food bound cobalamin from proteins, the release of pancreatic enzymes into the duodenum, the binding of cobalamin to intrinsic factor, or the binding of the intrinsic/cobalamin complex to receptor sites in the terminal ileum potentially decreases the bioavailability of B12 (Carmel, 1997; Festen, 1991). The most common cause of B12 deficiency, particularly in middle to late adulthood, is food bound B12 malabsorption. A lack of acid hydrolysis in the stomach, usually due to achlorhydria, limits the unbinding of B12 from food, and the ability for free B12 to attach to transport proteins for absorption in the small intestine (Carmel, 1997; King, Leibach, & Toskes, 1979).

Intrinsic factor mediated B12 malabsorption, historically considered to be the most common cause of B12 deficiency (Festen, 1991), is less common than food bound B12 malabsorption (Baik & Russell, 1999). There is a deficiency of functional intrinsic factor for binding and transport of B12 to the ileum for absorption, consequently, B12 is

unable to be absorbed via transport-mediated processes. In intrinsic factor mediated malabsorption, inhibition of absorption also affects the existing B12 stores excreted via bile into the GI tract ready for reabsorption via the enterohepatic-recycling pathway, so body stores of B12 deplete more rapidly than in other causes of deficiency. The term pernicious anaemia applies to this cause of deficiency; it is more likely to be severe if not treated, to cause macrocytic changes to red blood cells and to be accompanied by anaemia (Baik & Russell, 1999). The deficiency of intrinsic factor is either because of antibody mediated inhibition of parietal cell release of intrinsic factor, or antibodies against intrinsic factor binding to either B12 or against the cubilin receptors in the brush border of the terminal ileum (Festen, 1991; Stabler & Allen, 2004).

Low levels of transcobalamin are also associated with defects in absorption of B12 and transport of B12 to tissues. Medications may interfere with B12 absorption; metformin through blocking of the calcium mediated binding of cobalamin to transport proteins (Reinstatler, Qi, Williamson, Garn, & Oakley, 2012) and proton pump inhibitors (e.g. omeprazole) by either inhibiting acid hydrolysis of B12 bound to dietary proteins, or through some yet unidentified mechanism (Hirschowitz, Worthington, & Mohnen, 2008; Valuck & Ruscin, 2004). Tropical sprue or other GIT infections are another contributor to B12 malabsorption and deficiency (Refsum et al., 2001; Stabler & Allen, 2004).

In the South Asian population, the most common cause of B12 deficiency is insufficient dietary B12 intake (Krishnaveni et al., 2009; Refsum et al., 2001; Sharma et al., 2003; Yajnik et al., 2006). The intergenerational inheritance of low vitamin B12 stores combined with the accumulative effects of low B12 diet, lead to deficiency states much more rapidly than in a person with sufficient B12 stores who commences on a vegetarian diet (Refsum et al., 2001). B12 insufficiency and deficiency is also common in Asian Indian people with non-vegetarian dietary practices because even those who

eat meat, do so infrequently (Sharma et al., 2003). Generally B12 deficiency due to inadequate intake, or absorption-inhibiting medications, takes longer to become symptomatic and is not as severe as pernicious anaemia (Herbert, 1994; Herrmann, 2002). This is because existing B12 stores undergo efficient enterohepatic recycling, so it can take between 3 to 5 years to deplete existing B12 stores. However, for South Asian people, the long intergenerational history of inadequate B12 intake means that sufficient B12 stores are not passed onto subsequent generations, so B12 deficiency can be more profound.

The intergenerational effects of low body stores of vitamin B12, passed on from one generation to the next are suggested as one of the many contributory causes to the ballooning rate of NCD in the Indian population (Yajnik, 2009b). Studies have shown the phenotype for people of Indian origin differs from other populations. Indian people have a lower percentage of muscle mass, a higher percentage of body weight and a higher insulin resistance, factors that are positively associated with a higher incidence of T2DM (Rush, Freitas, et al., 2009; Rush, Plank, & Yajnik, 2007; Yajnik, 2004). The intergenerational effects of B12 deficiency may be a contributing factor to these differences (Yajnik, 2009a).

The links between B12 deficiency and T2DM susceptibility were supported in a study of 785 pregnant women in India (Krishnaveni et al., 2009). Findings highlighted that maternal B12 deficiency (< 150 pmol/L) was associated with increased abdominal adiposity, insulin resistance and impaired glucose tolerance. This risk was higher in those with low serum B12 and high folate concentrations. This effect existed even after adjusting for covariates such as age, socioeconomic status, parity and family history of diabetes. There was a negative association between B12 status and the development of gestational diabetes, with the latter being two times more likely in women with B12 deficiency. More than 40% of the women in this study were B12 deficient (Krishnaveni

et al., 2009). A longitudinal follow up of offspring of these women found that at nine years of age, the children, especially females, of mothers with T2DM or gestational diabetes had a higher level of adiposity, insulin resistance and a higher systolic blood pressure than those born to non-diabetic mothers (Krishnaveni et al., 2010).

The associations between B12 deficiency and CVD have been supported in some studies (Kumar et al., 2009; Pac et al., 2005; Sadeghian et al., 2006; Weikert et al., 2007), but there is still debate about the direct associations of B12 deficiency and NCD. The risks from B12 deficiency are proposed to be mediated through hyperhomocysteinaemia (Selhub, 2008), although a study by Kumar et al. (2009) found associations between B12 deficiency, and increased concentrations of another metabolite cysteine, rather than homocysteine. Kumar et al.'s (2009) case control study undertaken in 816 subjects (368 participants with coronary artery disease and 448 matched controls) in India assessed the association between dietary intakes of vitamin B12, concentrations of vitamin B12 and rates of coronary artery disease CAD (Kumar et al., 2009). The median age of subjects was fifty years. In the CAD group, 60% of subjects were found to be deficient in B12 compared to 48% in the non CAD group. A binary logistic regression analysis of factors that significantly affected CAD risk were age ($p < 0.001$), male gender ($p < 0.001$), body mass index ($p = 0.015$) and low B12 ($p = 0.032$). Over 50% of the participants were B12 deficient (median serum B12 was 145 pmol/L) (Kumar et al., 2009). Research support for lowering homocysteine, and reducing the risks of B12 deficiency for CVD have had mixed results (Brattstrom, Landgren, Israelsson, Lindgren, & et al., 1998; Lonn, Salim, Arnold, Sheridan, & et al., 2006), although the majority of studies have researched B12 supplementation in conjunction with other B vitamins, rather than the homocysteine lowering effects of B12 in isolation (Strain, Dowey, Ward, Pentieva, & McNulty, 2004).

**Section Three: People of South Asian Origin**

Women of South Asian origin, and of childbearing age, residing in Auckland, New Zealand are the focus of this research. When ethnicity is based by country of origin, people of South Asian origin are estimated to represent over one quarter of the world's population. They originate from the nations of India, Pakistan, Bangladesh, Nepal, Bhutan, Maldives and Sri Lanka (United Nations Department of Economic and Social Affairs, 2012). In addition, people of Indian origin also make up 37. 5 % of the Fiji islands (total population = 837,271) (Fiji Islands Bureau of Statistics, 2007). However, when analysing population statistics, the definition of who is South Asian depends on interpretation of the term ethnicity. Ethnicity can infer a self-defined membership of a particular group of people; membership based on geographical origins, a particular population group or biological categorization of race (MacLachlan, 2006).

Traditionally in New Zealand, statistics on ethnicity were based on race and did not take into account the social characteristics of a group with which one self-aligned. Important to an individual's and a community's identity and self-esteem is culture; the characteristics, social customs and norms of the group to which they relate or belong. Race as a definition of ethnicity is very limiting in that it ignores this self-identification and reduces the person to the percentage of biological inheritance attributed to each race (MacLachlan, 2006; Spencer, 2006; Taylor & Spencer, 2002). Geographical definition ignores the migrant patterns of populations and both geographical and population definitions ignore the process of culture and that as humans, people construct their own reality of 'who they are', based on the groups that they feel they most closely belong to (Statistics New Zealand, 2005a).

Traditionally, race was the primary definition of ethnicity in NZ with a person opting to specify the percentage of their self that could be attributed to each race. Since 2004, Statistics NZ have redefined the statistical parameters for ethnicity to be inclusive

of "*self-identification with the ethnic group or groups that one wishes to identify with"* (Statistics New Zealand, 2005a). The key component of ethnicity is cultural affiliation; defined by Statistics NZ as *"the social, historical, geographical, linguistic, behavioural, religious, and self-perceived affinity between a person and an ethnic group"* (Statistics New Zealand, 2005a).

This creates an anomaly because when population statistics are discussed within this thesis document, the figures cited will be derived from ethnicity data gathered by Statistics New Zealand using the definition of ethnicity as cited above. However, in order to capture the biological and intergenerational factors that may influence B12 status, the definition of South Asian for this study was that of South Asian origin; both geographical and biological origins from one of the South Asian countries listed above and at least two maternal and paternal generations of South Asian origin. In addition participants were characterized by the sub-ethnic and religious groups that they identified with.

**Statistics New Zealand categories of ethnicity.**

Statistics NZ categorizes ethnicities at various levels. Level one is a very broad group of European Maori, Pacific Peoples, Asian, Middle-Eastern/Latin American/African and 'Others'. Prior to 1996, the South Asian population was grouped along with the Asian population under the broad category of 'Other'. Since 1996, at level one collection of statistics, the South Asian population has been grouped with together with all other Asian populations under the category 'Asian'. The South Asian population are classified as 'Indian', and 'Other Asian' at level two of coding and then separated into subgroups at level three (Table 2) and four of coding (Table 3) (Statistics New Zealand, 2005a). People from Bhutan and the Maldives are still classified under Asian nec, even at level four of coding. This separation at different levels of

categorisation is significant as it can influence the way that population health statistics are collated and interpreted.

Table 2
*South Asian Ethnic Groups included in Statistics New Zealand Level Three Categories (Statistics New Zealand, 2005a)*

| |
|---|
| 431 Indian *nfd |
| 441 Sri Lankan |
| 444 Other Asian |
| *Not further defined (nfd)<br>A 'not further defined' (nfd) ethnic group category contains responses that are not specific ethnic group responses but are able to be placed in a broader category in the ethnicity classification. |

Table 3
*South Asian ethnic groups included in Statistics New Zealand level four categories (Statistics New Zealand, 2005a)*

| |
|---|
| 43100 Indian *nfd |
| 43111 Bengali |
| 43112 Fijian Indian |
| 43113 Gujarati |
| 43114 Tamil |
| 43115 Punjabi |
| 43116 Sikh |
| 43117 Anglo Indian |
| 43199 Indian **nec |
| 44100 Sri Lankan nfd |
| 44111 Sinhalese |
| 44112 Sri Lankan Tamil |
| 44199 Sri Lankan nec |
| 44412 Bangladeshi |
| 44413 Nepalese |
| 44414 Pakistani |
| 44499 Asian nec |
| *Not further defined (nfd)<br>A 'not further defined' (nfd) ethnic group category contains responses that are not specific ethnic group responses but are able to be placed in a broader category in the ethnicity classification.<br><br>**Not elsewhere classified (nec)<br>A 'not elsewhere classified' (nec) ethnic group category contains ethnicity responses that are infrequent or unanticipated. (US Library of Congress Studies, 2009) |

**Culture, religion, gender and dietary preferences.**

People of South Asian origin represent a wide range of different ethnicities, cultures social groups and religions. The main religious groups include Hinduism, Islam, Christianity Sikhism Buddhism, and Janism. The majority of people in India and

Nepal practice Hinduism, in Bhutan and Sri Lanka Buddhism, in Pakistan, Bangladesh and in the Maldives Islam (Fuller, 2004; Ibrahim, Ohnishi, & Sandhu, 1997). Vegetarianism is an intrinsic part of three religious groups in South Asian culture: Janism, Hinduism and Buddhism (Jayanthi, 2001). The practices vary across different subgroups, with vegetarianism being practiced by some subgroups and not others. In some cultures such as the Brahman culture in India, vegetarianism was traditionally linked to caste, with the higher castes eating only a vegetarian diet and shunning any animal slaughter, while the lower castes such as the Harijans slaughtered animals and were non vegetarians (Jayanthi, 2001). Multigenerational patriarchal families are highly valued in South Asian cultures, with several generations of one family living together (Fuller, 2004; Raine & Vallianatos, 2008). In India particularly and within other South Asian cultures, marriages are arranged by the family, with the bride joining her husband's family and adopting the dominant practices and customs of that family (Ibrahim et al., 1997). There are diverse religious practices, dietary preferences and customs that have been maintained across different cultural groups and sub groups for millenniums of generations (Jayanthi, 2001; Vaidyanathan, 1989).

The dietary patterns of South Asian people are influenced by religious beliefs and family dietary practices, region of origin and the traditional availability of food in that region (Fuller, 2004; Jayanthi, 2001). There are two main dietary patterns: vegetarianism and non-vegetarianism. Traditionally lactovegetarianism was the staple diet in the Indian culture with rice, chapattis and roti as the cereal main course and dahl preparations, buttermilk and yoghurt as the accompaniments (Jayanthi, 2001). In more recent times there have been variations to the vegetarian diet. These include lactoovovegetarians who consume eggs as well as dairy, cereals, grains, fruit and vegetables; lactovegetarians who include dairy but not eggs, and vegans who only eat

vegetables, fruits, cereals and grain (Jayanthi, 2001; Raine & Vallianatos, 2008; Sharma et al., 2003).

The non-vegetarian diet of South Asian people differs to that of western cultures. In the traditional Western non-vegetarian diet, meat is the main course and cereals and vegetables the accompaniment. Meat in the non-vegetarian Western diet may be eaten two to three times per day. In the Indian non vegetarian diet, cereal based foods still feature as the main course and meat dishes are an accompaniment to the main course alongside dal preparations and vegetables (Jayanthi, 2001). Meat tends to be fish, chicken, goat or lamb served in small portions and may only be eaten two to five times per month (Sharma et al., 2003). Socioeconomic status also influences the availability of meat. This means that even non-vegetarian Indian people may only consume small quantities of meat infrequently. A study exploring dietary habits in 1150 pregnant women in Delhi (Sharma et al., 2003) identified that 96 % of the women had iron deficiency anaemia and that rates for anaemia did not vary significantly between meat-eaters and non meat-eaters. The authors' concluded that this was because of the low rate of meat eating overall in India (Sharma et al., 2003). Since dietary practices, particularly vegetarianism are so interlinked with religion, family practices and culture, it is vital that any changes to dietary practices proposed in this VitB12 study are consistent with the participants' cultural dietary preferences.

**The health of the South Asian population in New Zealand.**

The way in which New Zealand population statistics have been collected has meant that the Indian population living in New Zealand has not been readily identified as a high risk group for T2DM or CVD (Gala, 2008; Ministry of Health, 2008; Rasanathan et al., 2006; Statistics New Zealand, 2005a). At level one statistical aggregation, the low rate of T2DM and CVD amongst other Asian groups has diluted

the high incidence amongst the Indian population in particular (Gala, 2008; Rasanathan et al., 2006).

A Health Needs Assessment (HNA) report undertaken by Counties Manukau District Health Board (CMDHB) provides valuable data on the health of Asian people within its catchment population (Gala, 2008). The level one Asian population demonstrated better outcomes than European on most indicators of health. However the subgroup of Indian in this level one 'Asian' group demonstrated some major areas of concern on health indicators, in particular the over representation of low birth weight babies and the high rates of obesity (using ethnic specific definitions of overweight and obesity), T2DM and CVD. Other South Asian ethnicities are not identified separately in the report. When compared against other non-Asian population groups, the Pacific Island population was the only group to have a higher prevalence (10.6%) of diagnosed diabetes mellitus than the Indian population (8.9%). Maori (6.6%), Chinese (2.6%) and European (4.7%) were all lower. These statistics represent diagnosed cases of diabetes mellitus and do not reflect the significant number of undiagnosed cases.

The Indian population in the CMDHB HNA ranked high on risk factors such as high cholesterol and high blood pressure and low on health protective factors such as participation in physical activity and consumption of vegetables (Gala, 2008). The health of new migrants to a country is generally better than second and subsequent generation migrants in terms of health determinants (Gushulak, 2007; McDonald & Kennedy, 2004). This is of particular concern for South Asian people who already demonstrate a significant health disadvantage.

These New Zealand statistics mirror international statistics that highlight South Asian populations as a particularly high risk group for T2DM and CVD (Ramachandran et al., 2010; Wild et al., 2004). Population projections in New Zealand are only shown for Indian and Sri Lankan populations. These highlight that these South Asian

populations are expanding rapidly, particularly in the four major city centres (Statistics New Zealand, 2008c). In 2006 (the most recent census in New Zealand), the Indian population in New Zealand was 104,583, and Sri Lankan 8,310. These population figures are expected to double in the next two decades (Statistics New Zealand, 2008c). This reinforces the priority that must to be given to the South Asian population in terms of primary prevention of risk factors contributing to CVD and T2DM.

**Section Four: Interventions to Manage Vitamin B12 Deficiency**

There is evidence for success of dietary interventions to increase serum B12 concentrations. Response to fortification with B12 food was evaluated in a 12-month milk or meat supplementation trial in 555 schoolchildren aged 5 to 14 years in Kenya (Siekmann et al., 2003). Children supplemented with 200 to 250 mL of milk per day (0 96 µg B12 in 200 mL) under controlled experimental conditions increased their serum B12 concentrations by an average of 66 ± 71 pmol/L from 164 to 236 pmol/L. Those supplemented with 60 to 85gm of meat per day (0.75 µg B12 in 60 gm) increased their serum B12 by 47 ± 66 pmol/L. It is difficult to know how well the results for increased dietary B12 intake would translate to individuals in a free-living situation, but this research provides proof of principal that increased dietary intake of B12 can be effective. At baseline, 68.2% of these children were either depleted or deficient in serum B12, so the response to increased dietary B12 intake may have been high in this group because of a physiological up-regulation of B12 receptors, enhancing dietary B12 absorption. In this group of Kenyan schoolchildren with significant B12 deficiency, it took two years of supplementation for nine months of each year, to virtually eradicate B12 deficiency (McLean, Allen, Neumann, Peerson, & et al., 2007). The results of this Kenyan research suggests that increased consumption of animal-derived or B12 fortified foods can be effectively used to increase and maintain sufficient B12 concentrations in people on predominantly vegetarian diets.

The World Health Organization and the NZ Government policy on micronutrient supplementation advocate increasing dietary intake of foods containing micronutrients in preference to tablet supplementation (Ministry of Health, 2003; World Health Organization & Food and Agricultural Organization of the United Nations, 2006). The proposed VitB12 study aims primarily to increase B12 concentrations in women pre-pregnancy by increasing B12 intake in commonly consumed foods. In this study, the supplement treatment group is the comparator group for the acceptability, efficacy and sustainability of B12 supplement compared with increased B12 dietary intake. The Ministry of Health guidelines acknowledge the challenge of obtaining sufficient B12 intake through vegetarian based diets alone, hence the trial of both supplement and increased dietary B12 intake in the VitB12 study.

At the commencement of the VitB12 study, there is a lack of published research on the prevention and management of B12 deficiency due to insufficient dietary B12 intake. There are general recommendations for those with strict vegan or vegetarian dietary practices to take a B12 supplement (Ministry of Health, 2003), but often the dose and frequency are not given, and if they are, then the recommendations are supported by review articles rather than research evidence (Antony, 2003; Panebianco, 2007). The sublingual route is recommended in some literature, even for those with deficiency due to inadequate dietary intake (Donaldson, 2000), indicating the perception of oral B12 supplements are not well absorbed even in those whose B12 deficiency is due to inadequate dietary intake. This highlights the need for more research on strategies to prevent and manage B12 deficiency due to insufficient dietary intake and to quantify the amount, type and frequency for consumption of B12 containing foods, or the dose, frequency and route of a B12 supplement.

**Vitamin B12 supplementation.**

Oral B12 supplements most commonly contain cyanocobalamin and intramuscular B12 supplements contain hydroxycobalamin which can then be converted into methylcobalamin and adenosylcobalamin, the formulations of B12 that enter the methylation cycle. A systematic review of B12 supplementation trials included two randomised controlled trials undertaken in older adults that compared high dose oral supplementation regimes (1000 to 2000µg) with high dose intramuscular regimes.(Butler et al., 2006; Vidal-Alaball et al., 2005). The review found that in the limited number of studies that qualified for the systematic review, oral supplementation was just as effective as intramuscular supplementation (Butler et al., 2006). In some countries, such as Canada and Sweden, oral B12 supplementation is more common than intramuscular with two thirds of supplement treatments prescribed as oral (Lederle, 1998; Loökk et al., 2001; Nilsson et al., 2005). In most countries however, there is an impression that oral treatments are not absorbed, so intramuscular treatments are used for symptomatic B12 deficiency, whether the cause is related to malabsorption or insufficient intake (Graham et al., 2007). This highlights a gap in understanding of the pharmacokinetics of B12 supplementation.

The dose of vitamin B12 supplement required to increase and maintain B12 concentrations in people with insufficient dietary B12 intake is not well established. To date, older adults with a recognized age-related decline in the GIT absorption of B12 have been the focus of B12 supplementation research. The majority of studies have researched supplementation for B12 malabsorption caused by insufficient gastric hydrolysis of food bound B12 or a lack of intrinsic factor required for B12 transport and absorption. Treatments have traditionally focused on B12 supplementation by intermittent intramuscular injection with hydroxycobalamin 1000 µg, to bypass the need to GI absorption (Graham et al., 2007). The assumption is that the lack of intrinsic

factor would inhibit the therapeutic efficacy of oral B12 supplementation. When vegetarian or non-vegan clients present with pernicious anaemia or neurological symptoms in response to B12 deficiency, intramuscular supplementation is also the treatment of choice to rapidly increase B12 concentrations in order to treat the symptoms (Carmel, 2008; Hudson, 2010). If dietary B12 intake could be increased enough to avoid deficiency and improve B12 status, then intramuscular B12 injections may not be required, except when insufficient intake is accompanied by significant B12 malabsorption, for example, in inflammatory bowel disorders.

There is one study undertaken in participants who were B12 deficient due to insufficient dietary B12 intake. A proof of concept trial for B12 supplements and folate rich green leafy vegetables was undertaken on 40 non pregnant women in Pune India (Yajnik, Lubree, et al., 2007). Trial treatments compared 1) 100gm cooked green leafy vegetables alone or 2) 500 µg B12 supplement alone, or 3) green leafy vegetables and B12 supplement together, or 4) a control group treatment. At baseline, there was significant B12 deficiency; 62% ($n$ = 26) had low serum B12 (< 150 pmol/L), 25% ($n$ = 10) had a low serum folate (< 3 ng/mL) and 24% ($n$ = 27) had raised homocysteine concentrations (≥ 15 µmol/L). Researchers administered the allocated treatments to participants every second day for six weeks. Outcome measures were changes in serum folate, homocysteine and serum B12 concentrations. There were no changes in serum folate, B12 or homocysteine in the folate fortified or control groups. However, the B12 supplement and combined B12 treatment group had a significant increase in serum B12 (from 125. to 215 pmol/L, $p < 0.001$) and a significant decrease in homocysteine (from 18.4. to 13.4.µmol/L, $p < 0.01$). This proof of principal trial supported that the B12 supplement is absorbed well and that taking high dose B12 supplements was effective in decreasing homocysteine in a population with low serum B12 and raised homocysteine concentrations (Yajnik, Lubree, et al., 2007). Adherence with taking supplements over a

prolonged period in free-living participants was not evaluated in the previously cited B12 supplementation study and requires further investigation.

Two RCTs, that compared the effectiveness of high doses of oral and sublingual cobalamin for increasing serum B12 and decreasing homocysteine, found no significant differences between either routes of administration (Sharabi, Cohen, Sulkes, & Garty, 2003; Yazaki, Chow, & Mattie, 2006). Cyanocobalamin 500 µg oral, 500 µg sublingual or an oral vitamin B complex of cyanocobalamin 250 µg, thiamine100 µg and pyridoxine 250 µg were the comparator treatments in one study (Sharabi et al., 2003).A vitamin B12 complex containing methylcobalamin 1000 µg, folic acid 400 µg and pyridoxine 5mg, either sublingual or oral were the comparator treatments in the other study (Yazaki et al., 2006). Both sublingual and oral supplements produced significant increases in serum B12 and a significant decrease in homocysteine. Authors of both studies concluded that if the dose of oral B12 was high enough, then sufficient amounts of oral cobalamin could be absorbed to reverse the effects of vitamin B12 deficiency and to treat hyperhomocysteinaemia (Sharabi et al., 2003; Yazaki et al., 2006).

In contrast to the majority of other vitamin B12 supplementation studies, Sharabi et al.(2003) included a younger age group in their study; the mean age in each treatment group was 44.5± 14.7, 50.2 ± 15.1 and 49.6 ± 11.6 years (Sharabi et al., 2003). This study population has relevance for the population in the VitB12 study, although 70% of the participants in the Sharabi et al (2003) study were male. Five of the participants tested positive for pernicious anaemia. Fifty percent of the participants in the study were vegetarian so insufficient dietary B12 intake was the suggested cause of B12 deficiency. There do not appear to be any published studies comparing low dose oral and sublingual B12 supplements.

**Low dose oral B12 supplementation.**

When risk of B12 deficiency exists due to inadequate dietary B12intake, absorptive mechanisms are intact so high pharmacological doses of B12 supplement should not be required. Physiological doses close to the recommended daily intake should be sufficient. The randomised controlled studies undertaken using low dose cyanocobalamin supplements, have also focused on older adults with B12 malabsorption. The BOSSANOVA study undertaken in France enrolled 89 older adults who had serum B12 concentrations of < 162 pmol/L and diagnosed food bound B12 malabsorption (Blacher, Czernichow, Raphael, Chade-faux, & Morinaeu, 2007). The 30-day study compared the effectiveness of six different doses of B12 cyanocobalamin supplement (2.5, 5, 10, 20, 40 and 80 µg respectively). The aim of the study was to determine an effective cyanocobalamin dose for use in B12 flour fortification and the desired change was an increase in serum B12 by 37 pmol/L over 30 days. All doses were significant in increasing serum B12 over 30 days, but had an insignificant effect on homocysteine or MMA. The 5.9 µg dose was effective at increasing serum B12 by 37pmol/L. The 30 day period may not have been sufficient time frame to produce a change in homocysteine or MMA (Blacher et al., 2007).

These findings differ from an RCT conducted in the Netherlands where 120 older adults were randomly allocated to receive either a 2.5, 100, 250, 500,or 1000µg cyanocobalamin supplement over 16 weeks (Eussen et al., 2005). Serum B12, holoTC, MMA and homocysteine were the main outcome measures. A high dose (647 to 1032µg per day) was required to reduce MMA to the desired outcome of less than 0.26 µmol/L, however a low dose (2.5 µg per day) was sufficient to produce a small but significant increase in serum B12 and holoTC over 16 weeks (Eussen et al., 2005).

The findings of the Eussen et al. (2005) study are similar to others undertaken in older adults with documented B12 malabsorption. In a study of 31 older adults in Australia

with diagnosed B12 deficiency, neither a 10 µg nor a 50 µg oral cyanocobalamin supplement taken daily over 4 weeks, produced any significant decrease in homocysteine (Seal, Metz, Flicker, & Melny, 2002). However, the 50 µg dose was associated with a significant increase in serum B12, but the 10 µg dose was not. None of the studies cited above included participants who were B12 deficient or depleted due to inadequate B12 intake, and in whom GIT absorption of B12 was intact. These findings are consistent with other studies investigating B12 supplementation in older adults with food-cobalamin malabsorption (Andrès et al., 2003; Andrès et al., 2001; Rajan et al., 2002; Seal et al., 2002; Verhaeverbeke, Mets, Mulkens, & Vandewoude, 1997). High dose B12 supplements (from 100 to 5000 µg) have been effective in increasing serum B12 concentrations over one to four months but there have been mixed results with the effectiveness of B12 supplements to decrease MMA or homocysteine. Factors such as the dose of supplement, the frequency and duration of supplementation, and the baseline metabolite MMA and homocysteine concentrations are suggested to influence these (Rajan et al., 2002; Seal et al., 2002; Yajnik, Lubree, et al., 2007).

There is a gap in the research literature where effective physiological dose strategies to improve B12 biomarkers in women of childbearing age need to be investigated. This includes the B12 supplement dose, formulation and dose frequency required to improve both serum B12 and holoTC concentrations. The proposed B12 supplement dose for the VitB12 study is 6µg per day, based on the optimum dose findings in the study by Blacher et al. (2007). This is also close to the dose that may be achieved thorough increasing dietary intake of vitamin B12, thus allowing a useful comparison between low dose vitamin B12 supplementation versus increasing dietary B12 intake.

## Section Five: Justification for the Current Vitamin B12 Research Project

The predominance of vegetarian practices in the South Asian population, the prevalence of low B12 concentrations and the high rates of NCD make South Asian women a priority for research (Gala, 2008; Krishnaveni et al., 2009; Krishnaveni et al., 2010; Ramachandran, 2005; Rasanathan et al., 2006; Rush, Chhichhia, et al., 2009; Xin, 2008; Yajnik, 2009a). In the long term, further research needs to investigate whether increasing B12 concentrations in women preconception and throughout pregnancy reduces the offspring risk for insulin resistance, abdominal adiposity and low muscle mass (Yajnik, 2009a). Prior to researching the effects of increased maternal B12 status on offspring health however, it is important to determine effective ways to increase B12 concentrations. In a non-pregnant population, an intervention trial comparing the effect of low dose B12 supplementation against placebo supplementation is acceptable ethically. In a pregnant population, a longitudinal study that includes a placebo treatment raises ethical and safety issues. An intervention study is needed in a non-pregnant population first, to determine effective strategies to raise B12 concentrations, with identification of any associated risks. Once identified as safe and effective, these strategies could be implemented in a follow-on longitudinal study in pregnancy. The strategies need to take into account the myriad of factors influencing B12 intake. It is important to investigate whether increased dietary B12 intake as advocated by the Ministry of Health is an effective way to increase B12 or whether low dose B12 supplementation is a more effective way (Ministry of Health, 2003). Research is needed to answer questions about the most effective way to work with the South Asian community and with health professionals working with that community to increase awareness of the issues around B12 deficiency and to develop strategies to increase B12 that are appropriate for the community as well as sustainable over time.

South Asian women are the focus of contemporary research into B12 and the effects on offspring because of the high incidence of low B12 status in this population, the concurrent high rates of NCD, and the research evidence suggesting metabolic reprogramming in the offspring of Indian women with B12 deficiency and high folate status (Yajnik et al., 2008). It is also important to see if the findings associated with low B12 status are replicated in other populations such as European, Asian, Maori or Pacific Island. Populations other than South Asian would offer a valuable comparison of changes that occur in situations of low maternal B12. Some factor that occurs in association with B12 deficiency in South Asian women may potentiate the metabolic changes in the offspring e.g. reduced consumption of protein. There is a scarcity of research on the B12 status of childbearing age women from different ethnic groups in New Zealand to be able to compare with South Asian women. With the current global recession, the threatening global food crisis and concerns about the impact of farming on our environment, dietary patterns may change towards lower meat intakes and vegetarianism. B12 is exclusive to animal based foods (or fortified products), therefore it is important for all populations to understand the risks and benefits associated with moving away from animal based foods and the precautions needed to avoid B12 deficiency (Jacobs, Haddad, Lanou, & Messina, 2009; Underwood, 2003).

**Focus of the current vitamin B12 study.**

The focus of the current VitB12 study is to measure the occurrence of low vitamin B12 status in a convenience sample of South Asian women of childbearing age and to investigate how to prevent vitamin B12 deficiency in women prior to conception. The VitB12 research methodology and methods have been developed to answer the following research aim and research.

**Research aim.**

To prevent vitamin B12 deficiency in South Asian women of child bearing age.

**Research questions.**

For South Asian women of childbearing age:

1. What are the factors that influence dietary intake of vitamin B12?
2. What is the prevalence of Vitamin B12 deficiency?
3. Is low dose supplementation or in increase in dietary intake of vitamin B12 containing foods more effective, acceptable and sustainable over time for increasing vitamin B12 biomarker concentrations?
4. What are appropriate approaches for working in partnership with the community on research to investigate and prevent vitamin B12 deficiency?

**Appropriate methodology and methods to answer the research questions.**

A mixed methods research approach is the most appropriate design to answer the above research questions. Mixed methods research is a research design with philosophical assumptions as well as methods of inquiry. As a methodology, it involves philosophical assumptions that guide the direction of the collection and analysis of data and the mixture of qualitative and quantitative approaches in many phases of the research process. As a method, it focuses on collecting analyzing and mixing both quantitative and qualitative approaches in a single study or a series of studies. Its central premise is that the use of quantitative and qualitative approaches in combination provides a better understanding of research problems than either approach alone (Cresswell & Plano Clark, 2007, p. 5).

The questions for the VitB12 study require in-depth community knowledge, perspectives and engagement, but they also require a well-controlled research design to accurately measure the effect of study treatments. A mixed methods approach can include methods from both the positivist and the interpretive paradigms that enrich the exploration of research problems and provide a better understanding than just one method alone (Cresswell & Plano Clark, 2007; Giddings & Grant, 2007; Grant &

Giddings, 2002). Community input and inclusion (from both South Asian women and their health professionals e.g. midwives) are critical when implementing any change in health related behaviours (Cresswell & Plano Clark, 2007). A qualitative study can provide a rich source of contextual and environmental factors that influence dietary practices and therefore the consumption of B12-containing foods. Community input provides guidance with factors that affect adherence with the prescribed dietary intake of B12 containing foods or B12 supplements to be implemented in the quantitative study, and critical insights on how to safely conduct research within the South Asian community. Quantitative methods allow for carefully controlled application of scientific methods to the design, implementation, analysis and interpretation of research, enabling findings to answer the research questions with a degree of confidence. The methodology and methods for the mixed methods research approach used in the VitB12 study are described in more detail in Chapter 3.

**Engaging with the South Asian community.**

The qualitative methods involving input from community focus groups and the involvement of the South Asian community to collaborate on the VitB12 research is underpinned by a participatory worldview and a joint generation of knowledge approach to knowledge development (2002) (Grant & Giddings, 2002). Here, the community guides the research process towards meeting the needs of the community with regard to the problem of low vitamin B12 concentrations within the South Asian community and the implications for women and their children. A community based participatory research approach (Minkler & Wallerstein, 2003) will be used in the VitB12 study to provide a model for collaborating in partnership with the South Asian community. This approach is important in order to conduct research that is appropriate for the community's needs, consistent with community philosophy, with outcomes that have relevance for the community and are able to be translated into meaningful changes in

health practices. The integration of CBPR into the VitB12 study supports answering the research question: What are appropriate approaches for working in partnership with the community on research to investigate and prevent vitamin B12 deficiency? CPBR is described in more detail in Chapter 3.

**Chapter Summary**

Concerns about the effects of maternal B12 deficiency on the future health of offspring make this research project a priority to undertake with South Asian women. The problems of anaemia, impaired cognition and neurological function that occur at deficient concentrations of B12 (< 150pmol/L) are well recognized health issues. It is the previously unrecognized risks that appear to be triggered and attenuated at the lower limits of normal range (150 to 222 pmol/L), when there are insufficient B12 stores available for one carbon metabolism that are a concern. This is particularly the case in pregnancy and breastfeeding where mothers can pass on risks associated with B12 deficiency or insufficiency to their offspring. In the life course model of the aetiology of chronic disease, it is the risks associated with low muscle mass, relative adiposity and insulin resistance that increase susceptibility to NCD such in later life. Lifestyle factors throughout the life-course of the offspring can heighten or decrease these already present risks. Effective health promotion strategies begin by addressing factors such as low maternal B12 that increase the offspring's intergenerational susceptibility to risk.

The long term research question raised by this literature review is; does increasing maternal B12 concentrations prior to and during pregnancy decrease the risks of abdominal adiposity, insulin resistance and low muscle mass in babies born to women at risk of low B12? In the short term, research is needed to determine effective strategies for increasing B12 concentrations in South Asian women of childbearing age prior to conception. Working with communities through a CBPR approach enables the community to be involved with the research so that appropriate strategies developed to

increase B12 intake are acceptable and sustainable for the community as well as being effective in increasing B12 concentrations. To address the long-term research question, these strategies can be evaluated in a future longitudinal study of women in pregnancy investigating the effects of improved B12 status on the growth and development of the child. The next chapter (Chapter 3) presents an overview of the methods and methodology for the VitB12 study, then a discussion of the focus group data collection and analysis.

# Chapter 3: Justification for the Mixed Methods Research Approach and Design of the Focus Group Interviews

This chapter provides an overview the research methods selected to investigate the research aim of preventing vitamin B12 (B12) deficiency in South Asian women of child bearing age. The research used an embedded experimental mixed methods design informed by a community based participatory research (CBPR) approach, in which community focus group interviews in phase one preceded and informed a larger randomized controlled trial (RCT) in phase two.

This chapter is organised into three main sections. **Section one** restates the research aim and questions, and the ideas from the literature review that justify the specific focus of the research. This includes an overview of the embedded experimental mixed methods research design implemented to meet the research aim. Descriptions include the methodology and researcher assumptions underpinning the mixed methods design, plus explanation of, and justification for the design. The CBPR approach to the research is briefly described with rationale given for community participation in the planning and conduct of the research, including detail on preparing for and conducting culturally competent research. Descriptions include ethical considerations and ethical conduct of the focus group interviews, plus the ethical approval for both phases of the research. While section one overviews all phases of the VitB12 study, section two and three include the qualitative methods for the focus group interviews only. The quantitative design and methods for the RCT are covered in Chapter 5. This flow of information is because methods for the RCT phase of the research are informed by focus group interview findings.

**Section two** describes the identification and recruitment of participants for the community focus group interviews. Descriptions include the selection of focus group participants and considerations around membership of each group, structuring of interview questions and the process of facilitating the interviews and recording the interview data.

The analytic processes used to explicate the focus group findings are covered in **Section three**. The approach was qualitative descriptive, using thematic analysis with both inductive (ideas seen as emerging from the data) and deductive (ideas in response to a specific question or theme) generation of codes, sub-themes and themes. This section includes steps taken to ensure the rigor of research processes in the focus group phase of data collection and analysis, including auditing of the decision making processes and steps for establishing the trustworthiness of the findings.

## Section One

### Study Design

The following research aim and questions were identified from the literature review in Chapter 2 as the focus for the VitB12 study. An embedded experimental mixed methods research design was the most appropriate design to investigate the research aim and the research questions.

**Research aim.**

To prevent vitamin B12 deficiency in South Asian women of child bearing age.

**Research questions.**

For South Asian women of childbearing age:

1. What are the factors that influence dietary intake of vitamin B12?
2. What is the prevalence of vitamin B12 deficiency?

3. Is low dose supplementation or in increase in dietary intake of vitamin B12 containing foods more effective, acceptable and sustainable over time for increasing vitamin B12 biomarker concentrations?
4. What are appropriate approaches for working in partnership with the community to investigate and prevent vitamin B12 deficiency?

**Mixed Methods Overview**

A mixed methods research design was used for the VitB12 study with data collection occurring sequentially in two phases; phase one using the qualitative methods of focus group interviews for data collection and phase two using quantitative methods of a RCT.

A critical element of mixed methods research design is the way in which the qualitative and quantitative data collection and analysis are mixed within the design. There needs to be a purpose for including different research methods in the design and for the way they are mixed, so that in combination the methods provide a more detailed answer for the research questions, than when the methods stand alone (Cresswell & Plano Clark, 2007). The data from different methods can be merged, connected or embedded, with the method used influenced by the philosophy underpinning the research as well as the research questions to be answered (Cresswell & Plano Clark, 2007; Teddlie & Tashakkori, 2009). The design for the VitB12 study was an embedded experimental mixed methods design, in which phase one (focus group interviews), was embedded within, and informed phase two (the larger RCT). One method is dominant (in this research, the RCT), and the results from the method that is not dominant (focus group interviews) are embedded within the dominant methods. The design used for the VitB12 study is an adaptation of Cresswell & Plano Clark's (2007) embedded experimental design. There was ongoing input throughout the research study from a

community collaborative research group (CCRG) and this is included in the mixed methods design.

The way that a researcher views reality and from this, the way they view knowledge and knowledge acquisition, influence the methodology that a researcher aligns with (Hughes & Dumont, 1993). The researcher's assumptions and worldviews on knowledge influenced the research design selection for the VitB12 study.

**Researcher's Perspective and Assumptions**

The researcher's 34 years' experience as a registered nurse, working with people from a diverse range of cultures and on wide range of health issues influenced, and continues to influence her assumptions about knowledge and knowledge acquisition around B12 deficiency and strategies for prevention in South Asian women. The first assumption was that any strategies to prevent B12 deficiency must be guided by a detailed and appropriate assessment of factors that affect B12 status. The next assumption was that collaboration with the people involved is vital, as explication of these factors and their importance must come from the South Asian community. The third assumption was influenced by a history of clinical nursing practice informed by research evidence. In contemporary health care environments, credible evidence is required to maximise the benefits of interventions and to minimise risks, particularly avoidable risks. The third assumption, informed by this experience was that research to promote change in the political, economic and social context of healthcare needs support with clinically significant research evidence that is measurable, valid and reliable. The fourth assumption was that while the scientific experimental method provides support for the strength of associations between interventions and outcomes, it does not explain the reality and complexity of individual or community factors that influence response to outcomes. The fifth assumption was that the impetus to change health behaviours needs to come from the community, and so active participation of the

community in determining the need for change was essential for translation of research evidence into revised health practices and behaviours. Based on these assumptions, an experimental method, supported by collaboration with, and subjective experiences from, the South Asian community and health care providers was considered the most appropriate to investigate how to prevent vitamin B12 deficiency in South Asian women of childbearing age.

**Methodology Rationale and Justification**

Methodology refers to the philosophical framework; the assumptions and values that underpin the methods used in research and the way that they are analyzed and interpreted, while methods refer to the techniques that are used to gather the empirical evidence, for example, RCT or focus group interviews (Cresswell, 2003; Cresswell & Plano Clark, 2007; Giddings & Grant, 2007). Methodology is influenced by paradigms for knowledge generation; how a researcher views reality and from this, the assumptions made about knowledge and knowledge acquisition (Hughes & Dumont, 1993). These guide the way that research is conducted, how methods are used, and in mixed methods research, which research method receives the most weighting as well as the analysis and interpretation of data and information collected (Giddings & Grant, 2007; Halcomb, Gholizadeh, DiGiacomo, Phillips, & Davidson, 2007). Paradigms for knowledge development are positivism and postpositivism, interpretivism, radical and poststructural (Grant & Giddings, 2002).

The predominant paradigm underpinning this VitB12 research was postpositivism, with knowledge generation focused on identifying strategies to prevent B12 deficiency in South Asian women (Cresswell & Plano Clark, 2007). In both the positivist and postpositivist paradigm, methodologies are oriented towards determining cause and effect relationships and the researcher is an objective observer in the research (Cresswell & Plano Clark, 2007; Giddings & Grant, 2007). Positivism assumes that

knowledge is able to be captured using methods that control for random or systematic error and that narrow in on a few selected variables. A deductive method for reasoning is used as data is collected, and findings analysed to support or reject an a priori formed hypothesis (Cresswell & Plano Clark, 2007; Grant & Giddings, 2002). However, unlike a very conservative positivist paradigm where the scientific method and determining cause and effect are the only way to conduct research, postpositivism acknowledges that research from this paradigm only suggests associations, it does not confirm the associations, and knowledge is much more complex with many interrelated factors and variables that cannot be controlled for (Cresswell & Plano Clark, 2007). Postpositivism also acknowledges that reality is constructed through a complex interplay of social and cultural factors and that human experience and the perception of that experience cannot be captured using traditional cause and effects experimental research methods (Giddings & Grant, 2007). The mixed methods embedded experimental design was an appropriate choice of research approach, as the mixed methods provide for a wider explanation of interactive factors that influence the outcome of the RCT.

An advantage of research from a postpositivist paradigm is that it provides measurable research outcomes, and these are currently needed in the social, economic and political reality of health care and services (Gabbay & Le May, 2011). Measurable outcomes of population costs and benefits can be used to provide evidence for practice, to justify funding and to support change. The postpositive paradigm influencing this research means that the qualitative method of focus group interviews provides a smaller supporting role in the development of the larger quantitative method of the RCT. The focus group interviews also helped to inform and expand on the RCT findings. The RCT investigates the effect of interventions to increase B12 concentrations, and associations between the dose/effect relationship with food or supplements, with analysis and interpretation determining the cause and effect relationship. Traditionally,

establishing cause and effect through tight control of reliability and validity is the domain of the positivist paradigm for knowledge generation. In this VitB12 research, the enrichment of these findings through the integration of qualitative findings from the focus group interviews extends knowledge generation to the postpositivist paradigm, with some influence from an interpretivist paradigm (Giddings & Grant, 2007; Halcomb et al., 2007). With the inclusion of the community focus groups, plus the community collaborative research group, the South Asian community guides the research process towards meeting the needs of the community with regard to the problem of low B12 concentrations within the South Asian community and the implications for women and their children

**Community Based Participatory Research**

The VitB12 research was conducted in collaboration with the South Asian community. CBPR, the model used for community collaboration in this research, is a collaborative socio-ecologic approach to research (Minkler & Wallerstein, 2003a). Emphasis is on collaborating in partnership with communities on social, health and environmental change. Partnership in the CBPR context involves community members, leaders of community organisations and researchers, meaningfully and equitably in the research process. Each shares their particular expertise and takes joint responsibility for the conduct of the research in order to enhance understanding of the research problem and to translate the knowledge learned from the research into behaviours that genuinely improve the health of community members. (Choudary, 1998; Minkler & Wallerstein, 2003a). CBPR methods are critical to ensuring that the priorities and processes for research are appropriate for the communities being researched (Minkler & Wallerstein, 2003a; Viswanathan et al., 2004). By working in partnerships with those communities, researchers compliment the strengths that already exist within the community (Fredericks, 2007; Minkler & Wallerstein, 2003a). For the South Asian community in

New Zealand, these strengths are the strong sense of togetherness, the close family bonds and the multiple community organizations that work together to build and maintain strong links between South Asian people and their communities (Personal observation from attending South Asian community forums, health days and community network groups).

The imperative of community consultation and collaboration when researching and implementing any health, social or environmental projects was highlighted in the report: "Engaging Asian Communities in New Zealand" (McGrath, Butcher, Pickering, & Smith, 2005). Many of the recommendations in this report echo the principles of CBPR. In particular, consultation with key community groups was vital to avoid repetition and "focus group fatigue". New Zealand is a small country, consequently some communities have been researched so often without the promised results that they are withdrawing from collaboration rather than engaging in it. Community groups can advise about this. Another key finding from the "Engaging Asian Communities in New Zealand" report was to ensure that representation was obtained from community members as well as community leaders (McGrath et al., 2005). The focus group interviews used on this research aimed to engage the community in collaborating on how to conduct the RCT that followed on from the focus group interviews.

One of the key messages of CBPR is to grant ownership of the project to key community members so that they are directly involved in the project; making decisions rather than being on the receiving end of decisions (Viswanathan et al., 2004) and this was a major consideration in the conduct of the investigation of this research. Community leaders, representatives and health professionals have been involved with the VitB12 research project from its inception. Although the research issue was identified by the research team, the issue of high rates of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) are well recognized among the South Asian

population, and community organizations are working with health professionals to prioritize, develop and implement strategies to address these risks (Gala, 2008). The current VitB12 study aligned with priorities for health strategies and was supported by the community leaders. The community group was set up to act as collaborators for the VitB12 research, the CCRG met prior to the start of the research, once during the early stages of the research, then again once the research was completed. Communication was also via email, and members of the group advised and guided the study, particularly on appropriate methods for, and support with participant communication, recruitment and retention. The CCRG are assisting with dissemination of the findings and any additional action to take. The CCRG included a health professional, health promoter and two women from the community with personal experience of vitamin B12 deficiency. As well as the CCRG, three general practitioners, two South Asian midwives and the community group The Asian Network Incorporated (TANI) were consulted about the research. These people offered support in terms of access to participants and use of their facilities if required for data collection. This also strengthened networks between the researcher and South Asian community organizations.

**Conducting Culturally Competent Research**

It was vital that the conduct of this Vitamin B12 study was culturally competent as the research involved a culturally and linguistically diverse group of people and the researcher was from a different culture to the participants. In this study, developing the trust of the community was facilitated by the researcher relationships with the CCRG and in particular with the research assistant who was a key member of many different organizations and boards within the communities and had an established position of trust. She was able to guide the researcher about conducting the research in a culturally appropriate manner and to facilitate the initial contact with people in the South Asian community. Strategies to guide cultural competence were also informed by Campina-

Bacote's (2002) *The Process of Cultural Competence in the Delivery of Healthcare Services Model* and by Suh et al.'s (2002) *Cultural competence in qualitative interview methods with Asian immigrants.* Consideration was given to the five major constructs of cultural competence proposed by Campinha-Bacote (cultural awareness, cultural knowledge, cultural desire, cultural skills and cultural encounters), and how these should be incorporated into this study.

Cultural awareness and knowledge were paramount for gaining trust of participants, conducting culturally safe research and being able to collect and interpret data appropriately (Bandesha & Litva, 2005; Huer & Saenz, 2003). In order to develop some cultural awareness and knowledge, the researcher immersed herself in a range of fiction and nonfiction literature exemplifying South Asian culture. Members of the South Asian community and the research assistant also provided guidance on cultural considerations and organised for the researcher to attend some home-based Diwali festivities so she could experience first-hand the Indian way of hospitality and the importance of traditionally prepared food for socially bonding family and friends. Although the above mentioned literature and experiences were only a preliminary encounter with South Asian and in particular, Indian culture, they did sensitise the researcher to be cognizant of cultural differences in roles and in meanings of everyday activities and the translation of these into research practices (Huer & Saenz, 2003; Suh, Kagan, & Strumpf, 2009). For example, when organising the focus groups, culturally appropriate foods and refreshments were offered as a gesture of encompassing hospitality.

Cultural desire is the motivation to strive for cultural competence while cultural encounter refers to the motivation of the researcher to engage in cross-cultural communication with the research participants (Campinha-Bacote, 2002; Suh et al., 2009). As the VitB12 study included South Asian women from a wide range of different

cultural and religious groups, it was a challenge to be familiar with the subtle differences for each cultural group. For the researcher, being receptive to and respectful of cultural diversity demonstrated cultural desire. When participants consented to take part in the focus group, they were asked if there were any considerations to be aware of when involving them in a group discussion. It was anticipated that this receptiveness to learn about cultural norms and practices would support participants to feel comfortable sharing their preferences beforehand and their experiences with the group. When visiting women participating in the RCT, the women were asked about any particular customs the researcher needed to consider when visiting them in their home. For example, one of the participants wanted her mother, a respected Indian elder to be intermittently present during data collection. The researcher demonstrated respect for the mother's status by ensuring that she remained at a lower level than the mother when interacting with her.

Receptive communication was critical so women felt comfortable sharing their preferences. For example, one of the younger women felt uncomfortable with the researcher touching her feet and she explained that it was usually younger women who touched the feet of older women as a mark of respect. As the researcher needed to apply electrodes to one foot for bioimpedance readings during RCT data collection, she checked with the women in the study first that they were comfortable with her applying the electrodes to their feet. For most of the women it was not a problem; for others they were reassured that the researcher was comfortable with touching a younger woman's foot. One participant preferred to apply the electrodes herself with instruction and supervision of the correct placement technique by the researcher.

Cultural skill refers to the skill of the researcher in being able to appropriately represent the views of the research participants. This included appropriate wording of interview questions for the focus group interviews, taking into account the cultural

perspectives of research participants. Cultural skill was also required to recognise cultural differences that influenced data and the way in which the data is interpreted or translated (Suh et al., 2009). The research assistant provided guidance for the researcher on cultural skills. Prior to commencing the focus groups, it was considered there may be difficulties with translation of questions for participants who were not comfortably conversant with English language. The research assistant was able to speak Hindi so it was proposed she could provide some translation services during the focus group interviews if required. This was not required as all of the participants in the focus groups were fluent in English. Clarification was required for the researcher however, as reference was made to certain foods, events, customs or sayings in the focus group discussions that the researcher did not understand, so the research assistant or focus group members would explain the meaning for the researcher. There was still however, a gap between the implicit, intuitive cultural understandings of the women advising on the conduct of the study, and the cultural naivety of the researcher. Clarification was still needed on situations as they arose during the research.

**Ethical Considerations**

All research participants have the right to protection and safety, to decline or withdraw from participation in a research study at any stage without disadvantage or penalty, to have their participation kept anonymous, and their details kept confidential. The next section addresses these principles of ethical conduct of a research project as outlined by Tolich and Davidson (1999)**.** This includes details of the ethical approval process, informed consent procedures, particular considerations around ensuring cultural competence and justice when involving participants, and maintaining participant anonymity and confidentiality (Tolich & Davidson, 1999). Discussions include consideration of the bicultural context of Aotearoa, New Zealand and the principles of Te Tiriti o Waitangi. Although the methods for the RCT are described in

Chapter 5, some of the ethical considerations that apply to the VitB12 study as a whole are discussed here, while those relevant to just the RCT only are outlined in Chapter 5

## Ethical Approval

The AUT University Ethics Committee (AUTEC) granted ethical approval for all phases of the research project on 23rd December 2008. During the early stages of the research, amendments were made to the approved ethics process (Appendix 1). Some of these amendments were made in response to changes in the research process prompted by the focus group findings.

14/09/2009. The approval of a recruitment advertisement in an Indian newspaper and The Asian Network Incorporated (TANI) newsletter

20/04/2010. Extension of recruitment to include South Asian women and the area for participant recruitment to include all of Auckland City

10/04/2010. AUTEC granted approval for the collection of blood samples by the researcher, an experienced registered nurse, or the research assistant (an experienced phlebotomist), in the participant's own homes or venue of their choice.

### Potential risks to participant.

For the focus groups, participants were invited to participate in the research and were advised both in the first verbal contact with the researcher and in the study information sheet that there was no obligation on their part to consent to participate and that they could withdraw at any stage without any disadvantage (Appendix 2).

Although recruitment was attempted through advertisement, this had very limited success, so the majority of participants learned about the research through South Asian community contacts. When recruited by word of mouth, South Asian community members were the first contact for the participants so that the researcher did not have access to details of who had been invited to participate. Once the researcher was

contacted by prospective participants, the voluntary nature of the research and the option to withdraw at any stage without disadvantage were reiterated. Participants were provided with details of the AUTEC consent and contact details for the VitB12 research project, and made aware that they could contact the researcher, research supervisor, or Executive Secretary of AUTEC if they were concerned at all.

**Informed consent.**

All participants considering participation in the focus groups interviews were provided with a copy of the focus group information sheet and the consent form prior to participation (Appendix 2 & 3). It was explained to potential participants on the focus group information sheet, the consent form, and verbally, that the interviews would be audiotaped and transcribed. Participants signed the written consent form confirming their willingness to participate in the study. Another explanation of the research processes was given prior to the focus group interviews and questions were invited and answered. Verbal consent was confirmed prior to each focus group interview and participants were made aware that they could decline to discuss any information in the focus groups that they felt was too sensitive to discuss.

**Confidentiality.**

Maintaining confidentiality was critical in this study. The South Asian community in Auckland is a small percentage of the larger population, and therefore it would be easier to identify individual members if care was not taken to protect identification. Signed participation consent forms were kept in a locked filing cabinet in a different location from the data. All identifiers such as location of work or specific roles were removed from the data. Only the researcher, research assistant and other focus group members of that particular group were aware of who had participated in the focus group interviews. Prior to each focus group interview, participants were advised not to divulge participation of fellow participants in the focus groups and to maintain

confidentiality of any information discussed in the interview. Pseudonyms were used in data and in the report to ensure anonymity. Further details on how identify was protected in the RCT are described in Chapter 5.

**Cultural and social considerations.**

The bicultural context of Aotearoa, New Zealand was considered and the principles of Te Tiriti o Waitangi. The treaty intentions of Te Tiriti o Waitangi provide people with the right to live in New Zealand, under partnership between Māori and the Crown. For South Asian immigrants accepted for residency under the Crown, this means they have right to protection under the Crown and the principles of Te Tiriti o Waitangi. This means that the research needed to benefit the South Asian population and not just be undertaken for the purpose of extending academic knowledge. To ensure that the research was meeting the stated aims, an interim data review was undertaken by an independent reviewer. As part of this interim review the preliminary risks and benefits were assessed, and evidence of benefit and none of harm confirmed. An important consideration throughout the research was that the researcher was from a different ethnic and cultural background than potential research participants, so the involvement of the South Asian CCRG in the research was critical to ensure the conduct of the research was culturally competent.

**Section Two**

Involvement of the South Asian community extended to the focus group interviews where data were collected on the conduct of the RCT and appropriate ways to recruit and retain women in the RCT. The interviews with South Asian community members and health professionals were also used to collect data on knowledge of dietary sources of B12 and B12 deficiency, factors that influence B12 intake in South-Asian women and suggestions on how to increase B12 intake.

## Focus Group interviews

### Objectives for focus group research.

The focus group interview methods for data collection and analysis addressed the following objectives.

a) To collaborate with the South Asian community on implementing a culturally appropriate randomised controlled trial comparing interventions for preventing vitamin B12 deficiency.
b) To identify factors that influence vitamin B12 intake (via diet or supplements)
c) To identify the knowledge and experiences that South Asian women have of vitamin B12 deficiency.

### Focus groups as a method for data collection.

Focus groups use facilitatied interview methods to obtain indivdual and collective data on a groups' values, beliefs and opinions on a particular topic, thus they support the collective consideration of issues and development of knowledge (Campinha-Bacote, 2002). In the context of this research, the focus group discussions were an economical and effective method of gaining insight into the opinions, beliefs and values of the group around knowledge and consumption of foods containing B12, experiences of B12 deficiency, dietary preferences, and the influence of religion, family, community, culture, economics and the New Zealand environment on these preferences. Open ended questions stimulated a wealth of information, more that would have been collected from single interveiws (Waldegrave, 2007). The open ended nature of the questions did not limit the information obtained in the way that other research methods such as surveys or questionnaires do.

The underlying assumption of focus groups for qualitative data collection is that indivduals of this group are valuable sources for information on the topic and that they are able to express these ideas within a group situation (Suh et al., 2009). To enable this,

careful consideration was given to the composition of the focus group, the development and asking of questions and the facilitation of the focus group discussions. Focus groups were structured so they were homogeonous, as common experiences enhance the group discussion and homogeneity reduces the risk that age, gender, or hierarchical power issues will inhibit members from contributing freely to the focus group discussion (Halcomb et al., 2007; Suh et al., 2009). Homogeneity was defined as participants from a similar religious and ethnic background, status in the community, age and gender while heterogeneity referred to differences with these (Krueger & Casey, 2000). While the composition of each focus group was homogeneous, across the six focus groups the representation was heterogeneous; each group involved a different cross section of the South Asian community in order to provide a broad range of opinion.

**Sampling and sample size.**

The size of each of the six focus groups was kept small (between five to seven members) in order to create a more personal environment for people of similar backgrounds (homogenous) for discussion (Krueger, 1998) (Table 4).

Table 4
*Membership of Focus Groups*

| *Focus group* | *Members* | *Participants* (*n*) |
|---|---|---|
| One | South Asian mothers of school aged children | Seven |
| Two | South Asian community support workers (mixed ages) | Six |
| Three | Mature South Asian women with adult children | Five |
| Four | Young South Asian mothers with preschool children and babies | Six |
| Five | Young professional South Asian women (no children) | Five |
| Six | South Asian health professionals; one general practitioner, three registered nurses practising in primary care, one health promoter, one nutritionist | Six |

**Recruitment of participants.**

Participant recuritement strategies included flyer advertisement through community groups, health care practices and the TANI newsletter (Appendix 4).

Despite the flyer advertisements in various publications, all of the participants were recruited through word of mouth by South Asian community contacts. When working with cultural minority communities, this appears to be the most effective way to recruit participants. The community contacts act as trusted intermediaries and the participants extend this trust to the researcher (Krueger & Casey, 2000; Suh et al., 2009). Potential participants contacted the researcher or research assistant by mail or telephone to signal their interest in participation. They were given a brief explanation of the study and the expectations of participation. Once participants verbally or by email consented to participate, then a potential date for the focus group discussion was negotiated. Participants were provided with copies of the participant information sheet and consent form by email or hard copy mail prior to taking part in the focus group discussions. Signed consent forms were either brought to the focus group interview by participants, or signed on arrival at the focus group interview. (Appendix 2 and 3).

**Inclusion criteria and geographical location of the research.**

South Asian community leaders, members and health professionals who lived in the Mt Roskill, Mt Albert, Sandringham, and Blockhouse Bay areas were included in the study population. The project was conducted in locations close to the participants' homes, for example, at local schools, community centres or a participant's home. This geographical location was selected because of the large number of South Asian people living in these communities and established contacts between members of the research team, key community members and health professionals in this area.

**Focus group procedures.**

Members were welcomed informally to the focus group as they arrived and were introduced to each other. Culturally appropriate food and drinks were provided to facilitate a relaxed environment and to encourage some mingling prior to the start of the group. The informal discussion before the group discussion helped to relax participants,

establish relationships and convey interest in participants and the knowledge that they had to share. If participants had not already done so, then they signed duplicate copies of the consent form (one for the study records and one for participant to retain).

At the start of each focus group, the research assistant welcomed participants and formally introduced the researcher. The researcher explained the neutral roles of both the researcher and research assistant in the focus group discussions, as well as the role of the researcher as facilitator of the discussion and the research assistant as host and note taker. The ground rules for the discussion were outlined and the requirement of confidentiality of any discussion, and not divulging the identity of fellow focus group members outside of the group (participants had also already signed the consent form acknowledging this). Explanations included the aim of the VitB12 study and the objectives of the focus group interviews. This was to provide participants with a clear picture of the information sought or expected (Suh et al., 2009). Participant consent was obtained to audio record the focus group interviews and to make written notes about the interview. Duplicate tapes recorders were used to record the interviews so in the event of equipment failure, a back-up audio recording was available.

The researcher asked the questions, directing the flow of discussion and keeping the conversation on track. The research assistant made written notes and diagrams of the flow of interaction across the group, noting nonverbal communication and indicating for later reference, any underlying values, norms or customs that may influence the responses given. At times, the research assistant was required to provide some clarification to information during the interview when there was some specific cultural aspect that the researcher was unfamiliar with and the participants could not clarify in enough detail. The researcher also made brief notes during the discussion, but only to note down key points to reflect back to the participants and on ideas for the ending question in the focus group discussion (Fontana & Frey, 2000). A brief outline of the

proposed follow-on RCT was given in order to provide context to the focus group discussions. Key concepts and terms as they related to B12 were explained as necessary at the outset of the focus groups to ensure a common basic understanding among participants.

One of the issues with cultural skill is appropriate interpretation of data. Language is a construct that implies shared meanings in social interactions and is therefore culturally defined (Suh et al., 2009). It was anticipated there could be problems with the interpretation of culturally defined subtle nuances, especially in response to specific questions. At the completion of each interview, the researcher and assistant would discuss the interview and notes made, most often for cultural clarification or elaboration of a comment (Patton, 2002). The assistant received the summary of each focus group interview so she could add details and context where required.

**Development of focus group questions.**

To obtain relevant data that would inform the follow-on RCT for B12 supplementation, a funnel approach was used to structure the focus group questions (Krueger & Casey, 2000). A broad opening question was used to make participants feel more comfortable about speaking out in a group situation (Appendix 5). The next question, the introductory question was a broad question on the topic that allowed the focus group facilitator to gain insight into the participant's knowledge and experiences around B12. Subsequent questions were transition questions that gradually narrowed down the topic, before asking the key questions seeking data that was crucial for informing the conduct of the intervention study. The questioning concluded with an ending question which was the key to the study and then a final question inviting participants to add any information they felt was relevant. Although designed to obtain similar information, the questions for the health professional focus groups were worded

differently to keep them in the context of health professional practice. Following each focus group interview, the interview was transcribed and analysed for codes. Codes and subthemes that were identified and needed elaboration or clarification were then followed up on in subsequent interviews or analysis (Strauss & Corbin, 1998).

The wording of the focus group questions took into account different cultural perspectives that may influence the answer (Krueger & Casey, 2000). The research assistant provided a 'cultural eye and ear' on the wording of the questions. In instances where the wording of the question was ambiguous or not well understood by focus group participants, then the questions were asked in a parallel format in order to elicit the same information but was asked in a clearer question format for those participants (Krueger & Casey, 2000). The purpose of each question and the nature of the information to be elicited was clearly documented prior to the focus group interviews in order to develop an appropriate parallel question if needed. Towards the end of the discussion, the research assistant was asked if she had anything else to add or to ask of the group members  This was to ensure that any cultural cues overlooked by the researcher were followed through during the interview if they needed to be (Fontana & Frey, 2000; Krueger & Casey, 2000).

**Facilitating focus groups.**

Successful and culturally competent facilitation of the focus group discussion is one of the keys to successful data collection (Krueger & Casey, 2000). It was also important for effective relationships with the participants in this VitB12 research so that the research was a worthwhile experience for them (Krueger & Casey, 2000). These strategies included:

- Planning the questions, being familiar not only with the questions but also how long should be spent discussing each group of questions (Patton, 2002). This was important when the discussion jumped ahead to a key question before

asking preceding questions. When that situation arose, notes were made to go back to preceding questions and to allow sufficient time to do so.

- Drawing out introverted group participants; using strategies such as eye contact and offering opportunities for contribution to draw out the opinions of less vocal participants (Fontana & Frey, 2000). Introversion of participants was an issue in just two of the focus group discussions.
- Establishing correct pronunciation of the participants' names prior to the start of the focus group interview. Using name labels and addressing participants by name throughout the interview.
- Showing respect for participants by listening actively even though some of the information was covered in earlier focus group interviews.
- Holding back on stating own personal views, but recognising where to probe or seek amplification or clarification.
- Using five-second pauses after a participant's comment to allow other participants time to elaborate and add further comment.
- Avoiding responses such as "correct" or "excellent" that imply a judgment; using responses such as "thank you" to acknowledge a contribution instead.

**Section Three**

**Analysing the Focus Group Data**

Thematic analysis was the qualitative descriptive method of analysis used for analysing the focus group interview data.

> *Thematic analysis is a method for identifying, analysing and reporting patterns (themes) within data. It minimally organises and describes your data set in (rich) detail. However, it also often goes further than this and interprets various aspects of the research topic* (Braun & Clarke, 2006, p. 79).

Thematic analysis was selected in preference to other qualitative descriptive techniques such as content analysis, because thematic analysis allocates codes initially

using inductive coding techniques, whereas content analysis uses more deductive techniques (Boyatzis, 1998; Braun & Clarke, 2006; Sandelowski, 2000). With inductive methods for data analysis, there are not preset frameworks into which to organise the codes, and the initial codes and subthemes are identified from the data itself (Braun & Clarke, 2006). In order to be culturally sensitive to the data and to appropriately represent the participant's perspectives, it was important to use inductive coding initially and allow the data to guide the identification of codes, rather than use a deductive framework that would organise codes into a framework preset by the researcher, and one influenced by the researcher's cultural perspectives (Suh et al., 2009). However, it is acknowledged that the researcher's perspectives, experience, intuition and sensitivity to the data still influenced the way that codes are identified and interpreted from the data (Braun & Clarke, 2006). While the data were obtained using some pre-set questions for the focus group interviews, these questions were not used to guide coding at this analysis stage.

Codes were words or phrases allocated to represent the meaning of a phrase or sentence from the interview transcript, while subthemes and themes were a collection of codes collated together to represent a repeated meaning or pattern (Boyatzis, 1998). The data were analysed, initially into codes, then into subthemes, then themes until all codes were allocated, and there were no assignable codes or subthemes that that could not grouped under a the key themes (Boyatzis, 1998; Braun & Clarke, 2006; Patton, 2002; Thomas, 2006). The coding initially used descriptions of the phrase or sentence, frequently using verbatim words from the phrase (this process is termed manifest coding) (Pope, Ziebland, & Mays, 2000). After the initial analysis, then coding became more interpretive, looking and coding for meanings behind the words (this process is termed latent coding) (Braun & Clarke, 2006; Pope et al., 2000). Transcripts from preceding focus groups were analysed before conducting subsequent focus groups in

order to generate any questions that needed elaboration or clarification, or to identify topics for follow-up in subsequent focus groups. An excerpt from the researcher's qualitative analysis log illustrates this process.

*9th Feb*

*Analysing focus group three*

*One thread to come through FG one and FG3 is concern about the cholesterol content of milk. I need to follow up with questions on this, as it appears to make some of the women reluctant to drink milk because of the cholesterol content. Is this just an issue with older age group women, or is it a concern for younger women as well? It may have quite an inhibitory effect on intake of B12 foods, especially for those with vegetarian preferences.*

After the initial analysis of the first three focus group interviews, some deductive analysis was used in subsequent group interviews and in reanalysis of existing transcripts, to follow up on any already developed sub themes and themes. The analysis then progressed to an interpretive stage where, meanings, key characteristics, contexts, and conditions were used to differentiate predominant themes (Halcomb et al., 2007; Huer & Saenz, 2003). The key three themes identified were those for which a prevalence of codes and subthemes grouped under them. Coding occurred cyclically: transcripts were read multiple times with notation of initial codes, questions or notes made in the margin. Much of the initial coding asked questions of the data in order to just get a feel for it. Following transcription and coding of subsequent interviews, previous transcripts were reviewed and recoded, and notes made about links to codes in other transcripts. With each new interview transcription and coding, the coding was reviewed on previous transcripts to identify patterns in the codes, and emerging subthemes between transcripts. This cyclical process was used to organise the data into meaningful patterns with links and explanations; to tell the story of the data (Suh et al.,

2009). Excerpts of the raw data are used to illustrate the key themes in Chapter 4 where findings from the focus group interviews are presented.

Analysis used manual coding methods, Microsoft Word 2007™ editing tools and NVivo version 9 software for coding. Initial coding was undertaken on hard copies of the transcripts, making hand written notes in the column. The coding was then transferred in Microsoft Word 2007™ using highlighting to identify the phrase, and the 'add a comment' feature to identify a code. Different interview transcripts were printed on coloured paper, to identify the source of the data. The transcripts were cut into code segments and manual methods used, organising codes into preliminary codes, subthemes and themes. Once a preliminary coding framework was established, then the latter three interviews were coded in NVivo using the coding framework, but also identifying any new codes. A qualitative log was kept of coding decisions so that these could be tracked to ensure they were guided by the data and not imposed from the researcher. Memos were used the track the research process (Halcomb et al., 2007). A summary of preliminary analysis of the data was sent back to focus group participants for them to check that the descriptions that emerged from the data were consistent with their inputs into the focus group discussion (Grbich, 1999; Lincoln & Guba, 1985; Richards, 2005; Sandelowski, 1993).

**Ensuring Rigor in Qualitative Methods**

Appropriate strategies to ensure rigour of qualitative research were considered (Browne & Sullivan, 1999; Grbich, 1999; Onwuegbuzie & Leech, 2007; Richards, 2005; Rolfe, 2006; Sandelowski, 1993). Sandelowski (1993) proposed that because of the nature of qualitative research, validity and reliability are not applicable measures. Instead, the most important criterion for research is that it is 'trustworthy', that it reflects the ideas of the research participants. Each research project is unique because of the interpretative nature and naturalistic setting of qualitative research; therefore the

quality is determined by the reader or consumer of the research and the degree to which they can track the research process and the decision making trail (Richards, 2005; Rolfe, 2006). For this reason the thematic analysis methods in this VitB12 research study built in trustworthiness checks through the research process.

The first of these trustworthiness steps was to track decision making to provide an auditable decision making trail (Richards, 2005; Rolfe, 2006). Decisions such as why datum was coded a particular way and how the codes were combined into subthemes and themes were tracked. All decision-making processes were documented in a qualitative research log.

Member checking is one traditional measure used to establish credibility of analysis (Lincoln & Guba, 1985; Onwuegbuzie & Leech, 2007) so preliminary analysis of the data was sent back to focus group participants for checking, thus ensuring that the descriptions identified from the data were accurate reflections of the focus group discussion (Lincoln & Guba, 1985). Sandelowski (1993) debates this process on the premise that some of the richness of qualitative research is lost if interpretation is limited to just what participants reflect in the data and this can detract from some meaningful conclusions from the data. In the focus group interview phase of this VitB12 research however, one intention of the research was to include and embed the communities' perspectives into the RCT research process and findings. In order to meet this criterion, it was important that the research participants checked the preliminary interpretation to ensure that it did reflect their perspectives and to prompt clarification where needed.

Coder checking was used to provide a trustworthy trail for data analysis (Rolfe, 2006). The analysis of data and identification of codes, categories and key themes was checked by a research colleague experienced with qualitative research, to ensure that the interpretation was consistent on the data analysed (Lincoln & Guba, 1985;

Onwuegbuzie & Leech, 2007; Patton, 2002; Strauss & Corbin, 1998). The conclusions and generalisations that are made from research findings also influence trustworthiness. Caution will be taken with focus group findings to ensure that findings are generalised appropriately and not beyond the naturalistic setting of the research (Patton, 2002).

**Chapter Summary**

The research design used for this VitB12 research was an embedded mixed method experimental model with a CBPR approach. A CCRG guided the research process, to ensure conduct of the research was culturally appropriate, and that the research remained relevant for the South Asian community. The objectives for this phase of the research were to identify factors that influence dietary B12 intake in South Asian women, explore women's experience of B12 deficiency, identify appropriate strategies for increasing B12 intake in Indian women aged 18 – 50 years, and to use these findings to inform the conduct and interpretation of the follow on RCT. Focus group interviews preceded the RCT, and were used in the subsequent planning of the RCT. This chapter described the methods for participant recruitment, data collection for, then analysis of the focus group interviews. The data were analysed using thematic analysis techniques from a qualitative descriptive approach, with manifest, then latent coding techniques. The next chapter (Chapter 4) describes the findings from the focus group interviews, their interpretation and the embedding of the focus group findings in the conduct of the follow-on RCT. The generation of new knowledge from the findings, plus their integration to expand existing knowledge around preventing B12 deficiency are described in Chapter 8, the discussion chapter.

# Chapter 4: Findings from the Community Focus Groups

This chapter presents findings from thematic analysis of the focus group discussions. The analysis was undertaken at two key stages of the VitB12 study. Primary analysis occurred prior to the randomised controlled trial (RCT) in order to answer questions around knowledge of vitamin B12 (B12) deficiency, dietary preferences and appropriate strategies for including women in the follow-on randomised controlled trial. Secondary analysis was undertaken after the randomised controlled trial (RCT) to aid interpretation of the RCT findings. Key themes from the primary analysis are described in this chapter. Discussions include how the findings are incorporated into the follow-on RCT. While key subthemes and themes from the secondary analysis are presented in this chapter, the application of these to add depth and aid interpretation of the RCT findings is discussed in Chapter 8. The methods used for qualitative data collection and analysis are described in Chapter 3.

**Research Questions**

The focus group discussions were to answer the following questions.

For South Asian women of childbearing age:

1. What are the factors that influence dietary intake of vitamin B12?
2. What are appropriate approaches for working in partnership with the community to investigate and prevent vitamin B12 deficiency?

**Key themes from Focus Group Discussions**

Key themes are; **dietary practices as integral to identity and belonging**, **managing B12 deficiency** and **study participation as a positive experience**. The meanings will be described for each of these themes, along with any conditions and context that influence the theme (Table 5).

Table 5
*Dietary Practices as Integral to Identity and Belonging- Subthemes and Codes*

| Theme | Dietary practices as integral to identity and belonging | | | |
|---|---|---|---|---|
| Sub themes | Being vegetarian | Preference for traditional foods | Collective dietary practices | Mothers and Mother-in- laws |
| Codes | More than just a food preference<br><br>Important to self and family identity<br><br>Given less recognition than non vegetarians<br><br>Resources for vegetarian food | Inside food<br><br>Outside food<br><br>Inside food as healthier and preferable to outside food | Rules<br>• flexibility<br>• adhering<br><br>Modifying traditional recipes<br>• collective approach<br>• making them healthier<br>• adapting to suit kiwi food<br>• occasions when recipes traditional<br><br>Increasing B12 intake | Females responsible for food preparation<br><br>Pleasing respected elders.<br><br>Passing down knowledge and wisdom |

**Dietary practices as integral to identity and belonging.**

Dietary practices were integral to the women's identity and feelings of belonging within their family and community. Dietary practices were complex because they were influenced by a variety of factors such as family traditions of dietary practices, perceptions that home prepared food was healthier, rules about vegetarianism, the collectivist nature of South Asian culture, the importance of celebrations and the influence of older generation women on the foods that South Asian women prepared for their families. Sub themes included ***being vegetarian***, ***preference for traditional foods,*** and ***collective dietary practices***. Intervening conditions were ***celebrating with food, mothers and mother in laws,*** and a ***collective approach to modifying traditional recipes***.

***Being vegetarian.***

For participants who identified as vegetarian, dietary practices were much more than just a food preference. ***Being vegetarian*** was integral to their identity "… *it* [being

vegetarian] *is not just what you eat, it is whole way of life- how you are, who you are and how you are with the world*" (Participant 3 [P3], Focus group 4[FG4]). Being vegetarian was closely linked with family identity; *"My family are vegetarian-it is that whole family thing"* (P2, FG2).The participants expressed frustration when information on vegetarian foods was included as a bottom-line addition on dietary pamphlets and brochures. This was viewed as giving vegetarian dietary preferences less importance than non-vegetarian based food preferences. *"Oh it's in meat, fish, eggs and milk. Doesn't say much for us. But they're* [non vegetarian] *alright, they're alright. There it is in sirloin steak."* [pointing to a B12 information leaflet]. "*Here we go at the bottom"* [pointing to a small paragraph dedicated to vegetarian diets] (P3, FG3).

The need for food information resources specifically written for vegetarians was another reoccurring code. Participants recounted examples where doctors did not explain food sources for B12 other than it was in meat and eggs (not useful for lactovegetarian practices) and they did not seem to have resources on nutritional sources for B12.

> *My sister in law went to the doctor the other day and he said "you need to eat vitamin" … no "you need to eat the vitamin B12 diet plan"…Well where do you get that from. He says "Google it". Well you go on Google and it is all about meat and protein and these are vegetarians so where do you find it?* (P3, FG3)

***Preference for traditional foods.***

It was particularly important to focus group participants that they and their families consumed traditional, home-prepared food. This was referred to as ***inside food*** and food prepared outside the home or purchased commercially was referred to as ***outside food***. Participants from all dietary preference groups viewed inside food as healthier and preferable to outside food. Non-traditional ready-to-eat foods such as processed cereals purchased from supermarkets were still outside food, but if consumed in small quantities inside the home, these were acceptable. Developmental stage was an influential factor on the amount of inside food consumed, with older women (age > 35

years), and women with families, placing the greatest emphasis on it. The focus group of young professional women (all aged between 25 and 29 years and childless), were asked a follow-up question about home-prepared food. All participants in this focus group acknowledged that although consuming home-prepared food was preferable, a lack of time limited its consumption.

> *Yoghurt, I eat yoghurt and could eat more- you know the home-set yoghurt. I know I should eat the home-set* [yoghurt] *but I don't have time to make it. I just grab you know, the pots of yoghurt. I don't even have time to make breakfast. I eat the yoghurt if* [colleague] *brings some in for me. She makes it for family and then brings some in for me* [laughs] (P1, FG5)

Older age women (age > 35 years) expressed concern about the quantity of outside food that the younger generation consumed.

> *But see in our day, our parents never ate anything from outside food... It wasn't processed you know, like fresh veges and things like that and then we've come along and we've carried on the same until the last few years, carried on the same to a certain extent and-we do, but we do eat a little bit of outside food as well. Our kids-really* [with emphasis] *into outside food, totally into it* (P1, FG3)

Older women viewed the problem of B12 deficiency as a recent health problem for South Asian women because it was not recognised as a problem among the older generation members in their family. This contributed to the perception that traditional inside foods were preferable to foods obtained outside the home, and that outside foods may have contributed to the problem of B12 and folate deficiency.

> *Fifty years ago our parents never did anything like that* [take folic acid supplements] *and we are quite normal. I've got children and up until I was 40. I never took anything and they're all quite normal. So why now is it so important to have B12 and folic acid and all that? Well firstly our food is more processed, so it seems a reverse process - our food is more processed so we have all of these deficiencies and our kids are the double banger syndrome kids. Have the foods that we make at home plus the processed outside foods. Yeah we ate at home and that was it* (P2, FG3).

***Collective rather than individual dietary practices.***

A recurring theme with participants from all dietary preference groups was the close relationship between the ethnic group that women belonged to and the ***rules*** that appeared to be the norms or guidelines to permit consumption of some foods but

prevent consumption of others. ***Flexibility*** of the rules depended on ethnicity, with different cultural nuances within different ethnic groups. "*I am vegetarian but we are allowed to eat fish"* (P1, FG4). Some women explained how they could increase their intake of B12 foods because of this flexibility. *"I could eat eggs. We are allowed to eat them but I don't, except baking - because they taste slimy"* (P3, FG1). Other women would not even eat baking that contained eggs, or foods that contained gelatin because of strict vegetarian practices. For women who were non vegetarian, there were restrictions on the meat they could eat. For some women ***adhering*** to the rules was important and took precedence over nutritional requirements, while for others there was flexibility to adjust foods consumed. For example, one of the women was explaining how she was vegetarian and had B12 deficiency when she was pregnant. The deficiency resolved when she followed her doctor's advice and ate meat. Another of the women in the focus group remonstrated that eating meat was only possible for this women because of the ethnic group she belonged to. "*But she is non Brahmin so she can do that* [eat meat] *but we can't because we are Brahmin"* [stated very emphatically]. (P3, FG2)

With the exception of the health professional focus group, a lot of focus group discussion was on traditional foods and recipes. Reasons for modifying recipes included ***making them healthier*** and ***adapting to suit kiwi availability of food.*** There was a ***collective approach*** to ***modifying traditional recipes***, with discussion and consensus on how to change traditional recipes in order to increase the B12 content, or to make recipes healthier by decreasing the cholesterol content. There were however ***occasions when recipes were kept traditional***.

The acknowledgment of a health issue for themselves or one of their family members was the antecedent for changing traditional foods to a healthier version. Some participants were unsure how to change. When other women from the South Asian community provided information on how to increase the B12 content or decrease the

cholesterol content of dishes without changing the flavour or traditional nature of dishes, this fostered confidence for participants to consider changes. For example, in one of the focus groups, two women had cut their intake of yoghurt because they did not want the high cholesterol, but they were unsure of how to make low fat milk yoghurt using traditional hot pot methods because the yoghurt did not seem to set. Discussions included how to successfully make the low fat yoghurt, so that an important source of B12 could be included in the diet without having to include high cholesterol from full cream milk

Women in the focus groups discussed changes they had to make to some of their traditional dishes. Lack of availability of some of the seeds, flower heads, herbs and spices traditionally used was an acknowledged problem in New Zealand, so participants reported being creative in changing traditional dishes. Changing the dishes to make them healthier applied to everyday foods. However, when it came to special occasions such as weddings, Diwali and Eid al-Fitr, then it was important that as much as availability of ingredients allowed, foods were prepared at home and using traditional methods without modifications. The women accepted this was a time when they over ate because of the collective cultural obligation. Even women who reported using healthier methods to prepare traditional foods (such as the avoidance of ghee and full cream milks), resorted back to eating high fat foods or using high fat traditional food preparation methods for special celebrations.

> *Some food is so rich. We have been eating for two weeks for my nephew's wedding and after eating low fat I am not used to the rich food so I feel bloated. My son said are we going to have* [rich dessert food] *again because they feel really sick too* (P1, FG1).

***Mothers and mother in laws as key influences.***

In families, ***females*** were ***responsible for food preparation***. This included not only women, but also older female children. Recurring codes from focus group discussions revolved around the role of ***mothers and mother in laws*** in ***passing down***

***knowledge and wisdom*** about traditional food preparation. Mothers and mother in laws were very influential on the foods that were prepared and served at home. ***Pleasing respected elders*** was important for younger women, especially daughter in laws; they wanted to please their elders by preparing food in the expected ways. This limited healthy adaptations to traditional recipes.

> *And then our traditional foods we get taught how to make. How many of you guys live with extended families? Mother in laws, son in laws, the expectation from them that if you don't make it with this, then it is wrong* (P3, FG2*).*

**Managing B12 deficiency.**

***Managing B12 deficiency*** was important for the women, particularly if they had a long history of deficiency. The subthemes that contributed to this theme were ***experience versus knowledge of B12 deficiency***, ***choices for managing deficiency***, and ***keeping cholesterol low*** (Table 6).

Table 6
*Managing B12 Deficiency- Subthemes and Codes*

| Theme | Managing B12 deficiency | | |
|---|---|---|---|
| Sub themes | Experience vs. knowledge of B12 deficiency | Choices for managing B12 deficiency | Keeping cholesterol low |
| Codes | Experience<br><br>Knowledge<br><br>Education | Lack of choice<br><br>Searching for answers<br><br>Alternatives for treatment<br><br>B12 injections | Avoiding dairy products<br><br>Traditional methods for preparing foods |

***Experience versus knowledge of B12 deficiency.***

***Experience*** of B12 deficiency was common, but experience did not necessarily mean accurate ***knowledge*** about contributors to B12 deficiency, dietary sources for B12 or choices for managing deficiency. The women with experience of B12 deficiency were either lactovegetarian or lactoovovegetarian. While some of the women had a better knowledge of the causes of B12 deficiency and the dietary sources for B12 than those without any previous experience of deficiency, other participants who reported

receiving intramuscular B12 injections were unable to explain the reasons for their deficiency or any dietary sources for B12. Some participants had misconceptions about foods that contain B12, erroneously citing spinach, dal, seaweed or spurilina as dietary sources of B12. "*I am a vegetarian that is why my B12 and iron are low. I do eat spinach and dal to bring them up but I don't eat meat, eggs or cheese"* (P4, FG1). Other participants could list B12 containing foods, but their information about the quantity of foods required to reach the recommended daily intake of dietary B12, was not sufficient. For example, one of the women with a history of B12 deficiency reported; *"Eggs. My doctor told me if I ate 5 eggs-a-week, I would not have a problem with B12 anymore."* (P3, FG1)

Knowledge of the dietary sources for B12 did not necessarily mean the participant would increase their intake of B12 containing foods if they were diagnosed with deficiency. Some participants understood that their risk of B12 deficiency was related to vegetarian dietary practices and they were able to explain some of the animal based foods that contain B12, but this did not result in an increased intake of those foods in order to manage their B12 deficiency. The following participant was a lactoovovegetarian, yet she did not increase her intake of the foods she described as containing B12. "*I know a lack of it* [B12] *causes pernicious anaemia and it comes from egg, milk and meat, so I am low and have to have B12 injections as well. My doctor calls me in when B12 gets low. I eat milk but not enough so I get low"* (P4, FG1). None of the women in any of the focus groups discussed fortified food sources for B12. Some women had no previous knowledge or experience or B12 deficiency, but requested more details on foods that contain B12. There was limited knowledge about food sources for B12 among the health professionals; only two members out of six in the health professional focus group correctly verbalised foods that contain B12. A frequently repeated code was the need for more ***education*** on B12 deficiency, treatment

options and dietary sources of B12. The women themselves acknowledged they generally knew little about dietary sources of B12. They also recounted experiences to support that GPs and practice nurses needed educating, particularly on treatment options for B12 deficiency and dietary sources for B12. In the health professional focus group, the nurses and health promoters in the group agreed they needed a more detailed understanding on the causes of B12 deficiency and options for treatment. Although nurses were responsible for the administration of B12 injections, they did not provide any advice about foods that contain B12.

***Choices for managing B12 deficiency.***

Participants wanted ***choices for managing B12 deficiency***; the ***lack of choice*** offered, meant that participants went ***searching for answers*** and trialled their own ***alternatives for treatment***. Participants recounted prescription of ***vitamin B12 injections*** for treatment of vitamin B12 deficiency, but when asked for clarification, could not recall being offered alternative treatments such as tablets, referral to a nutrition expert for advice or being given dietary advice (beyond stating that B12 was in meat and eggs and to eat these). Participants were concerned about inadequate explanations as to why they were B12 deficient, and the lack of choices offered for treatment.

> *Now I had been on these B12 injections for years until I started to query it and then he* [GP] *would still measure the B12 and it was sometimes down and sometimes up and I said "are you going to give me some tablets?". And he said "it doesn't come in tablets it's in your diet". "But I said I don't change my diet. I'm a vegetarian- I don't change my diet*" (P1, FG3).

There were participants who preferred to have a B12 injection to treat their deficiency. One participant struggled to swallow tablets so would opt for a B12 injection. *"Ever since I was small and I had to go to the doctors I would say don't give me medicine, just give me injection. I can't take any medicine"* (P4, FG2). Another participant attributed extreme tiredness to her diagnosed B12 deficiency and found these

symptoms abated more quickly if she had a B12 injection. In the health professional focus group, participants explained how some women requested the B12 injection for management of fatigue and neurological problems. Some of the participants who had received the B12 injections found it a convenient way to treat their deficiency, while some found the injection process inconvenient. None of the participants mentioned any discomfort associated with the injection. The ease with which participants obtained the prescribed B12 injections, the cost of it and the process of administration of B12 injections by the GP and practice nurse affected perception of convenience.

> *I don't pay for the injection. My doctor has it for me so I just go to the nurse. It's just easier for me to have it as the injection and I don't have to pay* (P4, FG1).
> *But you still have to pay when you pick it up from the chemists* (P3, FG1).
> *I don't pick it up as my doctor has her own supply. I used to pay but I don't anymore* (P4, FG1).
> *I get the prescription from my doctor and pick it up from the pharmacy and then I have to pay, then take it back to my doctor and pay the nurse for the injection. It is a real process* (P3, FG1).

Some participants described how they researched B12 deficiency to find out the causes for their deficiency. Older aged participants reported ***searching for answers*** more frequently than younger aged participants did, but the older participants also reported a longer history B12 deficiency, which may have influenced the need to seek out information.

> *Well my experience goes back to the 80's and one day out of the blue they said, "you are low in B12, you've got to have injections". And the question was "why am I low" and they could not answer that so you go on a course of injections. And this went on for years and then I thought "well, what am I doing" so I said "no I am not taking anymore" so they sent me up to the hospital and when they sent me up to the hospital- I can't remember exactly what part, but I had to collect my urine every day for the whole week and then take it up there. And they analyzed it or did something and they said "she doesn't need these B12 injections".* (P2, FG3)

Participants were very persistent and when one source of information, such as their general practitioner (GP) did not provide satisfactory answers, they would seek out other sources of information. The World Wide Web was one source for information,

although it returned some distracting options such as tumours as a cause of B12 deficiency. '*So I went to the doctors and said "have I got cancer?" I said "I Googled it. You couldn't give me an answer so I am giving myself an answer"* (P3, FG3)*.'*

A small number of participants found their own ***alternatives for treatment*** of their B12 deficiency. One participant recounted finding her own treatment.

> *So he* [doctor]*...said* [B12] *had dropped below the average so he said "you have to have an injection". So I said "Ok I'll have an injection". I took two. And then I stopped again and I thought " no something is not right" so I started questioning myself and I would bounce ideas off a lot and then I said what, I always go back to "what do people do in third world countries?" They don't have these supplements, they don't have these things so then I went to alternative medicine and I said to the guys: "I have a B12 deficiency, what can I take or what can I do?" And I just get this tablet from India and its got B12 and zinc and everything in it. It has everything and my things and B12 are perfect and I don't even take it every day* (P2, FG3).

Participants who had a repetitive history of B12 deficiency and B12 injections were more likely to search for alternative options to manage the deficiency themselves.

#### *Keeping cholesterol low*

The majority of participants expressed a preference for preventing B12 deficiency by increasing their dietary B12 intake. Given that the main natural B12 food source in a vegetarian diet is dairy products, this created a dilemma as some participants expressed concerns about increasing their consumption of dairy products because of the cholesterol content. Participants were aware of the increased risks associated with elevated cholesterol and some considered all dairy products to be high cholesterol and therefore consumed these in small or null quantities. ***Avoiding dairy products*** was more common among the older–aged participants in the study, who reported having high or borderline high cholesterol results, but was less frequent among the younger–aged participants in the study. For some participants, low fat dairy foods were not an option as they interfered too much with ***traditional methods for preparing foods*** such as

yoghurts and raita; dairy products were avoided in preference to changing food preparation methods, or purchasing low fat versions.

> *I don't drink milk because of the high cholesterol. Even the trim milk and don't have eggs because of the high cholesterol. I eat yoghurt but not too much because of high cholesterol from yoghurt. It doesn't set with the low fat milk because it doesn't set even with using the hotpots* (P5, FG1).

This aligns with the earlier theme of dietary practices as integral to identity and belonging.

**Study participation as a positive experience for women.**

The third theme collects subthemes and codes from questions on how to conduct the study with South Asian women, including how to recruit and retain women in the RCT Specific categories were ***recruit through relationships with South Asian community***, ***trusting the research*** and ***keep the participation process easy*** (Table 7).

Table 7
*Study Participation as a Positive Experience for Women-Subthemes and Codes*

| Theme | Study participation as a positive experience for women | | |
|---|---|---|---|
| Sub themes | Recruit through relationships with South Asian community | Trusting the research | Keep the participation process easy |
| Codes | Being on the inside<br><br>Foot in the door<br><br>Word of mouth<br><br>Use culturally specific media | Kept updated about study progress<br><br>Worthwhile study<br><br>Helping other people by participating | Fitting in with times<br><br>Visiting participants in their homes<br><br>Keep the study period short<br><br>Individualise options for keeping in contact |

***Recruit through relationships with South Asian community.***

Focus group participants described that using a research assistant or a contact person from within the South Asian community was important for successful recruitment of women into the randomised controlled trial. This was referred to as ***being on the inside***; because the trust extended to the researcher by the support person meant

that participants would similarly trust the researcher to conduct culturally appropriate research. ***Word of mouth*** advertisement to other community members about the study was described as the best method for recruitment, more effective than using flyers or advertisements.

> *Word of mouth, the way that you contacted us for this or being introduced by one of us. It's easier if you are on the inside and know someone who knows about it* [the study] *then you want to be part of it* (P2, FG1).

If advertisements were used, then participants advised the research team to use ***culturally specific media*** such as an Indian newspaper and South Asian radio and television stations to explain the study and advertise for South Asian participants. Linked to the above subtheme of being on the inside, participants explained that having the key South Asian community member as support when using culturally specific media for recruitment was essential, both as a ***foot in the door*** and to interpret.

> *The Indian radio station. There is two of them so get them together and do an interview so people would be really interested. You will need to interpret though.* (P3, FG1)
> *So I would need Ella with me*? (Researcher)
> *P.'Yea that would be perfect because then you would have a foot in the door. We listen to them and you would reach a lot of Indian people.* (P3, FG1)

***Trusting the research.***

Participants predicted that women would remain in the study if they were kept ***updated about study progress***; so that they could judge for themselves that, it was a ***worthwhile study*** and they were ***helping other people by participating***. Suggestions included creating an organisation in Facebook or Twitter and keeping that updated so women could check in themselves and see how the research was progressing, and make suggestions of how to help. Suggestions also included keeping women updated with their blood results throughout the study so they would be motivated to continue. Women could also then make suggestions on the study.

> *We like to see the proof, look at it so we can decide for ourselves about the studies are doing. It depends on how important it is compared to other things-*

> *Text or Facebook. Something visual. Face book or Twitter is a good idea. You could have a group. You could have a group where it is invite only to join.* (P2, FG1)

***Keep participation process easy.***

Factors such as the length of time in the study and having to travel to and from the data collection visits were factors suggested to influence whether women participate and remain in the study. Participants' predicted that the majority of women who volunteered to take part would be working mothers whose time was precious so participation needed to be as easy as possible. Codes such as ***fitting in with times*** that suited participants, ***visiting participants in their own homes*** and ***keeping the study period short*** were all recurring codes suggested to keep participation as easy as possible.

> *Sometimes the length of time could put people off. I did a study and you had to pop something too and that went off for years. And half way through I said "I am getting a bit bored with coming up here" and that and then they shortened the time. So if they are a long length of time I think you will get people turned off. But you said this is just six months. That is so good. Much better to be part of that study.* (P2, FG3)

Participants emphasised the importance of retaining women's interest over the course of the RCT. Some focus group participants had taken part in other studies so had experience to offer on factors that encouraged them to comply with study regimes. There was a divergence of codes from focus group participants about preferred methods of keeping in touch with RCT participants. The codes did not seem to differ according to focus group participants' age or whether they were a health professional or community member. The key finding was to *'**individualise options for keeping in contact'***; establish from each of the women in the RCT, their preferred methods for staying in contact and receiving information. Options offered ranged from phone calls, SMS, email or social networking group options.

## Interpretation of Focus Group Findings to inform Randomized Controlled Trial

This section discusses the interpretation of relevant qualitative findings used to inform the RCT. Qualitative findings are also discussed in Chapter 8 where they are triangulated with the RCT findings, to add depth and explanation to RCT findings.

### Planning dietary B12 advice.

One of the key themes to influence the conduct of the RCT was that dietary practices were more than about food, they were a critical part of the collective identity for South Asian women, underpinned by a tradition of history and family values. This is in contrast to the Western perspective of dietary practices as individual choice, influenced by family practices, but also by personal philosophy and application of life health practices. These key influences on dietary practices mirror the threads of each culture. Western culture focuses more on individual achievement and freedom of individual choice about career, relationships, family structure and living accommodation (Veenhoven, 1999). In contrast, Indian culture emphasizes commonality and collective identity (Choudhry et al., 2002; Vaidyanathan, 1989). The collective identity is reinforced through bonds with immediate and extended family members, the family and cultural meanings given to possessions, symbols, paintings, adornments and even achievement within the family (Mehta & Belk, 1991). Dietary practices are part of this commonality. The role of older generation females is to pass on this collective knowledge in the form of traditional methods for cooking foods (Choudhry et al., 2002; Jayanthi, 2001).

The collective nature of dietary practices had implications for the RCT when planning the dietary recommendations for participants in the dietary advice arm of the trial. The dietary arm of the RCT proposed to provide individualised counselling on how participants could increase their dietary intake of B12 containing foods. Given the social and cultural nature of collective dietary practices, it was important that this advice

was based primarily on the dietary practices that a participant was already socialized into. The FFQ that participants completed at baseline provided information on foods that participants commonly consumed. The dietary advice recommendations for each participant did not suggest any new foods or recipes for food preparation because changing dietary practices did not seem appropriate based on the focus group findings. Instead, participants were advised to eat the B12 foods currently consumed, but in larger quantities. To address concerns about increased cholesterol intake from an increase in dairy products and eggs, recommendations included low fat versions of food (low fat milk, yoghurt and cheese). Dietary counselling included advice from the focus group discussions for making yoghurt using traditional methods and low fat milk, complimented by fridge magnets that illustrated the fat content of different milk products. Participants in the focus groups consumed a predominantly vegetarian diet, so vegetarian (in preference to gelatine) capsules were prepared for the B12 supplement and placebo groups in the RCT.

**Approaches for working in partnership with the community.**

In order to recruit participants through relationships with South Asian community members as per the focus group findings, the research assistant for the focus group discussions continued in this research assistant role for the follow-on RCT, facilitating contact with South Asian community members. Along with members of the Community Collaborative Research Group (CCRG), the research assistant also provided support and guidance as to the appropriate conduct of the research. The importance of involving community members in recruiting participants is consistent with the findings of a UK based study investigating the barriers and facilitators to participation in research for South Asian people with asthma (Rooney et al., 2011). In this UK study, the lack of a direct personalised approach to participant recruitment was cited as a barrier for participation in research. Personalised approaches, especially those

made by fellow members of the community were more successful in recruiting participants (Rooney et al., 2011).

A Canadian study that used focus group discussions to investigate immigrant resettlement processes for ten South Asian women aged 59 to 78 years, employed a South Asian assistant who was known to the community being researched (Choudary, 2001). This was successful in supporting recruitment and in making the research a positive experience for participants because the research assistant already had the trust of participants. The employment of a community based research assistant was therefore identified as crucial, both in the focus group findings in this VitB12 research, and from published research undertaken on migrant cultures, where communities were hesitant to participate in research if they could not trust that the research would be culturally sensitive and conversant with the needs of the community (Bandesha & Litva, 2005).

The research assistant and members of the CCRG provided the initial link for the VitB12 study that enabled the 'foot in the door' so that participants who volunteered for the research felt that they could trust the researcher and the research. An Indian newspaper was used to advertise the study, in keeping with using culturally specific media. The role of the researcher as an invited visitor into the community was important to remember when undertaking the research; in particular, mindfulness of the collective identity and consideration of this at all times when asking women to take part as an individual in the study. Potential participants were encouraged to discuss taking part in the RCT with family remembers in order to gain a collective opinion on participation.

**Encouraging participation in the research.**

The themes from the focus group interviews about making participation easy for women were incorporated into the RCT methods. This involved strategies such as visiting the RCT participants in their homes or location of choice for collection of blood tests and measurement data, keeping times flexible to make participation easy for

particpants , delivering repeat bottles of supplements to RCT participants, or following up on dietary advice information. The suggestions that focus group participants gave around keeping RCT participants updated with study progress, such as Facebook or Twitter organisations, could not be used because of potential infringements of anonymity and confidentiality, and the double blinding for the RCT.

**Chapter Summary**

The dominant themes to emerge from focus group discussions were ***dietary practices as integral to identity and belonging, managing B12 deficiency*** and ***study participation as a positive experience***. Knowledge of the dietary sources for B12, the causes of B12 deficiency and the range of options for preventing and managing B12 deficiency were self-identified as limited among the South Asian and health professional focus group participants. South Asian women in the focus groups recounted experience with B12 deficiency and while some were able to identify the likely cause as insufficient dietary intake through vegetarian dietary practices, most participants could not accurately identify the foods in their diet that contain B12. The most common experiences for management of B12 deficiency were intramuscular B12 injections. Changing dietary preferences was not an option for women in the focus groups, particularly if they were vegetarian, because dietary practices were inextricably linked with family and cultural identity.

Focus groups participants wanted options other than repeated B12 injections to manage B12 deficiency. Some searched multiple sources for information on alternatives such as obtaining B12 supplements from India. Increasing intake of vegetarian based B12 foods had not been trialled as an alternative option, but on realising that B12 was contained in some of the foods that they consumed, focus group participants identified this as an option they would like to try. A dietary intervention group was included as one of the study treatments in the follow-on RCT to trial this option.

Focus groups described how keeping the RCT participation process easy was important for making study participation a positive experience for women. Potential participants would also need to know that they could trust the researcher to be culturally appropriate and that the research was a worthwhile study for participation. For these reasons, it was important to recruit for the RCT through word of mouth from South Asian community contacts. This was preferable to the use of media advertisements or study recruitment flyers. If the latter two were used, culturally specific media such as Indian newspapers, radio or television stations were recommended.

Where relevant, focus group findings were incorporated into the research design for the RCT. These findings included the employment of a South Asian research assistant to facilitate contact with potential participants from the South Asian community, and planning of a participant focused RCT study design where the researcher visited participants in their own homes or a venue of their choice for data measurements and blood sampling. Based on focus group findings of dietary practices as integral to identity and belonging, dietary recommendations in the RCT remained congruent with a participant's dietary practices. The RCT study design is described in more in detail in the next chapter (Chapter 5).

# Chapter 5: Methods for the Randomised Controlled Trial

This chapter describes the methods for the quantitative phase of the trial, a randomized controlled trial (RCT) which aimed to enrol 72 South Asian women (18 to 50 years). The trial was to compare the effectiveness, acceptability and sustainability of three treatments for improving serum vitamin B12 (B12) concentrations: daily 6µg B12 supplement capsule, or daily placebo supplement capsule or advice to increase consumption of foods containing B12. The aim of the study was to investigate ways to reduce vitamin B12 deficiency in South Asain women aged 18 to 50 years. The hypotheses and specific objectives for the RCT are detailed along with the allocation and treatment concealment processes, calculation of sample size, ethical considerations, and participant recruitment. Descriptions of the RCT include data collection and analysis methods, and justification for the statistical tests used. Ethical considerations specific to the RCT are outlined in this chapter, but the process of obtaining ethical approval and ethical considerations across all phases of the trial are detailed in Chapter 3. The RCT was registered with the Australian New Zealand Clinical Trials Registry ACTRN12610000262000.

**Aim**

To prevent vitamin B12 deficiency in South Asian women of childbearing age.

**Research Questions to be answered by the RCT**

For South Asian women of aged 18 to 50 years:

1. What is the prevalence of Vitamin B12 deficiency?

2. Is low dose supplementation or in increase in dietary intake of vitamin B12 containing foods more effective, acceptable and sustainable over time for increasing vitamin B12 biomarker concentrations?

**Objectives and Hypotheses**

The RCT researched the following specific objectives:

a) Calculate the prevalence of vitamin B12 deficiency, insufficiency and sufficiency in the sample population (using the B12 biomarkers serum B12 and holotranscobalamin [holoTC]).
b) Measure the effectiveness, acceptability and sustainability of treatments (6µg B12 supplement capsule, placebo capsule or dietary B12 advice) at two months post commencement of treatment and at six months (trial completion).
c) Measure the relationships between self-reported intakes of foods containing vitamin B12 and B12 biomarkers.
d) Identify relationships between B12 biomarkers and biochemistry biomarkers (folate, glucose, insulin, and lipids).
e) Identify relationships among B12 biomarkers, blood pressure, waist to hip ratio, waist to height ratio, grip strength, body mass index, body fat percentage and body fat-free percentage.

**Primary hypothesis and outcome.**

For South Asian women aged 18 to 50 years;

- Individualised advice to increase consumption of vitamin B12 containing foods will be more effective than taking a daily 6µg vitamin B12 supplement capsule, which in turn will be more effective than a daily placebo supplement capsule for

increasing serum vitamin B12 over six months (predicted increase over six months of 66 +/-71 pmol /L).

**Secondary hypotheses and outcomes.**

For South Asian women aged 18 to 50 years;

- Insulin resistance will be inversely related to serum vitamin B12 concentrations.
- Grip strength will be lower, and body fat percentage, waist to height ratio and waist to hip ratio higher in women with serum B12 concentrations less than 222pmol/L, when compared to women with concentrations greater than 222pmol/L

## Ethical Considerations for the Randomised Controlled Trial

The considerations outlined here include those specifically for the randomised controlled trial. For additional detail on ethical considerations and ethical approvals, refer back to Chapter 3.

**Informed Consent.**

Potential RCT participants were advised verbally, and in the written RCT information sheet and RCT consent form, that participation was voluntary; that there was no obligation to take part, and they could voluntarily withdraw at any stage without any penalty or questions (Appendix 6 and 7). This advice was noted as important in the focus group discussion as it was considered that some women would take part in the RCT out of obligation to the community, rather than because they wanted to participate.

**Potential risks to participants.**

Approval for blood sampling in the participant's home or venue of choice was granted in an ethics amendment to the AUT University Ethics Committee (AUTEC) (see list of amendments to the ethical approval process in Chapter 3). There was the

potential for participant injury and infection from the venepuncture technique. To reduce risks to the participants, and to the researcher or research assistant, the venepuncture was conducted as per the blood testing protocol approved by AUT University Ethics Committee (Appendix 8).

Participants were not advised of their results until the end of the trial unless the blood test measurements were abnormal and a threat to well-being. There was the risk of harm from withholding participant blood biochemistry and haematology measurements that were outside the normal or recommended parameters. During and following the study, Dr Janet Rowan, (Physician, National Women's Hospital, Auckland City Hospital, Auckland) was consulted by a third party if laboratory measurements were outside the normal reference range defined by the laboratory (the researcher was blinded to this consultation unless the participant needed to be excluded from the study). Iron studies were requested when deemed necessary (as per notation on ethics approval form and on the participant information sheet). Participants were advised to use the contact details provided to contact the research team in the event of any concerns. Participants with serum B12 <110 pmol/L were to be excluded from the study and advised to consult their general practitioner immediately. This was not necessary as all serum B12 measurements reported were above this concentration.

**Confidentiality and anonymity.**

Participant's details were kept confidential so that only the researcher, the primary supervisor, the research assistants and a third party member of the research team were aware of who had participated. Hard copy questionnaire and data collection sheets (identified by a participant ID code and no mention of name), are kept in a locked cabinet in the Body Composition and Metabolism Research Centre (BCMRC) research room at AUT University. The consent forms are kept in a separate locked filing cabinet

in the primary supervisor's office and will be kept for six years before being destroyed by shredding. Electronic data is stored in a password protected folder, with a copy stored on a memory stick, also kept in the locked filing cabinet in BCMRC research room.

## Design of the Randomised Controlled Trial

### Sampling and recruitment.

Convenince sampling was used and participants recruited by advertisement through community groups, health care organizations and by word of mouth. Advertisements were placed in an Indian community newspaper and The Asian Network Incorporated (TANI) newsletter.(Appendix 4) Advertisements were also placed on notice boards and left on leaflet tables in community centres and health centres within the geographical location of the study.

### Inclusion/exclusion criteria.

Apparently healthy women of South Asian origin, aged 18 to 50 years at the time of recruitment, and living in Auckland City. Criteria for exclusion included women with chronic disease, major health conditions, malabsorption syndromes, pregnant women or planned pregnancy in the following 6 months, women who were already taking B12 supplements or who had received a B12 injection in the past 18 months, and women on medications that may interfere with B12 absorption from the gastrointestinal tract, for example, metformin.

### Sample size.

The calculations for the number of participants required in each of the VitB12 study treatment groups were based on evidence from the daily provision of milk (0.96μg of B12 in 200mL) to 120 children in Kenya (Siekmann et al., 2003). The mean change in serum B12 for the milk group in the Kenyan study was 66±71 pmol/L over twelve

months, and for the control group -13±65 pmol/L (Siekmann et al., 2003). Sample size was calculated using the difference between the means of the milk and control groups from the Kenyan study and the within-group variability of the milk group. It was assumed that for the VitB12 study, the mean observed change in serum B12 would occur over six months. To achieve this change using B12 dietary advice or supplements, 20 participants were required for each of the intervention groups in the study (80% power, α level of 5%). At a dropout rate of no higher than 20%, 24 participants per group or a total sample size of 72 participants were required (Reasoning for this determination of sample size was confirmed through verbal communication with Associate Professor Lindsay Plank, Auckland University, New Zealand). As collected, the medical laboratory information was sent to an independent expert for an interim data monitoring review of the two-month outcomes. The interim data review indicated that a substantive difference was seen among groups with a clear outcome for treatment of 60 participants. Given the difficulties recruiting and the intensity of research interactions, the decision was made (by the research supervisor) to stop recruiting and concentrate on achieving timely completion of the study.

**Randomisation, Group Allocation and Treatment Concealment Processes**

Once women expressed an interest to participate in the study, they were emailed or posted a copy of the RCT invitation and information sheet (Appendix 6). This was followed by an email or phone call from the researcher to explain and discuss the research process and to respond to any questions. Women answered preliminary screening questions about ethnicity, medical history, medications, and vitamin supplement use in order to assess eligibility. If they appeared to meet inclusion criteria, then the first of a minimum of three research visits was scheduled for a morning and participants were requested to fast (nothing but water), from the evening before. The

face-to-face interactions for measurements were scheduled to occur either in the participant's homes, or workplace, or at the AUT University Body Composition and Metabolism Research Centre (BCMRC) research room.

At the first visit, the informed consent process was completed and participants then signed the consent form (Appendix 7). When the baseline questionnaire was completed (Appendix 9), the researcher reviewed the questionnaire for participant eligibility and if eligible, then baseline blood samples were taken. A third party person not directly involved with the RCT, randomly allocated participants into a research treatment group using a stratified random assignment process with the sequence of randomisation determined by a sequence-matched random number allocation table. Participants were initially divided into one of two strata by meat eating practices (non-meat-eating and meat eating), then randomly allocated from each stratum in equal numbers to one of the three treatment groups. The third party person kept a confidential record of the participant number matched to the participant name, so allocation to either the placebo or B12 supplement group remained concealed from the researcher and participant. That number matching information was kept in a locked drawer. The researcher received notification of the B12 dietary advice group membership though and advised participants of their allocation to this group treatment. The researcher did not receive the participant blood test measurements until completion of the RCT.

## Rationale for Data Collected

### Anthropometry and bioelectrical impedance analysis.

Anthropometry and bioelectrical impedance analysis were used alongside medical history and laboratory data to determine health risks related to body composition and to identify any associations between body composition and B12 biomarker status (Rush, Freitas, et al., 2009; Rush, Puniani, Valencia, Davies, & Plank,

2003; Yajnik et al., 2008) Muscle strength and function were evaluated by right and left grip strength, and used to identify associations between low muscle strength, body composition and B12 concentrations (Liang, Su, & Lee, 2000; Yajnik et al., 2008).

**Blood tests.**

Folate sufficiency was measured by assessment of serum folate and to determine any relationships with B12 biomarkers, body composition and grip strength. Fasting lipid results were used conjunction with blood pressure readings to characterise cardiovascular risk based on the New Zealand Guidelines Group cardiovascular risk assessment charts (New Zealand Guidelines Group, 2009). Fasting glucose provided a rudimentary guide to glucose tolerance. It was also a preliminary test to ensure participants met study inclusion criteria for no major disease or history of diabetes mellitus. Fasting insulin was measured, and used along with fasting glucose to calculate insulin resistance using the homeostatic model 2 assessment of insulin resistance (HOMA2 IR), Beta cell function (HOMA2β%) and cellular insulin sensitivity (HOMA2S%) (Wallace, Levy, & Matthews, 2004). A validated computerised HOMA2 IR calculator from the University of Oxford (Diabetes Trial Unit, 2004), was used to calculate this. HOMA2 IR was compared with serum B12 and holoTC to determine any significant relationships (Yajnik et al., 2008). A ratio of 1 reflects insulin sensitivity, where beta cell function and cellular insulin sensitivity are balanced. There is no universal agreement of the HOMA2 IR value that defines insulin resistance or increased risk for T2DM, metabolic syndrome, and CVD. Ratios of 1.8 have been identified as cut-offs above which insulin resistance exists (Esteghamati et al., 2010; Geloneze et al., 2009), and a ratio of 1.4 as the cut-off for metabolic syndrome (Geloneze et al., 2009). These cut-off values were calculated in studies of non South Asian populations so although they are the cut-offs used in this study, there are limitations with their use.

These values refer to HOMA2 IR rather than the traditional HOMA1 IR calculation (Levy, Matthews, & Hermans, 1998; Wallace et al., 2004).

**Treatments and data collection**

Participants were allocated to one of the following group treatments:

- Group One. 6 µg B12 Supplement (labelled as supplement A)
- Group Two. Placebo (labelled as supplement B)
- Group Three. Dietary B12 advice

Placebo and cyanocobalamin supplements were custom prepared by Pharmaceutical Compounding New Zealand Limited for this RCT. The B12 supplement capsule contained cyanocobalamin 6mcg (Vitamin B12 0.1% water soluble) with microcrystalline cellulose as the filler, filled into a Capsugel® made of Hydroxypropyl Methylcellulose (HPMC), a cellulose-based raw material that is Halal certified and contained no animal products so was suitable for vegetarians. The placebo contained microcrystalline cellulose as the filler and red food colour powder as colour, filled into the vegetarian Capsugel® and was visually identical to the B12 supplement capsule.

Participants allocated to the supplement A or B treatment groups were advised to take one capsule with a full glass of water every day for a period of six months. Repeat bottles of capsules were hand delivered to, and previous bottles collected from, participants at two, and then four months of treatment. Any remaining capsules from the previous period were manually counted to measure adherence with the capsule intake. At the two and six month visits, participants were asked about any adverse effects experienced that they attributed to the group treatment. At the completion of the six-month study period, and after the final measurement visit, all participants were offered

individualized B12 dietary advice, following the advice guidelines that the dietary B12 advice group received.

Following the baseline measurements, and notification of allocation to the B12 dietary advice group, participants in that treatment group received verbal advice along with personalised written information on how to increase their consumption of B12 containing foods to an estimated 2.4 µg per day. This advice was based on the B12-containing-foods identified in their baseline B12 food frequency questionnaire (B12FFQ). The B12FFQ was repeated at two and six months for all research participants, and for the dietary B12 advice group, the dietary advice they received was reinforced, or modified where necessary based on the results of the two-month B12FFQ. If participants reported consuming 2.4 µg or more of dietary B12 per day, then the advice was tailored to reinforce this dietary pattern. In between the data collection periods, the researcher was available to the B12 dietary advice group participants by telephone or email if any clarification of the dietary B12 advice was required. Adherence to dietary recommendations was estimated by comparing dietary B12 calculated on the current B12FFQ to the amount reported on previously completed B12FFQs. Participants from the dietary advice or placebo group who demonstrated low B12 biomarker stores were offered a 60-day course of the 6µg B12 supplement capsule once the study was completed.

**Measurement protocols.**

At baseline, participant demographics and characteristics were recorded from the interview-administered questionnaire (Appendix 9). All blood tests, physical measurements and dietary assessments were made at baseline, then two and six months post enrolment in the study**.** Duplicate measures of body composition derived from hand to foot bioelectrical impedance (±5 Ω and phase >4°) and body size by height (±0.5cm),

weight (±0.1 kg), waist circumference (±0.1cm), hip circumference (±0.1cm), mid upper arm circumference (±0.1 cm), systolic and diastolic blood pressure (±10 mmHg) and pulse (±10 bpm) were made. All physical measurements were recorded at least twice and the average calculated. If any of the measurements exceeded the tolerance limits cited above, they were repeated a third time and the closer of the two measures averaged. The hip, waist and mid upper arm circumference were all measured with a Figure Finder® tape measure (Novel Products Inc. Rockton, IL).

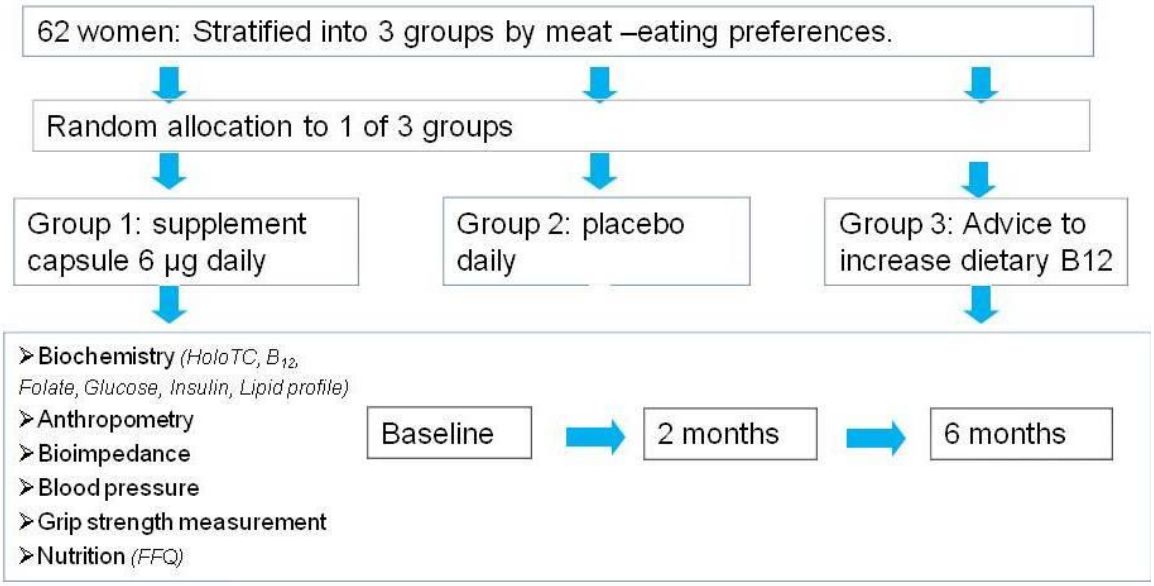



*Figure 2*. Overview of the RCT data collection process

**Questionnaire data.**

Questionnaire data was collected using the questionnaire and data collection form (Appendix 9) and included demographic data, personal and family dietary patterns and health information, migration history, menstruation pattern, and any medications or supplements currently taken.

**Blood sampling and analysis.**

The researcher, assisted where necessary by a phlebotomist research assistant, sampled 18 ml of venous blood from the antecubital foassa. If there was any difficulty

(such as difficulty locating veins due to adiposity) then the participant was requested to attend a community blood collection centre. The processes for blood sampling are detailed in Appendix 8. Fasting blood samples were analysed for serum folate, vitamin B12, lipid profile, glucose and full blood count by Diagnostic Medlab (DML), which is accredited with International Accreditation NZ and the Royal College of Pathologists of Australasia.

Samples for insulin, holoTC, homocysteine and methylmalonic acid (MMA) were transported on ice after sampling and centrifuged within four hours of being taken. The samples were centrifuged at AUT University BCMRC laboratory for 10 minutes at high speed (5,800rpm) using the Z150A compact lab centrifuge. Samples were then stored temporarily at -4 degrees freezer at AUT BCMRC research laboratory, before being transferred to – 85 degrees freezer at Auckland University.

The samples collected, and testing methods are as follows:

- Glucose (2ml) in Fluoride tube: Measured by Diagnostic Medlab using Roche/Hitachi automated chemistry analyser.
- Lipids, folate and serum B12 (4 ml) in SST tube: Lipids measured using Roche Hitachi automated chemistry analyser. Serum B12 and folate measured using the Modular Analytics E170 immunoassay analyser.
- Full blood count (4ml) in EDTA tube: Measured using the haematology Sysmex-XE-2100 analyser.
- Homocysteine and MMA 4ml in EDTA tube: The samples were separated and stored frozen at -85degrees Celsius. Measurement of serum MMA and homocysteine, may be analysed at a later date if funding becomes available
- Insulin and HoloTC (Active B12) 4 ml in heparin tube:

- Insulin samples were stored at -85 degrees Celsius until required, then forwarded to Waikato District Health Board laboratories in two batches for measurement (at 3 months post study commencement and following completion of the study). They were measured using Abbott IMxInsuilin immunoassay (list No2A10, Abbot Laboratories, Japan). One sample of aliquoted plasma was used as a test control and aliquots of the same sample forwarded in both batches to test for inter-sample consistency.
- HoloTC samples were stored at -85 degrees Celsius until forwarded in two batches to Middlemore Hospital Laboratory for measurement of Active B12 using AxSYM® Active-B12 microparticle enzyme immunoassay analyser. A test control sample of plasma was forwarded in both batches to measure inter-batch consistency.

**Height.**

Height was measured using a portable stadiometer with metal base to which a movable measuring tape attached. The tape measure was secured at one end to the metal base and at its other end to a horizontal headboard and spirit level. A locking device on the tape measure locked the tape measure at the measured height once the headboard rested horizontal on the vertex of the participant's head. The portable stadiometer was used on a flat non-carpeted surface against a blank wall. Participants removed shoes and were asked to stand with their feet together, heels flat against the back of the stadiometer base with arms by their sides, shoulders relaxed, head in a neutral position and eyes looking straight ahead so that the base of the ear was level with the zygomatic arch. Heels, buttocks, shoulder blades and the back of the head were maintained against the wall. The tape measure was pulled tight so there were no kinks or bends. Participants were asked to inhale, stand tall, and then the headboard was bought down

to rest on the vertex of the head. When the spirit level indicated that the head board was horizontal, the locking device was used to secure the tape measure at the recorded height. Height was recorded in cm at the baseline measurement point only and was recorded to the nearest 0.1 cm.

**Weight.**

Weight was measured using an electronic digital scale (Soehnle, Germany). Shoes were removed prior to weighing and participants were also requested to remove any heavy garments, belts and objects from their pockets. Participants were requested to empty their bladder prior to weighing. The scale was placed on a flat, non-carpeted surface and zeroed prior to weighing. Participants were then asked to step onto the centre of the scale and stand still with their weight distributed evenly between both feet. Weight was measured in kg to the nearest 0.1kg and the two weights were recorded in quick succession to avoid the time effect bias on weights. As the scale was transported from participant to participant, the accuracy of the scales was checked over one weight measure each two weeks using a standard weighing scale in the BCMRC laboratory at AUT University.

**Blood pressure and pulse.**

The arterial blood pressure was measured using digital sphygmomanometer (Omron T5, Omron Corporation, Japan). The mid upper arm circumference was used to determine cuff size. If mid upper arm circumference was 22-32cm, then the medium cuff was used. If mid arm circumference was 32-42cm, then the large cuff was used. Participants were requested to sit with their left arm and hand relaxed and at the level of their heart. Systolic and diastolic arterial blood pressure was measured in mmHg and pulse rate in beats per minute.

**Mid-upper arm circumference.**

The participant was advised to stand with the left arm flexed at a 90 ° angle. The point midway between the lateral projection of the acromium process at the shoulder and the inferior surface of the olecranium process at the ulna was marked (Lustig & Strauss, 2003). The participant then extended their arm into a relaxed position and the mid-point mark was used to measure the MUAC. The tape was checked to ensure that it was not twisted and that it was parallel to the midpoint line.

**Waist circumference.**

Waist circumference was measured as the mid-point between the superior iliac crest and the inferior lateral margin of the ribs, looking from the anterior view (Kohli, Min, & Lear, 2009; Lustig & Strauss, 2003; Moreno et al., 2003). Participants were advised to stand tall with their abdomen relaxed, arms relaxed at their sides and their feet together. They were asked to breathe in, and then exhale and waist measurement was taken at the end of gentle exhalation. The tape was checked to ensure it was parallel to the floor and not kinked or twisted. The waist to height ratio was calculated (cm/cm)

**Hip circumference.**

The point of measure was taken horizontal to the point of maximal gluteal protuberance from the lateral view over one layer of clothing (Kohli et al., 2009; Lustig & Strauss, 2003). To aid consistent measure for subsequent visits, the distance of the hip from the point of waist measure was noted on the participant data form. Participants stood upright, buttocks relaxed and feet together. Measurement was taken after gentle exhalation. The waist to hip ratio was calculated (cm/cm).

**Grip strength.**

Muscle grip strength was tested using a Smedleys hand held dynamometer (Smedleys Dynamo Meter, 100kg, TTM, Tokyo). Prior to each test, the dynamometer

dial was set back to zero. Participants were shown how to hold the dynamometer in their pronated dominant hand. The opposing arm was held relaxed by the side. Participants were instructed to stand tall with their feet shoulder width apart for balance, raise the hand holding the dynamometer directly above the head with a the elbow fully extended, squeeze the hand trigger of the dynamometer and simultaneously bring the dynamometer forward in a 180 ° arc, and down to their side, then relax the grip on the dynamometer bar. The maximum grip strength was recorded on the dynamometer in kilograms (kgs) to the nearest ±1kg. The elbow was kept extended through the procedure. Participants then repeated the procedure with the non dominant hand. The process was repeated twice with each hand and the maximum grip strength for each arm was the measure recorded.

**Bioelectrical impedance analysis (BIA).**

Bioelectrical impedance analysis (BIA) is a doubly indirect measure of body composition that uses the relationship between the bioelectrical impedance of the body and the conductivity of total body water content. From the bioimpedance recordings obtained, previously validated prediction equations allow the calculation of fat free mass (FFM) and therefore, fat mass (FM). Lying BIA is traditionally considered the most valid posture in which to measure bioimpedance, with the comparison tables for bioimpedance developed using lying bioimpedance values (Lukaski, Johnson, Bolonchuk, & Lykken, 1985). A trend in recent studies is to use standing BIA because it is a more convenient measurement technique than lying BIA (Rush, Crowley, Freitas, & Luke, 2006). It is not reliant on participants having to find somewhere to lie down, and it does not exclude those who are unable to lay flat e.g. those with respiratory or back problems. The use of standing BIA as a measure of body fat and water percentage was validated in an Auckland University of Technology based study of 205 volunteers

(Rush, Crowley, et al., 2006). Compared with lying BIA values, standing BIA values were found to be a valid and reliable measure of body fat and water content, provided that standing BIA values were multiplied by a correction factor of 1.021 for average age group 15 to 59 years (Rush, Crowley, et al., 2006). In this VitB12 study, a single frequency 50 kHz hand-to-foot bioelectrical impedance instrument (Imp DF50, Impedimed Ltd, Queensland, Australia) was used to record BIA. Accuracy of the instrument was the checked against a test calibrator (resistance measurement recorded 999.9±9Ω) prior to each use.

Constant environmental temperature, time-period of standing, avoidance of recent food and fluid intake plus recent voiding of urine are required for reliable measures (Rush, Chandu, & Plank, 2006; Rush, Crowley, et al., 2006). When measuring bioimpedance in the VitB12 study, participants were kept standing for no more than three minutes to avoid changes to bioimpedance from fluid shifts arising from prolonged elevated venous hydrostatic pressure in the legs (Rush, Crowley, et al., 2006). The BIA recordings for each participant were taken twice, and repeated if agreement between two measures was outside the expected precision.

Participants were instructed to withhold food and fluids for at least four hours prior to measurement and to void just prior to the measurement. Environmental and body temperature affects bioimpedance measures; ideally the room temperature should be 25 (± 5) degrees (Liang et al., 2000). It was not possible to control for this when measuring participants in their own homes, but participants were asked to ensure their home was warm for the measurements.

To measure bioimpedance, participants were instructed to stand with their feet hip distance apart to avoid contact between thigh (may cause short-circuiting of the current if thighs touch). The skin was prepared using an isopropyl alcohol wipes

(Kendall, Webcol®, Alcohol Preps) and electrodes (3M Red Dot$^{TM}$, 2330) attached to the following sites on the right side of the body:

a) Proximal hand site. Dorsal aspect of right wrist at the level of the ulna head (yellow connector)
b) Distal hand site. Dorsal surface of right hand just proximal to the third metacarpophalangeal joint (red connector)
c) Distal foot site. Dorsal aspect of the right foot just proximal to the second metatarsophalangeal joint (blue connector)
d) Proximal foot site. Dorsal anterior aspect of the right foot between lateral and medial malleolus (black connector)

A distance of at least 5 cm was kept between the distal and proximal electrode site to prevent short-circuiting of current and participants stood very still while the BIA was taken. Impedance (Z ohm), resistance (R ohm), reactance (Xc ohm) and phase angle (P°) readings were used to calculate fat mass (FM), fat free mass (FFM) and body fat % (BF %), using bioimpedance resistance formulae derived from validation studies on body composition in Asian Indian women (Rush, Chandu, et al., 2006). This is more appropriate than formula used to calculate body composition in European as studies show that people of Asian Indian origin have different body morphology than comparison populations such as European (Rush, Freitas, et al., 2009; Rush, Goedecke, et al., 2007)

FFM = 0.456 $H2/R$ + 0.127 $W$ + 0.0746 $X$ + 5.959

FM (kg) = $W$ (kg) – FFM (kg)

BF% = FM(kg)/$W$ (kg) × 100%

$H$ = height (cm); $R$ = resistance; $W$ = weight (kg); $X$ = reactance

Standard estimating error (SEE) = 2.0kg

**Measuring B12 dietary intake.**

Participants recorded their consumption of selected foods containing B12 in a three-month food-frequency recall questionnaire (B12FFQ) at baseline, two months and six months. Foods and quantities reported were entered into a spreadsheet created in Microsoft Office Excel 2007 ™ (12, 0, 6654, 5003), and average daily intake of dietary B12 calculated in micrograms consumed per day. The development, validation and application of the B12FFQ are discussed in detail in Chapter 6.

**Contact with participants throughout the study.**

At recruitment, participants were asked their preferred mode of communication throughout the study (emails, texts, mobile phone calls or landline phone calls to work or home). In the week following baseline measurements and blood tests, participants allocated to either the B12 supplement or placebo group were contacted and the capsules delivered by the researcher.

Participants in the dietary advice group were contacted and advised of their group allocation. They were visited or telephoned plus emailed or hard copy delivered their individualized B12 dietary advice guidelines (Appendix 10), depending on their preference and availability.

All participants were contacted again three weeks into the study to check on progress and answer any questions. A preliminary date was made for the next visit for measurements and blood tests at two months duration into the study. At the two month visit, participants were provided with another bottle of capsules or a review of their dietary B12 guidelines. Further contact was made with participants at four months and additional capsules provided to the supplement/placebo participants if required. Participants were not contacted again until just prior to study completion to organize a date for the six-month visit for measurements and blood tests. Participants were advised

that they could contact the researcher at any stage if they had any questions or problems. On study completion, participants were provided with a report on their blood assays, physical measurements and estimated dietary B12 intake results

**Statistical analysis**

Unless specified otherwise, data were analysed using Predictive Analytic Software (PASW) Edition 18 software. Questionnaire data (Appendix 9), laboratory results, physical and anthropometric data plus adherence with supplements and reported dietary B12 intake were entered into a PASW 18 spreadsheet. Where PASW 18 could not provide statistical tests e.g. 95 % confidence intervals on Pearson's and Spearman's correlation coefficients, or on analysis of variance (ANOVA) test, a Microsoft Excel™ algorithm from the *'A New View of Statistics'* website calculated these (Hopkins, 2007). Once data were entered, the PASW 18 spreadsheet was double-checked to ensure that the data were correct.

**Intention to treat analysis.**

An intention to treat approach was used to maintain the integrity of randomisation during data analyses. This approach includes all of the participants in the treatment group to which they were assigned, whether or not they have been lost to follow-up during the study (Fergusson, Aaron, Guyatt, & Hebert, 2002; Overall, Tonidandel, & Starbuck, 2009; Polit & Gillespie, 2009). No participants crossed over intervention groups during this RCT, but participant data was not 'missing at random' as four participants were withdrawn after vitamin B12 injection supplements prescribed by their General Practitioner; all had serum B12 results in the lower quartile of participants in the study. Participants lost to follow-up were included in the analysis using the Last Observation Carried Forward (LOCF) method for imputation of missing

values (Overall et al., 2009). LOCF was selected because it would continue the low serum B 12 results for those participants whose results were not 'missing at random' and with the exception of the B12 dietary advice group, the number of participants missing from each group was small (Overall et al., 2009). Computer generated methods of multiply imputing missing data are potentially problematic in small samples or when data is missing for non-ignorable reasons (Huang & Carriere, 2006). Analyses were repeated with and without missing data and results compared to determine any differences in inferences drawn.

**Descriptive characteristics.**

Descriptive statistics were used to characterise the study population and to distinguish between treatment group characteristics. Continuous variable data were explored for normality; normally distributed data are reported as mean, standard deviation, minimum and maximum ranges, and non-normally distributed data reported as median with $25^{th}$ and $75^{th}$ percentiles (Field, 2009; Peat & Barton, 2005).

**Analysis for differences between treatment group characteristics**

For normally distributed characteristic variables, univariate ANOVA models were used to determine any influential differences in treatment group characteristics that could affect the primary group treatment outcomes. The limitation of introducing a familywise Type 1 error through using multiple one way ANOVAs is acknowledged (Field, 2009), but this was a preferred option over multiple regression because a sample size of 60 participants was not sufficient to allow for the multiple predictors in a regression model (minimum sample of 15 per predictor required) (Pallant, 2010; Tabachnick & Fidell, 2007). For ANOVA, continuous variables of age, anthropometry, biochemistry, and haematology characteristics were fitted as the response variables,

with the treatment group as the predictor variable. Post hoc tests with Tukey's honestly significant difference (HSD) and a Bonferroni adjustment were used to identify which group, if any, were different on that characteristic. Both significance and 95% confidence intervals were reported with test significance accepted at $p \leq 0.05$. For non-normally distributed continuous variables, non-parametric analysis of variance using the Kruskal-Wallis test was used, with treatment group entered as the predictor (fixed factor), and the characteristic variable entered as the response (dependent) variable. Reporting of results included asymptomatic significance, with comparison of median, $25^{th}$ and $75^{th}$ percentiles to determine the locus of treatment group differences. The variables for which there were significant group differences were controlled for as covariates in the analysis of covariance (ANCOVA) testing the primary study outcomes.

**Change in percentage B12 deficiency between RCT groups**

To categorise the extent of baseline B12 deficiency between groups, baseline serum B12 and holoTC variables were organised into literature derived constructs of low (deficient) (De Benoist, 2008; World Health Organization, 2008), borderline low ( insufficient) or sufficient based on the following criteria (L. H. Allen, 2009; Herbert, 1994; Molloy et al., 2009)

- Low serum B12 < 150 pmol/L
- Borderline low serum B12 150 to 222pmol/L
- Sufficient serum B12 > 222pmol/L
- Low holoTC < 35pmol/L
- Borderline low holoTC 35 to 44pmol/L
- Sufficient holoTC >44 pmol/L

Descriptive statistics were used to report the change in the percentage and number of women in each treatment groups who were low in serum B12 or holoTC (deficient), borderline low ( insufficient) or sufficient at baseline, two months and six months

**Justification for statistical methods**

The primary outcomes for analysis in the RCT were the serum B12 and holoTC response to each of the three treatments over the six-month treatment period (change over time). There were outliers in the serum B12 and holoTC results at baseline, two and six months, so these variables were all (natural) log transformed to achieve a normal distribution (Field, 2009; Hopkins, 2007). However, the log transformed variables still violated the assumption of sphericity required for a robust general linear model (GLM) repeated measures ANOVA (the assumption of a null hypothesis for Mauchley's test for sphericity was $< 0.05$ for both B12 biomarkers) (Field, 2009; Miller & Chapman, 2001; Peat & Barton, 2005). Analysis of residuals plots against predicted values on log transformed data revealed a random pattern suitable for linear regression, so univariate ANOVA and ANCOVA were appropriate as tests for statistical analysis, with the adjusted coefficient of determination ($R^2$) used to measure the magnitude of association between variables (Bewick, Cheek, & Ball, 2005; Kvalseth, 1985; Leach & Hensen, 2007). Even though serum B12 and holoTC are interdependent, multivariate analysis of variance was not used as the study sample size was not sufficiently powered for this (Field, 2009; Meyers, Gamst, & Guarino, 2006).

The change in B12 biomarkers over time was captured using the variables of change scores on log-transformed data, calculated as the difference between baseline and two month, two month and six month, then baseline and six month B12 biomarkers (serum B12 and holoTC). (O'Brien & Kaiser, 1985; Tabachnick & Fidell, 2007). The

use of the change scores has limitations when the average baseline scores are not matched between groups, but even with this risk, the use of change scores is preferable to using repeated measures ANOVA that violates sphericity assumptions (O'Brien & Kaiser, 1985).

With any repeated measures analyses, regression to the mean can occur where low baseline scores are more positively correlated with change, therefore the low scores tend to improve more than high scores (Vickers & Altman, 2001). While some researchers recommend entering baseline scores as a covariate in ANCOVA to control for these baseline differences (Vickers, 2005), other experts caution against this as it violates the assumptions of independence of covariates in the analysis (Field, 2009; Miller & Chapman, 2001). Analysis of baseline data in this study highlighted a lower baseline holoTC for the B12 supplement group, compared to the other two treatment groups, however baseline scores were not included as a covariate in the ANCOVA model for this research because of violation of this prerequisite assumption of covariate independence. (Field, 2009; Miller & Chapman, 2001; Pallant, 2010). The use of change scores and calculation of magnitude of difference as a percentage of the previous score was used instead to adjust for the lower baseline holoTC (O'Brien & Kaiser, 1985; Peat & Barton, 2005)

**Calculating change scores and magnitude of change**

The following variables were calculated in turn for each of the B12 biomarkers (serum B12 and holoTC) to derive the change score (*diff*) variables.

- Difference between six month and baseline results
- Difference between two month and baseline results.
- Difference between six month and two month results

The *diff* variables were then back transformed using the exponential function in PASW 18. The exponential of the *diff* variable was subsequently used to calculate the geometric mean percentage change over time in B12 biomarkers in an Excel™ spreadsheet using the formula $100(e^{diff} - 1)$ (Hopkins, 2007). Reported B12 dietary intake as measured by the B12FFQ at baseline, two and six months were also skewed with outliers, so these were also log transformed prior to analyses, difference scores calculated, then back transformed to derive the geometric mean percentage change in dietary B12 intake over time, using the above formula. Effect sizes from the percentage change in B12 biomarker variables and dietary B2 intake were interpreted using Cohen's effect size classification of 0.2 as small, 0.5 as moderate and 0.8 as large (Field, 2009)

**Analysing for efficacy of group treatments using ANOVA and ANCOVA**

An analytical model was constructed using sequential univariate ANOVA, then ANCOVA to explore significant relationships between the change scores (*diff* variables) for serum B12 and holoTC, comparing these between treatment groups. The interdependence of serum B12 and holoTC means that the measurement of each of these variables in separate ANOVA tests could potentially inflate the familywise type 1 error rate (Field, 2009; Miller & Chapman, 2001; Tabachnick & Fidell, 2007). To reduce this likelihood, a Bonferroni adjustment was applied to interpretation of statistical significance. This accounts for the related serum B12 and holoTC variables in the ANOVAs with the traditional $p \leq 0.05$ significance divided by two to give an adjusted significance level of $p \leq 0.025$ (Field, 2009; Pallant, 2010). When reporting ANOVA results, the F statistic, degrees of freedom (*df*) and significance (*p*) values were reported from PASW 18 outputs, and the coefficient of determination ($R^2$) was used to report the percentage variance in B12 biomarkers that group treatment accounts for. The 95%

confidence intervals for the F statistic were calculated from an Excel™ algorithm (Hopkins, 2007) and the exponential of the back transformed log of difference scores were used to calculate the geometric mean percentage change in B12 biomarkers over time (Field, 2009; Hopkins, 2007).

Post hoc multiple comparisons were made to determine which intervention treatment, if any, contributed to the significant ANOVA test result. Post hoc tests included Tukey's HSD and Bonferroni. The B12 supplement treatment group was used as the reference group against which to compare the other two treatment groups in post hoc tests.

The model was extended by controlling for related variables that may influence the change in serum B12 and holoTC over time, using ANCOVA. Pearson's correlation coefficient tests were used first to highlight significant associations between the change scores for B12 biomarkers and other potentially related continuous variables such as age, intake of dietary B12, and supplement adherence. Variables with a moderate ($r \geq 0.5$) correlation were then included as covariates in ANCOVA. When another variable was added as a covariate, a Bonferroni adjusted significance was applied to account for a third variable added to the model ($p \leq 0.05$ divided by 3 variables equals a statistical significance adjustment level of $p \leq 0.017$). When reporting the effect of variables in the ANCOVA model, the adjusted coefficient of determination (adjusted $R^2$) was used as this reduces the potential over inflation of effect size that occurs when multiple variables are introduced into an regression model (Kvalseth, 1985; Leach & Hensen, 2007; Menard, 2000). Post-hoc tests are not available using ANCOVA in PASW 18, so planned contrasts were used to determine which intervention group contributed to any significant ANCOVA results. Simple contrasts were used with the B12 supplement

group as the reference group against which to compare the placebo and B12 dietary advice groups.

Where Pearson's correlation coefficients were used for analyses, significance was reported from PASW 18 outputs and the 95% confidence intervals for the correlation were calculated from an Excel$^{TM}$ algorithm (Hopkins, 2007). The coefficient of determination was calculated by squaring the correlation coefficient and this was used to report the percentage of variation explained by one variable on another (Field, 2009).

**Measuring acceptability and sustainability of interventions**

Acceptability of interventions was measured by persistance with study interventions and by qualitative data on their acceptability, collected at the two and six month measurement points respectively (Lachaine, Petrella, Merikle, & Ali, 2008). Complete cessation of taking capsules, or declining to take capsules was viewed as non-persistance for the supplement and placebo groups. Non-persistance with dietary advice was more difficult to ascertain and would only have been noted if a particpant had declined to accept the B12 dietary guidelines. Sustainability was measured by adherence with interventions over the study period (Lachaine et al., 2008). For the placebo and supplement groups, percentage adherence was calculated from the remaining capsule count at the two and six month data measurement points. As these variables were skewed, median and $25^{th}/75^{th}$ percentiles were reported.

For the dietary advice group, adherence was measured by an increase in the reported B12 dietary intake between the baseline, two and six month B12FFQs for participants whose intake was < 2.4µg/day, or by the mainteneace of estimated B12 dietary intake for those whose intake was ≥ 2.4 µg/day. The change in B12 intake variables were not normally distributed so median percent change with $25^{th}/75^{th}$

percentiles were reported. To measure the effect size for response to dietary advice in the B12 dietary advice group, the (geometric mean) percentage difference in recorded B12 dietary intake from B12FFQ was reported (from *diff* variables), with percentages derived from the exponential back transformed values.

**Chapter Summary**

The second phase of this body of research was a RCT comparing the efficacy, acceptability and sustainability of three different treatments on biomarkers of B12 (serum B12 and HoloTC). It was calculated that a total sample size of 72 participants would be required; 24 per each of the three intervention groups, with allowance for an attrition rate of 20 % over the six month study period. An interim data review after the two month data collection was collated, found substantive effects from a sample size of 62, so the recruitment was capped at that number of participants.

Participants were stratified by meat or non-meat-eating dietary practices when recruited into the study, then randomly allocated one of the three treatment groups; 6 µg B12 supplement capsule, placebo capsule or advice to increase dietary B12 intake. The randomisation process for the capsule groups was double blind with neither the researcher nor the participants knowing if they had been randomised into a B12 supplement or placebo group. It was not possible to conceal participant allocation to the dietary advice group, but the data obtained from these participants was initially analysed without the researcher being aware that these participants belonged to the dietary advice group.

The primary outcomes of serum B12 and holoTC results were not normally distributed. Once (natural) log transformed, distribution was normal. Data did not meet assumptions for repeated measures ANOVA testing, so to capture the time versus group interaction on B12 biomarkers, ANOVA and ANCOVA were conducted for outcome

variables calculated from the change between the baseline, two, and six month B12 biomarker measurements. Pearson's correlation coefficient analysis was used for continuous variables to identify influential variables moderately associated ($r \geq 0.5$) with group treatment. These influential variables were controlled for by inclusion as covariates in the ANCOVA model. Literature derived constructs of low (deficient), borderline low (insufficient) and sufficient B12 biomarkers were used to identify any reduction in the percentage of women B12 deficient in each of the treatment groups over the six-month RCT treatment period. The findings from the randomised controlled trial are presented in Chapter 7. Chapter 6 follows next with descriptions on the validation of the vitamin B12 food frequency questionnaire used in this study and associations of estimated dietary B12 intake with B12 biomarkers.

# Chapter 6: Development and Validation of a Vitamin B12 Food Frequency Questionnaire

A simple, nutrient-specific food frequency questionnaire (FFQ) was developed to assess dietary vitamin B12 (B12) intake at each of the measurement points in the study because any change in dietary B12 intake over the six-month study period could potentially influence the results of the randomized controlled trial (RCT) treatments. This chapter presents the process of FFQ development and how data were collected and analysed. The process of participant recruitment, selection and classification by dietary preference group is described in the RCT methods discussion in Chapter 5.

Reported dietary B12 intake and baseline B12 biomarkers (serum B12 and holotranscobalamin [holoTC]) are compared, and the strength, direction and significance of associations presented using correlation coefficients. The estimated dietary B12 intake is reported by dietary practice with comparisons made between meat and non meat-eating dietary practices and baseline B12 biomarkers.

**Dietary instrument to measure dietary B12 intake**

The purpose for developing a questionnaire to measure dietary B12 intake in the VitB12 study was to (1) identify associations between dietary B12 intake and B12 deficiency and (2) to measure changes in B12 intake over the six-month study period that could influence outcomes in the RCT.

This information was important to support answering one of the research questions in the VitB12 study.

For South Asian women of childbearing age:

1. What are the factors that influence dietary intake of vitamin B12?

There are many different methods for measuring nutrient intake over time such as multiple-day food diaries (closed and open ended records), 24-hour recall of food eaten, or FFQs. The FFQ was selected as the dietary instrument of choice in the VitB12 study because it provides the ability to rank individuals within a group and therefore link dietary intake with risk for disease (Gibson, 2005). Furthermore, FFQs are recognized as providing a moderately valid representation of nutrient intake over time, especially when estimating intake of specific nutrients, rather than quantifying total or absolute nutrient intake (Bingham et al., 1994; Cade, Thompson, Burley, & Warm, 2002; Kipnis et al., 2003; Willett et al., 1985). Other dietary instruments such as seven day diet diaries (7DDD) or open ended food dairies are a better representation when detailed quantification of the total nutrient intake for an individual is required, and caloric intake is used to adjust for quantities of nutrients (Bingham et al., 1994; Cade et al., 2002). In comparison to these methods, the FFQ collects less detail about food brands, methods for food preparation and exact quantities of foods (Subar et al., 2001). However, the more detailed food dairies are time consuming to complete and because occasional consumption of foods exceptionally high in B12, such as liver, can make a significant difference to average dietary B12 intake over time, it was necessary to capture habitual and episodic dietary B12 intake over a prolonged period such as three months (Cade et al., 2002). A FFQ is able to capture that and has the added advantage of being relatively easy to administer with a low respondent burden because it is timely to complete (Willett et al., 1988).

The optimum nutrient recall period for a FFQ varies and depends on the biological half life of the nutrient; recall periods in published studies varied from seven days (Braakhuis, Hopkins, Lowe, & Rush, 2011), one month (Subar et al., 2001), six months (Munger, Folsom, Kushi, Kaye, & Sellers, 1992), one year (Bingham et al.,

1994; Patterson et al., 1999) and four years (Willett et al., 1988). For the VitB12 study, a three-month recall was selected as the period that balanced considerations like capturing occasionally consumed B12 rich foods and seasonal changes in foods, while limiting recall bias. No dietary instrument can measure nutrient intake without error (Cade et al., 2002). Accurate recording relies on the participant's memory, their calculation of serving size and frequency of food consumption; perception or recall errors affect all of these. The dietary instruments themselves may omit important sources of the nutrient intake and essential storage or cooking methods that affect the quantity of nutrient in a given food (Cade et al., 2002; Kipnis et al., 2001; Kipnis et al., 2003; Rush, Plank, Laulu, Mitchelson, & Coward, 2004b; West & Van Staveren, 1997; Willett et al., 1985). Bioavailability of nutrients such as the B12 content in foods, varies across different foods and is affected by factors such as gastrointestinal transit times, gastric acidity, concurrently consumed foods, and in the case of B12, whether it is naturally contained in the food or added as fortification (Tucker et al., 2000; Vogiatzoglou et al., 2009; Watanabe, 2007). Errors may occur when inappropriate foods are substituted for foods from the data base to derive nutrient values, or the nutrient or food composition data bases from which nutrient values are derived are subject to error (Cade et al., 2002). For example, the 1997 New Zealand National Nutrition Survey (NNS97) included cereals as a source of B12 because the food composition tables from which the nutrient values for the NNS97 were derived, included B12 fortified cereal. However, cereals in New Zealand are not fortified with B12, and were not therefore, an active B12 food source. The conclusion of the 1997 NNS97 was that dietary B12 intake appeared to be satisfactory in the New Zealand adult population (D. G. Russell, Parnell, & Wilson, 1999). Xin (2008) from AUT University reanalyzed the nutrient database used for the 1999 NNS97 database, with cereals excluded as a source of B12. On

reanalysis, the total percentage of adults who did not meet the 2005 estimated average requirement (EAR)of 2.0 µg per day for B12 (Ministry of Health, 2005), increased from 12.5% to 27%, highlighting errors that can occur if food composition database information is not accurate (Xin, 2008).

The methods for testing validity and reliability of a dietary instrument need careful consideration in order to improve the accuracy of the instrument for measuring nutrient intake. Appropriate methods for determining validity (the ability of a dietary instrument to accurately measure nutrient intake) take into account factors that influence nutrient intake and bioavailability, the accuracy of food database listings for nutrients, and any biomarker measurements that reflect nutrient intake and absorption (Cade et al., 2002). Appropriate processes for determining reliability (the consistency of the dietary instrument for measuring nutrient intake) consider factors that influence reproducibility of the dietary instrument results such as changes in nutrient intake over time or over familiarity with the dietary instrument so that the food intake recorded on previous completions is reproduced subsequent completions (Cade et al., 2002).

Concurrent validity of a dietary instrument can be determined if there are known biomarkers of a selected nutrient in the blood or urine (Bingham et al., 1997; Braakhuis et al., 2011; Cade, Burley, Warm, Thompson, & Margetts, 2004; Rush et al., 2004b; Willett et al., 2001). Although this method provides the best assurances about the accuracy of data collected from instruments such as FFQs, factors such as malabsorption of the nutrient, length of time a nutrient can be stored in the body, and sensitivity or specificity of the biomarker for measuring that particular nutrient, may reduce valid associations (Kipnis et al., 2002; Kipnis et al., 2003; Rush et al., 2004b; Willett et al., 2001). Convergent validity is another method where dietary intake of a selected nutrient is measured using different types of dietary instruments and results

compared for associations. Detailed open ended 24-hour and seven-day dairies are frequently used as comparison methods, for which participants weigh foods and record exact amounts of all foods and beverages consumed within the specified time (Cade et al., 2002; Rimm et al., 1992; Subar et al., 2001; Willett et al., 2001). This method brings its own limitations because there is an assumption that the FFQ and the alternate dietary instrument are measuring the same nutrients when sometimes they are not. Comprehension issues with different dietary instruments can influence nutrient results and there is a risk of augmenting errors if the measures in the two dietary instruments are interrelated (Cade et al., 2002; Sevak et al., 2004).

The comparisons used to measure associations for the FFQ developed for the VitB12 study, the B12FFQ, were correlations between dietary B12 intake and B12 biomarker concentrations (serum B12 and holoTC). The few studies that specifically measure the relationship between dietary B12 intake and B12 biomarkers were used to derive construct validity of serum B12 and HoloTC as biomarkers against which to compare dietary B12 intake. The Hordaland Homocysteine Study (HHS) in Norway, compared the relationships between B12 biomarkers and dietary B12 intake, with the intake compared as total intake from all B12 containing foods, and then the dietary B12 intake from different food groups (Vogiatzoglou et al., 2009). The study of 5937 subjects (both genders) from the 47- 49 year and 71-74 year age groups, found a small but significant correlation between serum B12 concentrations and total dietary B12 intake ($r_s$ =0.11), with a breakdown of the relationship by food group showing as more significant for milk and fish and less significant for meat, cheese or eggs. Only 4% of people in this study did not meet the RDI of 2.4µg per day (Vogiatzoglou et al., 2009). The HHS supports findings from the Framingham Offspring Study of 2999 participants aged 26 to 83 years, where significant relationships were also identified between dietary

B12 intake from dairy foods and B12 biomarkers (serum B12 and holoTC), but not for B12 biomarkers and dietary B12 intake from meat (Tucker et al., 2000). Both of these studies included older adults, so the increased likelihood of age- related malabsorption of dietary B12 may have been a confounder in the relationship between dietary B12 intake and B12 biomarkers.

Two other studies support the findings of significant relationships between reported dietary B12 intake via dietary instrument and B12 biomarkers. A Danish study of 98 women aged 41 to 75 years, identified significant relationships between total daily B12 intake as determined from seven day food diaries and either serum B12 ($r = 0.4$, $p < 0.001$) and holoTC ($r = 0.3$, $p < 0.02$) (Bor, Lydeking-Olsen, Moller, & Nexo, 2006). Only four women in this Danish study reported a dietary B12 intake of less than the recommended 2.4 µg per day (Bor et al., 2006). A Florida study of 299 men and women aged 18 to 50 years also reported significant correlations between a diet history questionnaire and serum B12 ($r = 0.23$, $p < 0.0010$) or holoTC ($r = 0.23$, $p < 0.0010$) (Bor et al., 2010). No one biomarker can attain absolute specificity and sensitivity for measuring B12 status and absorption, however in the studies cited above, serum B12 and holoTC are supported as a valid representation of B12 absorption from supplements and therefore an indirect reflection of dietary B12 intake.

Test–retest is the most common method used to determine reliability of results from a dietary instrument and usually involves repeating the dietary instrument test at discrete time intervals and comparing the responses (Cade et al., 2004). This can be problematic because second and subsequent completions of the same instrument are influenced by memory of first completion if the time between applications of the FFQ is too short (testing threat), and on the other hand, dietary intake may change over time (Cade et al., 2004).

In the VitB12 study, reliability of the FFQ was not measured, even though the FFQ was administered at both two and six months into the study. This was because the subsequent RCT treatments to increase B12 biomarkers were likely to change dietary B12 intake over time. The lack of established reliability for the B12FFQ is acknowledged as potential source of bias in the retesting of the dietary instrument at two and six months in the RCT. Results for subsequent measures of dietary B12 intake using the FFQ are reported with the RCT findings in Chapter 7.

**B12 food frequency questionnaire**

The B12FFQ instrument used in the VitB12 study comprised 30 questions on consumption of food and fluids containing B12 and took 10 to 15 minutes for a researcher to administer. Foods containing B12 that were commonly consumed by South Asian women were identified in focus group discussion that preceded development and testing of the food frequency questionnaire. The quantity of B12 contained in these foods was calculated from the New Zealand Institute for Plant & Food Research Food Composition Tables (Lesperance, 2009), and only foods and fluids containing > 0.1µg B12 per minimum serving size were included in the B12FFQ. Women were requested to recall foods eaten in the previous three months in order to capture both habitual and infrequent consumption of B12-containing foods; questions were asked about the listed food and fluid items, with the quantities estimated using standard household measures such as cup, plate, segment of a plate, teaspoon and tablespoon. In order to reduce size perception errors in reporting, participants were shown examples of the measures. The questionnaire recorded nine different frequencies of consumption of a particular food ranging from ‘never’, ‘one time per month or less’ through to ‘six or more times per day (Appendix 11). The format of the questionnaire and frequency of consumption options (but not the foods), were based on a validated

FFQ used to determine antioxidant intake in athletes (Braakhuis et al., 2011). Any specific methods of food preparation were noted in the B12FFQ (particularly for eggs and milk), although quantification of the effect of food preparation on B12 bioavailability was not possible, therefore cooking methods were not factored into the calculation of dietary B12 intake (Watanabe, 2007). The researcher administered the FFQ so that detail could be clarified if required, for example, detailed questioning on brands to determine intake of natural B12 containing and B12 fortified foods. Recipes and dishes consumed are heterogeneous across South Asian cultures (Jayanthi, 2001; Sevak et al., 2004), so developing a FFQ based on commonly consumed recipes was difficult; instead the FFQ listed separate B12 containing food items such as chicken or yoghurt or eggs, and participants were required to estimate quantities of these food items in the dishes that they consumed.

During development, the B12FFQ was tested by five volunteers to check for comprehension, the logic of food order, and ability to accurately record the quantities and frequency of foods and fluid consumed from the category options in the B12FFQ. The B12 content for the foods and quantities listed in the B12 FFQ were calculated using the software program FoodWorks® (Xyris Software, Australia, 2007) and then downloaded into an Excel ™ spreadsheet, which was also tested during the development phase. Frequency of food consumption was further calculated to provide a daily frequency and this was multiplied by the portion size to derive the sum of B12 that the food item contributed to the average daily B12 intake. The final version of the B12FFQ and the Excel ™ B12 spreadsheet generated from the FoodWorks® program were critiqued and approved by a Professor Elaine Rush, a Professor of Nutrition.

**Data analysis**

Dietary B12 intake, serum B12 and holoTC variables were all positively skewed so were log transformed (using the natural log [ln]) to a normal distribution curve prior to analysis. Data are reported as medians, with interquartile ranges (IR) reported as the measures of central tendency and spread across each of four dietary preference groups. The groups are lactovegetarian (consume milk and sometimes cheese, but no meat or fish), lactoovovegetarian (as for lactovegetarian but also eat eggs), white meat-eating (eat chicken and/or fish but no red meat), and red meat-eating (eat white and red meats). Unless otherwise stated, data analysis were undertaken using Predictive Analytic Software (PASW) Edition 18 (IBM Corporation). Associations for the baseline B12FFQ were evaluated using Pearson's product moment correlation coefficients on log-transformed variables; baseline dietary B12 intakes from the B12FFQ were correlated with baseline serum B12 and holoTC results to determine the strength and direction of associations between them. PASW 18 does not provide 95% confidence intervals on correlation coefficients, so the correlation and probability values from PASW 18 were used to derive 95% confidence intervals from a separate World Wide Web published algorithm (Hopkins, 2007). The effect size of associations are reported using Cohen's effect size classification of 0.2 as small, 0.5 as moderate and 0.8 as large (Field, 2009; Peat & Barton, 2005). Bonferroni adjustments were applied to the analyses with a probability value of 0.025 or less accepted as significant in order to reduce the familywise error risk associated with separate correlation analyses undertaken on two interrelated B12 biomarkers (Field, 2009).

To calculate the likelihood of B12 deficiency or insufficiency from dietary (meat or non meat-eating) practices, binary variables of insufficient / sufficient serum B12 (0 < 222 pmol/L, 1 ≥ 222 pmol/L) and holoTC (0 < 45pmol/L, 1 ≥ 45pmol/L) were

computed. The relative risks of B12 deficiency from non meat or meat-eating practices (binary variables 0 and 1 respectively) were calculated in an Excel $^{TM}$ spreadsheet using the Relative Risk formula (RR) = (a /(a+b)) / (c / (c+d)) applied to a 2 x 2 odds ratio table (Table 8 and Table 9) (Bland & Altman, 2000; Estellat, Torgerson, & Ravaud, 2009; Peat & Barton, 2005). The 95 % confidence intervals were calculated and the odds ratio double-checked, using MedCalc for Windows, version 9.5.0.0 (MedCalc Software, Mariakerke, Belgium). The meat eating dietary practice group includes women who ate white meat only as well as those who ate red meat.

The application of the B12FFQ as a screening tool for B12 deficiency was evaluated by calculating the sensitivity and specificity of the B12FFQ. Sensitivity was derived from the number of true positives (low dietary B12 intake and low B12 biomarkers), while specificity was calculated from the number of true negatives (adequate dietary B12 intake and B12 sufficient on biomarkers) (Altman & Bland, 1994; Cade et al., 2002).

**Relationships between dietary intake of B12 and B12 biomarkers**

Women who ate red meat were the only dietary preference group to achieve the recommended daily B12 intake of 2.4 µg per day (Ministry of Health, 2003). Forty percent of women in the study reported a dietary B12 intake of less than 2.4 µg per day, with the median dietary B12 intakes for the lactoovovegetarian, lactovegetarian and white meat eating groups all below this. Those who ate white, but not red meat, reported a similar dietary B12 intake to women with non-meat-eating dietary preferences (Table 10). The women who ate both red and white meat were the only dietary preference group with a sufficient median serum B12 and holoTC concentrations. The groups with lactovegetarian, lactoovovegetarian and white meat-eating only dietary practices all had median serum B12 or holoTC concentrations that were either insufficient or deficient

(Table 11 and Table 12). The median serum B12 for the combined non-meat-eating group was 199 pmol/L ($25^{th}$/ $75^{th}$ percentiles [154, 263]), and for the combined meat-eating group 313pmol/L [209, 432].

The relative risk of low B12 biomarkers was compared across categories of non-meat and meat-eating dietary preferences. The risk of a low serum B12 was 2.2 times greater ($p$ = 0.02, 95% CI [1.2, 4.4]), and for a low holoTC 2.8 times greater ($p$ = 0.005, 95% CI [1.4, 5.9]) for women who did not eat meat (Table 8 and Table 9).

Table 8
*Insufficient/ Sufficient Serum B12 by Meat or Non Meat–Eating Practices 2 x 2 Table*

| Meat-eating Practice | Insufficient Serum B12[a] | Sufficient Serum B12[b] |
|---|---|---|
| Non Meat-Eating | 22 (63%) | 13 (37%) |
| Meat-Eating | 7 (28%) | 18 (72%) |

Note. Data reported as number of participants and percentage of each meat or non meat eating group
[a]Serum B12 < 222 pmol/L, [b]Serum B12 ≥ 222 pmol/L

Table 9
*Insufficient/ Sufficient HoloTC by Meat or Non Meat–Eating Practices 2 x 2 Table*

| Meat-eating Practice | Insufficient HoloTC [a] | Sufficient HoloTC [b] |
|---|---|---|
| Non Meat-Eating | 24 (69%) | 11 (31%) |
| Meat-Eating | 6 (24%) | 19 (76%) |

Note. Data reported as number of participants and percentage of each meat or non meat eating group
[a]HoloTC < 45 pmol/L, [b]HoloTC ≥ 45 pmol/L

Table 10
*Baseline Reported Dietary B12 Intake by Dietary Preference Groups*

| | n | [a] Median | [a] $25^{th}$ percentile | [a] $75^{th}$ percentile |
|---|---|---|---|---|
| Lactovegetarian | 26 | 1.8[b] | 1.0 [b] | 2.8 |
| Lactoovovegetarian | 7 | 1.6 [b] | 1.2 [b] | 2.8 |
| [c]All non-meat eating | 33 | 1.8 | 1.0 | 2.9 |
| White meat eating only | 5 | 1.6 [b] | 1.0 [b] | 2.7 |
| White and red meat eating | 22 | 5.5 | 3.3 | 6.8 |
| [d]All meat-eating | 27 | 4.7 | 2.7 | 6.8 |
| All participants | 60 | 2.7 | 1.2[b] | 5.0 |

[a]Dietary B12intake measured in µg/day. Tukey Hinges interquartile ranges
[b]Less than the RDI of 2.4 µg/day. [c] Includes women with lactovegetarian and lactoovovegetarian dietary preferences. [d]Includes women who eat white meat only or both red and white meat.

Table 11
*Baseline Serum Vitamin B12 Concentrations by Dietary Preference Groups*

| | n | Median[a] | 25th percentile[a] | 75th percentile[a] |
|---|---|---|---|---|
| Lactovegetarian | 26 | 202[c] | 149[b] | 265 |
| Lactoovovegetarian | 7 | 165[c] | 145[b] | 276 |
| [d]All non-meat-eating | 33 | 199 | 154 | 263 |
| White meat eating only | 5 | 216[c] | 148[b] | 236 |
| White and red meat eating | 22 | 375 | 230 | 456 |
| [e]All meat eating | 27 | 313 | 209 | 432 |
| All participants | 60 | 229 | 164[c] | 365 |

[a]Serum B12 measured in pmol/L. Tukey Hinges interquartile ranges
[b]Serum B12 deficiency (<150 pmol/L). [c] Serum B12 insufficiency ( 150 – 222 pmol/L). [d] Includes women with lactovegetarian and lactoovovegetarian dietary preferences. [e] Includes women who eat white meat only or both red and white meat.

Table 12
*Baseline HoloTC Concentrations by Dietary Preference Groups*

| | n | Median[a] | 25th percentile[a] | 75th percentile[a] |
|---|---|---|---|---|
| Lactovegetarian | 26 | 33[b] | 25[b] | 60 |
| Lactoovovegetarian | 7 | 30[b] | 28[b] | 49 |
| [d]All non-meat-eating | 33 | 30 | 25 | 51 |
| White meat eating only | 5 | 28[b] | 19[b] | 44[c] |
| White and red meat eating | 22 | 70 | 55 | 94 |
| [e]All meat eating | 27 | 64 | 45 | 88 |
| All participants | 60 | 44[c] | 29[b] | 70 |

[a]HoloTC measured in pmol/L. Tukey Hinges interquartile ranges
[b]HoloTC deficiency (<35 pmol/L). [c] HoloTC insufficiency (35 – 44 pmol/L). [d] Includes women with lactovegetarian and lactoovovegetarian dietary preferences. [e] Includes women who eat white meat only or both red and white meat.

The B12FFQ was a valid measure of dietary B12 intake, supported by moderate positive associations with (ln) serum B12 ($r$=0.50, $p$ <0.001, 95 % CI [0.28, 0.67]) and (ln) holoTC ($r$=0.55, $p$ <0.001, 95 % CI [0.34, 0.71]). Analysis of correlations were repeated using Spearman's correlation on the non-log transformed values, and similar results were obtained for serum B12 ($r^s$=0.54, $p$ <0.001, 95 % CI [0.33, 0.69]) and holoTC ($r^s$=0.57, $p$ <0.001, 95 % CI [0.37, 0.72]).

The relationships between the B12 biomarkers and dietary B12 intake, differentiated by meat/non meat-eating dietary preferences are shown in Figure 3 and Figure 4. Note the cluster of participants with non-meat-eating dietary preferences at the intersection of low serum B12 results and low B12 dietary intake (Figure 3). A similar

pattern occurred for the holoTC/dietary B12 intake relationship (Figure 4). Using the dietary B12 intake cut off point of < 2.4 µg, the B12 FFQ demonstrated a high degree of sensitivity and specificity for determining risk of B12 deficiency or insufficiency (Altman & Bland, 1994). Sensitivity for serum B12 deficiency (cut off point of B12 < 150 pmol/L) was 100% with nine out of nine true positives identified, but only 45% for serum B12 insufficiency (cut off point of B12 < 222 pmol/L) with nine out of twenty true positives identified. This gives an overall sensitivity of 62% (18 out of 29 cases) for detecting low or borderline low serum B12. Specificity was 74% with 23 out of 31 true negatives identified for low or borderline low serum B12 (Figure 3). The B12FFQ was sensitive for identifying holoTC deficiency (< 35 pmol/L) with 64% of true positives identified (25 out of 30 cases) and 100% (five out of five cases) of true positives identified for holoTC insufficiency (< 45 pmol/L). This gives an overall sensitivity for low/borderline low holoTC of 71% (21 out of 30 cases). Specificity of the B12 FFQ for detecting low/borderline or low holoTC was 77% with 23 out of 30 true negatives identified (Figure 4).

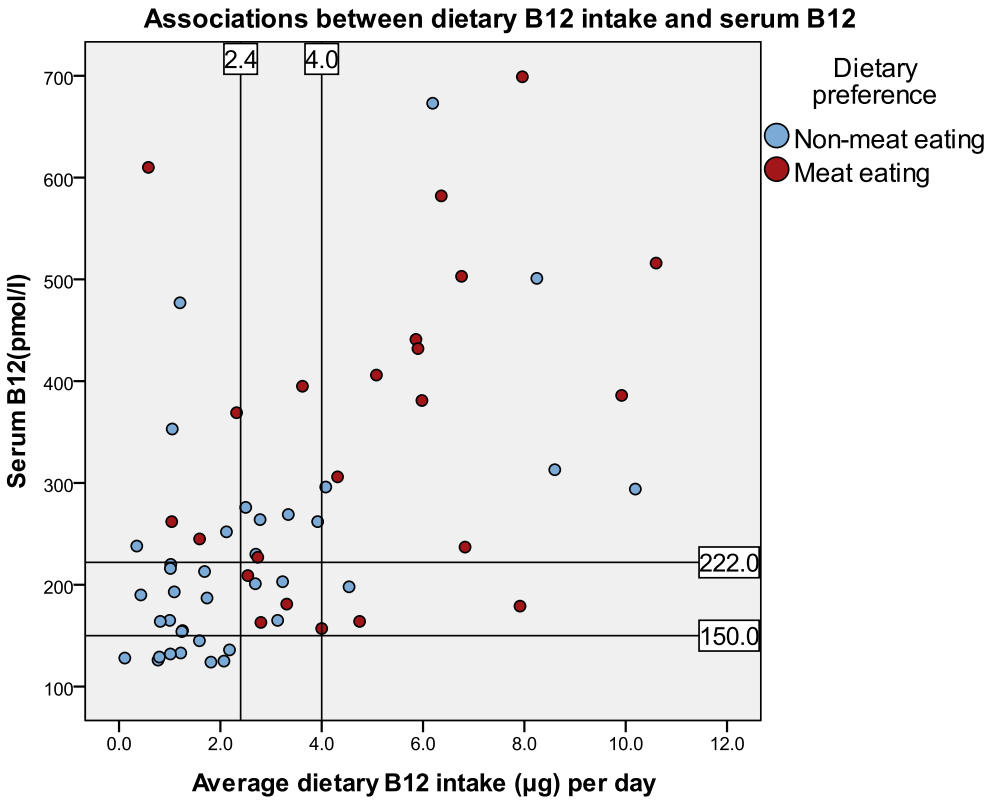



*Figure 3*. Associations between dietary B12 intake from B12FFQ and serum B12

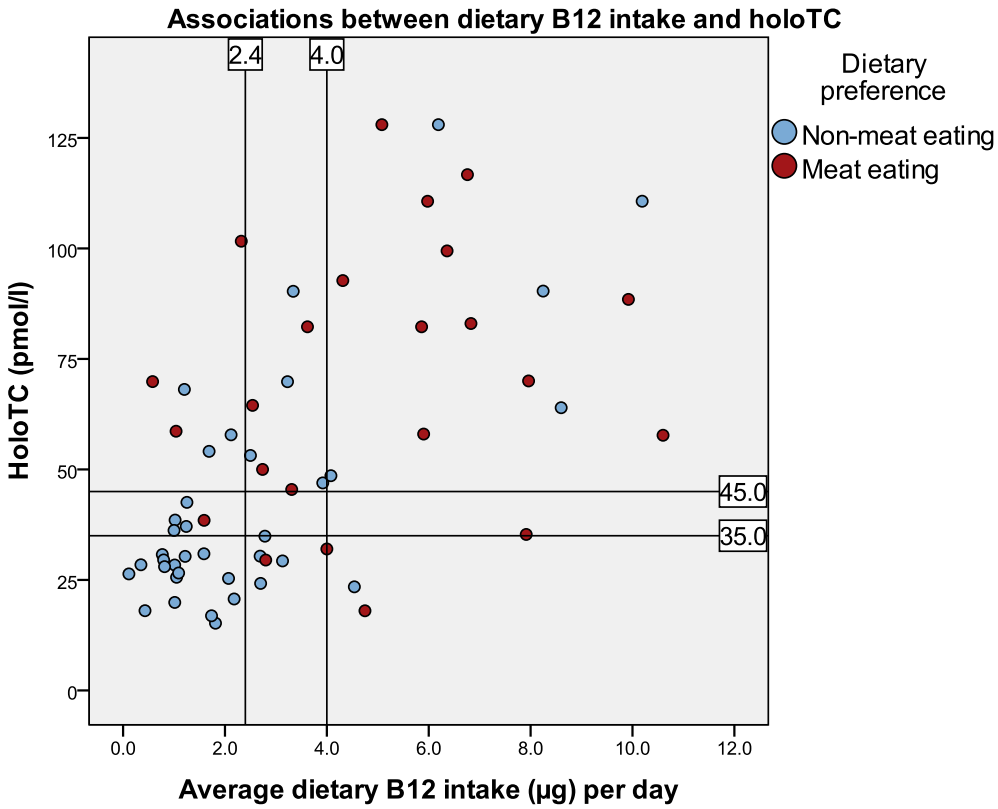



*Figure 4*. Associations between dietary B12 intake from B12FFQ and holoTC

**Chapter Discussion and Conclusion**

A 30-item, researcher administered, B12 nutrient specific FFQ, developed to measure dietary B12 intake, is a tool that may have concurrent and predictive validity to identify individuals with dietary patterns associated with inadequate intakes of vitamin B12. The B12 FFQ demonstrated greater than 62% sensitivity and specificity for detecting B12 biomarker deficiency or insufficiency.

Non-meat eating was the major risk factor for insufficient dietary B12 intake and for low B12 biomarker concentrations. Compared with women who ate meat, those with non- meat eating dietary preferences were 2.2 times more likely to be low in serum B12 and 2.8 times more likely to be low in holoTC.

While the purpose for developing and administering the B12FFQ was to identify relationships between dietary B12 intake over the subsequent six-month RCT that could influence the RCT treatment results, evidence is provided that the B12FFQ could be used as a rapid screening tool in clinical practice. Administration of the B12 FFQ could help identify those at risk of vitamin B12 deficiency or insufficiency and in need of dietary advice or supplementation.

Reproducibility of the B12FFQ was not tested and is acknowledged as potential source of bias as the test-retest reliability of the B12FFQ was not established as per expert recommended guidelines for the development of a FFQ (Cade et al., 2002). Further work is required to measure the test-retest reliability of the B12 FFQ. The reliability and the concurrent and convergent validity of the B12 FFQ, also needs testing in other populations, across ethnicities' and ages and with different dietary practices.

Effective screening and action for B12 deficiency requires health professionals such as midwives, nurses, nutritionists, dieticians and doctors to firstly be aware of the risk for B12 deficiency or insufficiency, secondly to screen, and thirdly to take action as

required. A simply administered tool to screen for risk is one-step in the prevention of B12 deficiency or insufficiency (Cade et al., 2002). Results for the two and six month measurements of dietary B12 intake using the B12 FFQ are reported with the RCT findings in Chapter 7.

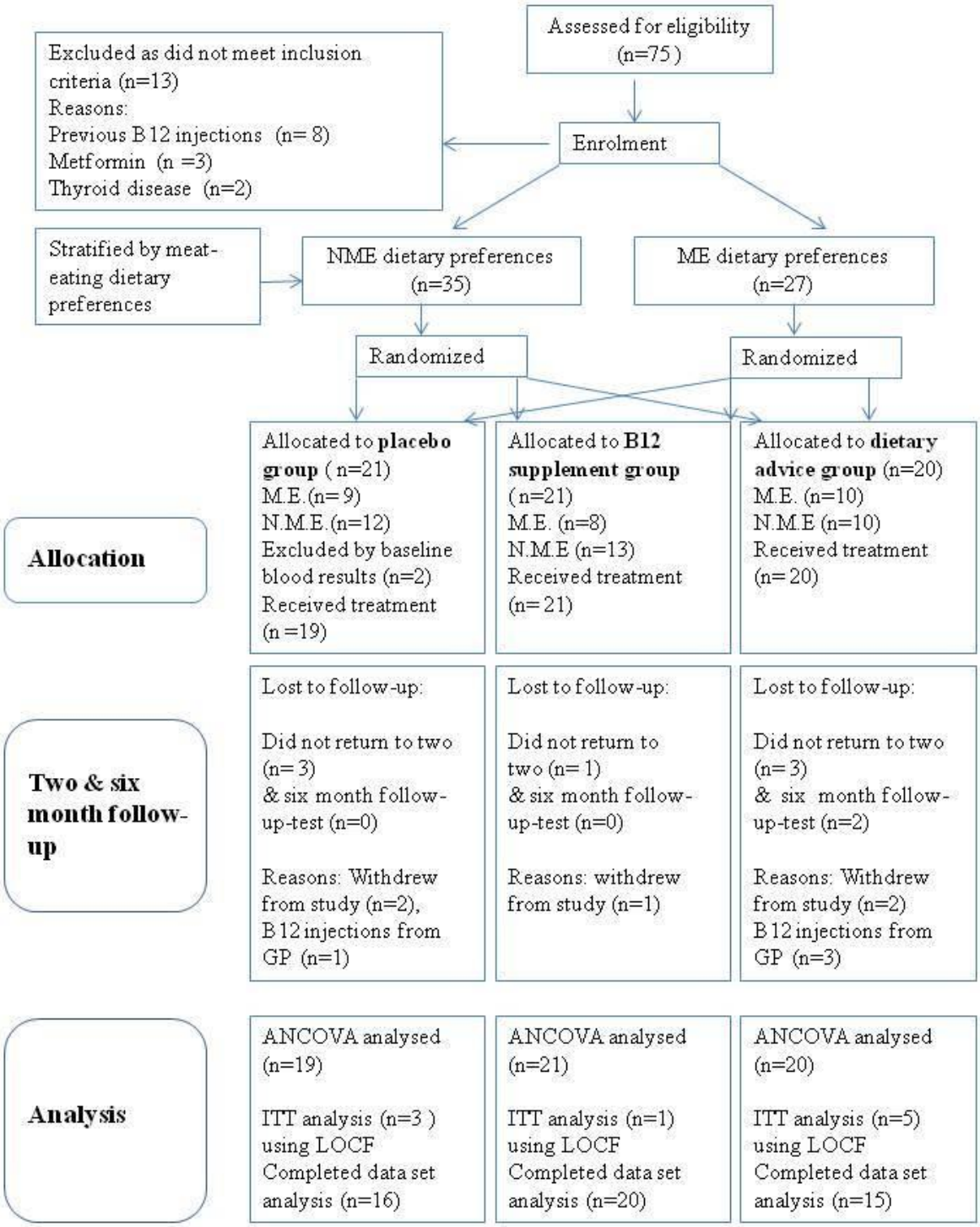
# Chapter 7: Findings from the Trial of Vitamin B12 supplementation and dietary advice on Vitamin B12 status in South Asian Women of Childbearing Age

Chapter 7 presents key findings from the randomised controlled trial (RCT) to investigate increasing vitamin B12 (B12) stores in women of South Asian origin, aged 18 to 50 years. Three treatments were trialled; (a) daily 6µg B12 dietary supplement, (b) daily placebo, or (c) advice to increase dietary B12 intake. Primary outcomes were B12 biomarker concentrations (serum B12 and serum holotranscobalamin [holoTC] holoTC concentrations) and the prevalence of B12 deficiency and insufficiency measured at two and six months. Secondary outcomes included relationships among B12 biomarker status, with concurrent measures of insulin resistance, haematology and anthropometry characteristics. Consolidated Standards of Reporting Trials (CONSORT) are used to guide the presentation of findings and the discussion of participant recruitment and flow through the trial. The use of a non-pharmacological research treatment (dietary B12 advice) alongside pharmacological treatments (B12 supplement and placebo), means that trial reporting incorporates elements of both the pharmacological and the modified non-pharmacological CONSORT reporting guidelines (American Psychological Association, 2010; Boutron, Moher, Altman, Schulz, & Ravaud, 2008a, 2008b; Schulz, Altman, & Moher). The statistical analysis methods were presented in Chapter 5.

**Recruitment and Participant Flow**

Between March 2009 and March 2010, 75 women accepted the invitation to participate in this vitamin B12 (VitB12) RCT and 62 met the initial inclusion criteria. Eight out of thirteen exclusions were based on receipt of high dose B12 supplement injections within two years of the trial, another three were for metformin prescription,

and two for a history of thyroid disease (Figure 5). Following baseline testing, two more women were excluded: one for an abnormally elevated serum B12 result (> 1480 pmol/L, presumably related to a B12 injection received two to three years prior), and the second woman for a fasting serum glucose concentration greater than 6.1 mmol/L. The remaining 60 women (75% of those who originally volunteered to take part) were categorised to either a meat-eating or a non-meat-eating dietary preference, and then randomly allocated from each category into one of the three treatment groups. At trial completion, the attrition rate for the supplement group was 5%, the placebo group 16%, and the dietary B12 advice group 25%, giving an overall attrition rate of 15%. With intention to treat applied, the analysis included 21 participants in the B12 supplement group, 19 in the placebo, and 20 in the dietary B12 advice group (Figure 1).




*Notes:* Flow diagram based on Consolidated Standards of Reporting Trials (CONSORT) guidelines.NME= Non- meat-eating dietary preference; ME= meat-eating dietary preference; GP = General practitioner; ANCOVA = Analysis of covariance; ITT=Intention to treat analysis; LOCF=last observation carried forward.

*Figure 5*. Flow of participants through the VitB12 randomised controlled trial

**Description of Study Population at Baseline**

There were no major differences in immigration history between RCT treatment groups. Most typically, participants had lived in New Zealand for six to ten years

(Figure 6). All of the women had at least 2 generations of family with South Asian origins and the women that participated in the study had origins from India, Fiji, South Africa or Sri Lanka (54 were of Asian Indian origin, four of Fijian Indian origin, one of South African Indian origin and one of Sri Lankan origin).

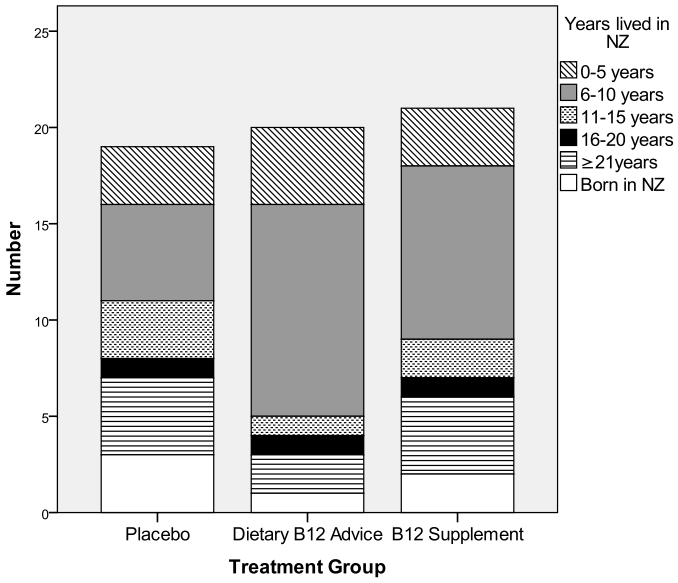



*Figure 6.* Immigration history (years lived in New Zealand) by treatment group

**Religion and dietary preference.**

The majority of participants were Hindi (n = 49) then Christian (including Catholic) (n = 8) religion. Religion was not specified for three of the participants. Among the Hindi women dietary patterns varied; 41% (n = 24) were lactovegetarian, 14 % (n = 7) lactoovovegetarian, 27% (n = 13) ate red and white meat and 10% (n = 5) ate white meat only. Participants who described their religion as Christian ate both red and white meat. Of the three participants who did not report religion, two were lactovegetarian and one ate both red and white meat.

**Dietary preference by group allocation.**

Vegetarian dietary practices were common in the study, with 56% (n = 33 out of 60) of participants reporting either lacto-vegetarian or lacto-ovovegetarian dietary

preference; 36% (n = 22) consumed both red and white meat and 8% (n = 5) women reported eating white meat only. Treatment group differences existed for dietary preference (Figure 7). The ratio of meat-eating dietary preferences to non-meat-eating dietary preferences was 1.1: 1 in the Placebo Group and 1.0: 1 in the Dietary B12 Advice Group but the B12 Supplement Group had more non-meat-eating participants than meat-eating (ratio 1.7: 1).

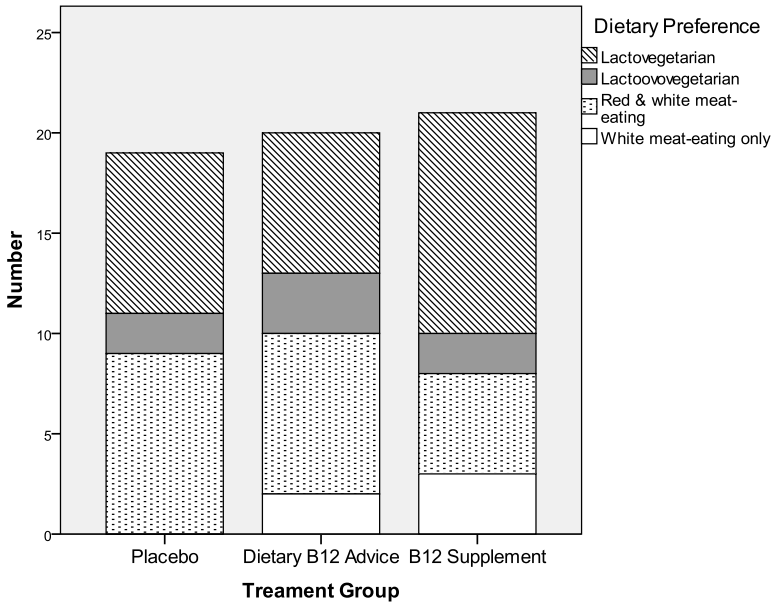



*Figure 7.* Dietary preference of participants by treatment group

**Age and physical characteristics of trial participants.**

The study sample was relatively homogenous in age and physical composition characteristics. At baseline, there were no statistically significant differences among treatment groups (Table 13).

Table 13
*Baseline Age and Physical Characteristics by Treatment Group*

| | B12 supplement (n=21) | Placebo (n=20) | Dietary B12 advice (n=19) | ANOVA [a] | | 95 % CI[b] |
|---|---|---|---|---|---|---|
| | M(SD) [R] | M(SD) [R] | M(SD) [R] | F(*df*) | *p* | |
| Age | 39 (7.3) [25, 50] | 34(9.7) [21, 49] | 37 (9.7) [18, 50] | 1.77 *(2, 57)* | 0.18 | [-0.8, 4.4] |
| Diastolic BP | 76(10) [62, 97] | 77(8) [63, 92] | 77(10) [60, 103] | 0.06 *(2, 57)* | 0.94 | [-1.6, 1.7] |
| Systolic BP | 119(15) [98, 152] | 115(11) [96, 145] | 117(15) [93,152] | 0.25 *(2, 57)* | 0.78 | [-1.5, 2.0] |
| Weight(kg) | 66.4 (11.1) [46.3, 87.0] | 63.7(13.5) [46.7, 103.4] | 62.9(10.6) [47.3,87.2] | 0.60 *(2, 57)* | 0.56 | [-1.4, 2.6] |
| Height(cm) | 159.3(4.6) [151.4,163.7] | 159.0(6.1) [143.6,171] | 159.5(5.0) [151.2, 168.5] | 0.00 *(2, 57)* | 0.99 | [-1.6, 1.6] |
| WHR | 0.75(0.06) [0.65, 0.84] | 0.77 (0.05) [0.69, 0.87] | 0.77(0.05) [0.68, 0.86] | 0.56 *(2, 57)* | 0.57 | [-1.4, 2.5] |
| WtHR | 0.49 (0.06) [0.39, 0.62] | 0.47 (0.07) [0.37, 0.62] | 0.49 (0.07) [0.39, 0.63] | 0.30 *(2, 57)* | 0.75 | [-1.6, 2.2] |
| BF % | 53 (4.2) [44.4, 59.4] | 51 (5.1) [43.0, 60.7] | 50(4.6) [41.5, 61.2] | 2.1 *(2, 57)* | 0.13 | [-0.7, 4.9] |
| BMI | 26.2 (4.1) [17.9, 34.7] | 24.6(4.5) [18.6, 36.8] | 25.3(4.8) [17.3, 36.4] | 0.61 *(2, 57)* | 0.55 | [-1.4, 2.6] |
| Grip strength R | 22.7(4.8) [12, 32] | 21.2(3.6) [14, 27] | 22.3(5.2) [12, 31] | 0.32 *(2, 57)* | 0.73 | [-0.8, 1.4] |
| Grip strength L | 19.8(4.9) [12, 31] | 20.8(4.7) [12, 32] | 21.5(4.8) [13, 29] | 0.65 *(2, 57)* | 0.53 | [-1.4, 2.7] |

*Note*. ANOVA with post hoc Tukey's honestly significant difference test
Data all normally distributed so presented as mean and standard deviation. M = mean, SD = standard deviation, R = range [upper limit, lower limit], , *F*= ANOVA, df= degrees of freedom, *p* = significance, BP = blood pressure in mmHg, WHR = waist to hip ratio, WtHR = waist to height ratio, BF% = body fat%, BMI = Body Mass Index (kg/m$^2$)
R = right hand, L= left hand
ANOVA of differences between groups[a], 95 % CI of ANOVA [b]

**Baseline laboratory studies.**

There was an unequal distribution of women with B12 biomarker deficiency across treatment groups. Baseline holoTC was lower for the B12 Supplement Group (median 30 pmol/L) than for the Placebo (47 pmol/L) and Dietary Advice Groups (58 pmol/L) (Table 14). Nonparametric analysis of variance (Kruskall-Wallis) identified this as a statistically significant difference ($H(2) = 6.9$, $p = 0.003$, 95% CI [1.5, 12.3]). Although the B12 Supplement Group baseline serum B12 was also lower (median 198 pmol/L) than for the Placebo (230 pmol/L) or Dietary Advice (267 pmol/L) groups, the difference was not statistically significant (Kruskall-Wallis; $H(2) = 3.8$, $p = 0.15$, 95 %

CI [-3.4, 11.0]) (  2). An alternative method of analysis using Generalized Linear Model (GLM) univariate ANOVA on (log) baseline serum B12 also confirmed insignificant B12 differences between treatment groups (F(2,57) = 2.1, p =  0.133, 95 % CI [-1.6, 5.8]). The remainder of baseline biochemistry biomarkers were not statistically significant among intervention groups (Table 14).

Table 14
*Baseline Biochemistry Results by Treatment Group*

| | B12 supplement (n=21) | Placebo (n=20) | Dietary B12 advice (n=19) | ANOVA [a] | | 95 % CI[b] |
|---|---|---|---|---|---|---|
| | M(SD) [R] | M(SD) [R] | M(SD) [R] | *F*(df) | *p* | [LL,UL] |
| Serum folate | 25(7.7) [11, 39] | 29(8.9) [15, 45] | 26(7.9) [12, 40] | 1.55(2, 57) | 0.22 | [-1.0, 4.1] |
| Glucose (mmol/L) | 4.9(0.42) [4.0, 5.5] | 4.7(0.48) [3.8, 5.6] | 4.6(0.50) [3.6, 5.8] | 1.95(2, 57) | 0.15 | [-0.7, 4.6] |
| LDL (mmol/L) | 2.7(0.61) [1.1, 4.1] | 2.3(0.73) [1.1, 3.6] | 2.5(0.76) [1.2, 4.1] | 2.00(2, 57) | 0.14 | [-0.7, 4.7] |
| HDL (mmol/L) | 1.4(0.35) [1.0, 2.4] | 1.5(0.33) [1.0, 2.2] | 1.5(0.44) [0.80, 2.6] | 0.52(2,57) | 0.60 | [-1.4, 2.5] |
| | Mdn ($25^{th}$, $75^{th}$) | Mdn ($25^{th}$, $75^{th}$) | Mdn ($25^{th}$, $75^{th}$) | *H* (df) | *p* | |
| HOMA %B | 87 (60,114) | 85 (75, 114) | 78 (59, 98) | 0.95(2) | 0.62 | |
| HOMA %S | 180 (76, 262) | 121 (48, 268) | 148 (92, 261) | 1.8(2) | 0.40 | |
| HOMA2 IR | 0.69 (0.33,1.42) | 0.38 (0.23, 1.79) | 0.46 (0.22 0.92) | 1.5(2) | 0.47 | |
| **HoloTC** | **30 (21, 58)** | **47 (31, 85)** | **58 (36,76)** | **6.9(2)** | **0.03*** | |
| **B12[d]** | **198 (165, 245)** | **230 (156, 396)** | **267 (192, 414)** | **3.8(2)** | **0.15** | |
| TC | 4.7 (4.2, 5.2) | 4.4 (3.5, 4.9) | 4.4 (3.9, 5.1) | 2.0(2) | 0.37 | |
| TG | 0.93 (0.77, 1.4) | 0.83 (0.66, 0.98) | 0.90 (0.62,1.08) | 2.6(2) | 0.28 | |
| C-HDL ratio | 3.3 (2.9, 4.1) | 2.9 (2.3, 3.6) | 3.1 (2.6, 3.8) | 3.8(2) | 0.15 | |

*Note*. ANOVA reported for normally distributed data
Kruskal-Wallis reported for non-normally distributed data
*F*= ANOVA, df= degrees of freedom ,*p* = significance; LL = lower limit; UL = upper limit; LDL = Low Density Lipoprotein (mmol/L); HDL = High Density Lipoprotein (mmol/L); HOMA %B= Estimate of Beta cell function calculated from fasting glucose and insulin; HOMA%S = Estimate of insulin sensitivity calculated from fasting glucose and insulin; HOMA IR =Estimation of insulin resistance based on ratio of HOMA%B to HOMA%S; holoTC = holotranscobalamin (pmol/L);TG = Triglycerides (mmol/L); C-HDL (mmol/L) = cholesterol: high density lipoprotein ratio
ANOVA of differences between groups[a], 95 % CI of ANOVA[b] .Kruskal Wallis for analysis of variance of non-normally distributed data[c] .Serum folate and B12 measured in pmol/L[d].
*$p < 0.05$. B12 supplement group baseline significantly lower than placebo or dietary advice groups.

In general, the women's haematological results were within the reference range (Table 15). No women met the criteria for macrocytic anaemia (haemoglobin [Hb] ≤ 120 g/L, mean corpuscular volume (MCV) ≥ 99fL). Microcytosis (MCV ≤ 80 f/L) was present in 17% (n = 10) of participants and follow up blood tests of iron stores revealed iron deficiency in 80% (eight out of ten) of these women. One of the remaining two

women with microcytosis, but normal Hb and iron stores, had a history of thallasemeia minor, which could account for the microcytosis, but the other participant's microcytosis was unexplained. Significant anaemia Hb ≤ 100g/L) was present in 5 % (n = 3) of the women, while borderline anaemia (Hb 100 to 120 g/L) was present in 8 % (n = 5) (Table 15). The mean corpuscular haemoglobin concentration (MCHC) was higher in the Dietary B12 Advice group than the B12 Supplement and Placebo Groups, but the MCHC for all groups were still within the normal reference range.

Table 15
*Baseline Haematology by RCT treatment group*

| | B12 supplement (n=21) | Placebo (n=20) | Dietary B12 advice (n=19) | ANOVA [a] | | 95 % CI[b] |
|---|---|---|---|---|---|---|
| | M(SD) [R] | M(SD) [R] | M(SD) [R] | F(*df*) | *p* | [LL,UL] |
| Hb | 128 (13.7) [93, 145] | 125(11.4) [99, 144] | 132 (7.05) [119, 142] | 2.56 | 0.09 | -0.9, 6.0 |
| RBC | 4.7(0.26) [4.3, 5.3] | 4.8(0.43) [4.2, 6.0] | 4.6(0.25) [4.2,5.0] | 0.27 | 0.76 | -3.1, 3.7 |
| PCV | 0.40 (0.03) [0.32, 0.43] | 0.39 (0.03) [0.33, 0.44] | 0.40 (0.03) [0.32, 0.45] | 0.66 | 0.52 | -3.0, 4.3 |
| MCHC | 27.2 (2.6) [22,30] | 26.3(3.2) [20, 32] | 28.5(1.6) [25, 31] | 3.57 | 0.04* | 0.6, 6.2 |
| MCV | 83.8(5.5) [74, 93] | 81.8(8.1) [64, 93] | 86.5(4.0) [74, 92] | 2.79 | 0.07 | -0.6, 6.2 |
| WBC | 6.6(1.7) [3.9, 11.6] | 7.0 (1.9) [3.6, 1.6] | 6.7(1.6) [4.2, 9.5] | 0.24 | 0.78 | -3.1, 3.6 |

*Note.* All data normally distributed. ANOVA with post hoc Tukey's honestly significant difference. LL = lower limit; UL = upper limit; Hb = Haemoglobin (g/L); RBC = Red blood cell (x10e 12/L), PCV = Packed cell volume (%) MCHC = Mean Corpuscular Haemoglobin Concentration (pg); MCV: Mean Corpuscular Volume (fL); WBC= White blood cell (x10e 9/L)
ANOVA of differences between groups[a]. 95 % CI of ANOVA [b].
*p < 0.5 Significant difference for dietary B12 advice group.

**B12 deficiency in sample population.**

B12 deficiency was common in the sample population at baseline. One out of two of the women were low or borderline low in B2 biomarkers (Figure 9).

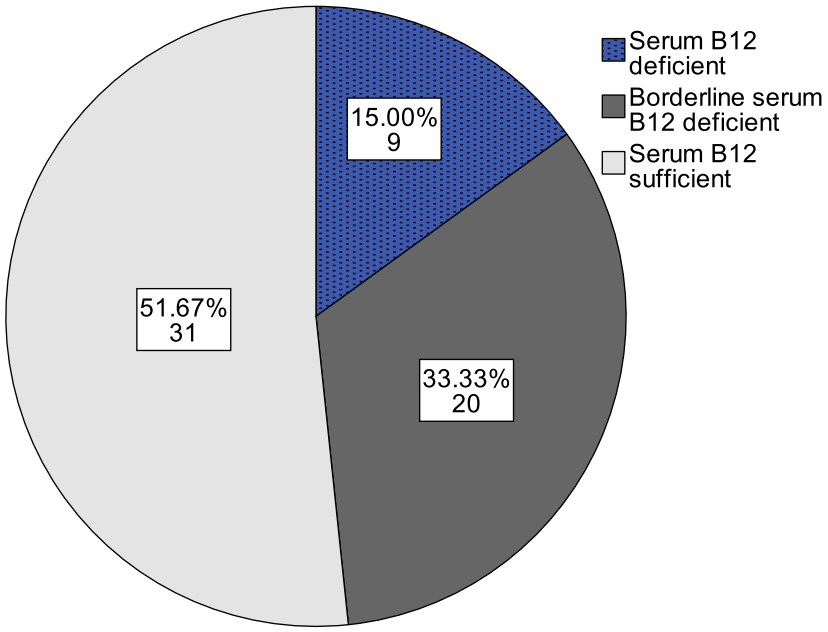



*Figure 8*. Baseline percentage of serum B12 deficiency, borderline deficiency and sufficiency

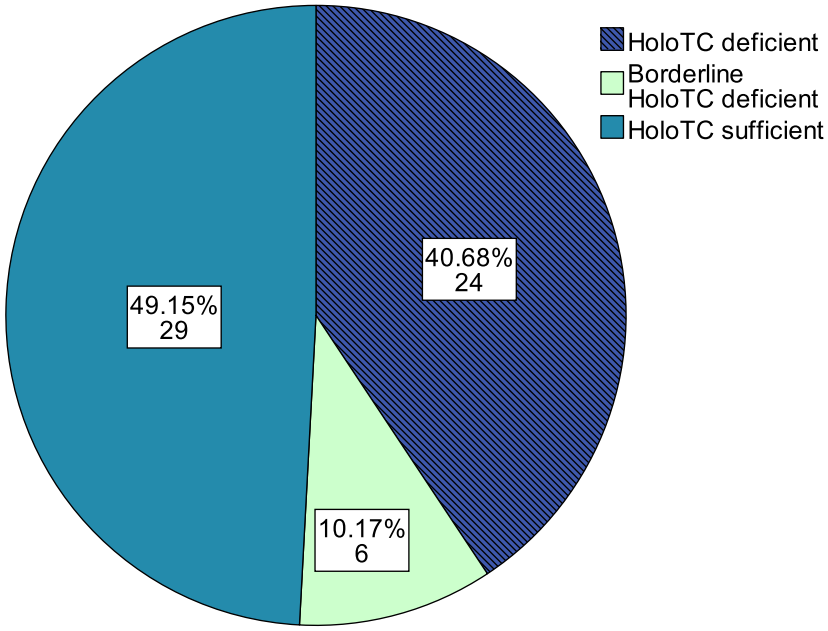



*Figure 9*. Baseline percentage of holoTC deficiency, borderline deficiency and sufficiency

**Response to RCT Treatments**

The following results report response to the RCT group treatments:

- The percentage of women in each RCT treatment group who were B12 biomarker (serum B12 and holoTC) deficient, insufficient and sufficient at baseline and six months.
- Analysis of variance results comparing the change scores between baseline, two and six month measurement of (logarithmically transformed [log]) B12 biomarkers and the strength of any associations among RCT treatment groups.
- Analyses of covariance (ANCOVA) results to determine the strength of associations among RCT group treatments while controlling for covariate influences.
- The percentage increase or decrease in the geometric mean of B12 biomarkers among RCT treatment groups from baseline to two months, and then from two to six months to measure effect size of the RCT treatments.
- Correlations (associations) between capsule adherence and the change in (log) B12 biomarkers over six months for the B12 supplement and placebo RCT treatment groups to determine the influence of capsule adherence.
- Correlations (associations) of the change in (log) dietary B12 intake, and the change in (log) B12 biomarkers over six months among treatment groups to determine the influence of dietary B12 intake.

**Change in percentage of women in each RCT treatment group with B12 deficiency**

For the B12 Supplement Group only, there was a reduction over six months, in the percentage of women who were B12 deficient or insufficient. The percentage of women in the B12 supplement group who were either serum B12 deficient or

insufficient decreased from 67% (baseline) to 24% (at six months) (Table 16). The improvement was less for holoTC; 71% were holoTC deficient or insufficient at baseline and 57% at study completion (Table 17). The improvement was most noticeable after the first two months of B12 supplementation, with moderate reductions only in the percentage of women with serum B12 insufficiency from two to six months, and a small increase in the percentage with holoTC deficiency from two to six months. In contrast to the B12 supplement group, for the Dietary Advice and Placebo groups, over six months, there was a small increase in the percentage of women with serum B12 deficiency or insufficiency. There was no change in the percentage of women with holoTC deficiency for the Placebo group, while the Dietary Advice group had a slight decrease in the percentage of women with holoTC deficiency.

Table 16
*Change in Percentage of B12 Deficiency (Serum B12) by Treatment Group at Baseline, Two and Six Months*

| Baseline measurement | B12 supplement group | | Placebo group | | Dietary B12 advice group | |
|---|---|---|---|---|---|---|
| | % | n | % | n | % | n |
| B12 deficient[b] | 14 | 3 | 21 | 4 | 10 | 2 |
| Borderline deficient [c] | 53 | 11 | 26 | 5 | 20 | 4 |
| B12 sufficient | 33 | 7 | 53 | 10 | 70 | 14 |

| Two month measurement | B12 supplement group | | Placebo group | | Dietary B12 advice group | |
|---|---|---|---|---|---|---|
| | % | n | % | n | % | n |
| B12 deficient[b] | 0 | 0 | 16 | 3 | 15 | 3 |
| Borderline deficient [c] | 38 | 8 | 31 | 6 | 25 | 5 |
| B12 sufficient | 62 | 13 | 53 | 10 | 60 | 12 |

| Six month measurement | B12 supplement group | | Placebo group | | Dietary B12 advice group | |
|---|---|---|---|---|---|---|
| | % | n | % | n | % | n |
| B12 deficient[b] | 0 | 0 | 26 | 5 | 15 | 3 |
| Borderline deficient [c] | 24 | 5 | 26 | 5 | 30 | 6 |
| B12 sufficient | 76 | 16 | 47 | 9 | 55 | 11 |

*Note.* Deficiency or borderline deficiency highlighted in grey.
[b]Serum B12 deficiency (<150 pmol/L). [c] Serum B12 insufficiency (borderline deficiency)( 150 – 222 pmol/L)

Table 17
*Change in Percentage of HoloTC Deficiency by RCT Group at Baseline, Two and Six Months*

| Baseline measurement | B12 supplement group | | Placebo group | | Dietary B12 advice group | |
|---|---|---|---|---|---|---|
| | % | n | % | n | % | n |
| HoloTC deficient[b] | 57 | 12 | 37 | 7 | 25 | 5 |
| Borderline deficient[c] | 14 | 3 | 10 | 2 | 5 | 1 |
| HoloTC sufficient | 29 | 6 | 53 | 10 | 70 | 14 |

| Two month measurement | B12 supplement group | | Placebo group | | Dietary B12 advice group | |
|---|---|---|---|---|---|---|
| | % | n | % | n | % | n |
| HoloTC deficient[b] | 34 | 7 | 42 | 8 | 25 | 5 |
| Borderline deficient[c] | 19 | 4 | 5.3 | 1 | 5 | 1 |
| HoloTC sufficient | 48 | 10 | 53 | 10 | 70 | 14 |

| Six month measurement | B12 supplement group | | Placebo group | | Dietary B12 advice group | |
|---|---|---|---|---|---|---|
| | % | n | % | n | % | n |
| HoloTC deficient[b] | 38 | 8 | 42 | 8 | 25 | 5 |
| Borderline deficient[c] | 19 | 4 | 5 | 1 | 0 | 0 |
| HoloTC sufficient | 43 | 9 | 53 | 10 | 75 | 15 |

*Note.* HoloTC deficiency or borderline deficiency highlighted in grey.
[b]HoloTC deficiency (<35 pmol/L). [c] HoloTC insufficiency (borderline deficiency)(35 – 44 pmol/L)

**ANOVA of the change in B12 biomarkers and strength of associations.**

Univariate ANOVA of the (log-transformed) change in B12 biomarkers over six months showed that group treatment predicted 21% (adjusted coefficient of determination [$R^2$] ) of the change in serum B12, $F(2,57) = 8.9$, p = 0.001, 95% CI [3.8, 14] (Table 18), and 18% of the change in holoTC, $F(2,57) = 7.4$, p = 0.001, 95% CI [3.1, 12] (Table 19). Post hoc tests using Tukey's Honestly Significant Difference (HSD) and a Bonferroni correction of *p* as significant at ≤ 0.025 found that B12 supplement was the only treatment group where the change (increase) in (log) B12 biomarkers was significant (serum B12, $p = 0.001$ to 0.005; holoTC, $p = 0.001$ to 0.018). There was no apparent change for the Dietary Advice or Placebo Groups (serum B12, $p > 0.99$ and holoTC, $p = 0.60$). Analysis with missing data excluded made no difference to this outcome.

**ANCOVA model, strength of associations, and controlling for covariates.**

Age, change in dietary B12 intake and capsule adherence were identified from correlation analyses as variables with an influence on change in (log) B12 biomarkers, so these were controlled for in an ANCOVA by entering them as covariates. There was a weak but significant association of age and B12 biomarkers. The strength of association between treatment and change in (log) B12 biomarkers was slightly stronger with age controlled for (25% of the change in serum B12 associated with group treatment, and only 21% when age was not controlled for) (Table 18).

Increases in (log) dietary B12 intake, and capsule adherence, were significantly associated with increases in (log) B12 biomarkers (changes significant for serum B12 only in the latter). After controlling for change in B12 dietary intake, 36% of the relationship between treatment and the change in serum B12 (Table 18) and 22% of the change in holoTC were explained at six months (Table 19). For the B12 Supplement and Placebo Groups only, the relationship between group treatment and serum B12 was stronger when capsule adherence was controlled for, with treatment accounting for 38% of the change in serum B12 and 22% of the change in holoTC at six months (Table 19).

Planned simple contrasts for each of the ANCOVA analyses using the B12 supplement as the reference group identified that B12 supplement was the group treatment associated with the significant changes (increases) in both serum B12 and holoTC over the study duration. Placebo and B12 dietary advice treatments were not significantly associated with change in either of the B12 biomarkers. The results reported above are for the intention to treat (ITT) analysis, however analyses with (ITT) and without missing values (MV) show similar results with an unequivocal and strong association between B12supplement treatment and a significant increase in B12 biomarkers over six months (Table 18 and Table 19). There was no significant

association between change and B12 biomarkers for the placebo and dietary advice groups between either the ITT or the MV analysis.

Table 18
*ANOVA and ANCOVA of Change in Serum B12 over 6 Months using Intention to Treat and Missing Values Datasets*

| | ITT data set | MV data set |
|---|---|---|
| [a] Serum B12, | 8.9(*2, 57*) | 8.1(*df2, 49*) |
| *p*[95% CI][b], $R^2$ | <0.001** [3.8, 14]), 0.21 | 0.001** [3.5, 12.7), 0.25 |
| [c] Serum B12 with age as CV, | 7.2 *(2, 57)* | 6.4 (*2,49*) |
| *p*[95% CI] [d], $R^2$ | 0.002* [2.8, 11.5]), 0.25 | 0.003* [2.3,11], 0.24 |
| Influence of CV age | 4.0 *(2, 57)* | 2.6 (*2, 49*) |
| *p*[95% CI]) | 0.05[0.0, 8.0] | 0.11 [-0.63, 5.8] |
| [c] Serum B12 with dietary B12 as CV | 10.2 *(2, 57)* | 11.5 (*2, 49*) |
| (*p*[95% CI]), $R^2$ | <0.001**[4.3, 16], 0.36 | <0.001**[4.9, 18], 0.40 |
| Influence of CV B12 dietary intake | 12.9 *(2, 57)* | 15.5 (*2, 49*) |
| (*p*[95% CI]) | <0.001**[6.4 ,20.3] | <0.001**[6.6, 24] |
| [c] Serum B12 with capsule adherence as CV | 19.3 *(1, 37)* | 16.9 (*1, 33)* |
| (*p*[95% CI]), $R^2$ | <0.001**[8.2, 30.4], 0.38 | 0.001** [7.1, 26.5], 0.44 |
| Influence of CV capsule adherence | 7.7 *(1, 37)* | 7.6 (*1, 33)* |
| (*p*[95% CI]) | 0.009* [2.1,13.3]) | 0.009*[2.0,13.2] |

*Note.* ANOVA /ANCOVA on log transformed change in serum B12 over 6 months with post hoc Tukey's honestly significant difference or simple contrasts. Bonferroni adjustment applied so significant at $p \leq 0.025$ ITT = intention to treat data with last observation carried forward for missing values. MV =missing values data set with missing values excluded pairwise from analysis, CV = covariate. ANOVA/ANCOVA reported as F (degrees of freedom), (significance), [95% CI], $R^2$ = adjusted coefficient of determination. Dietary B12 intake analysed as change in intake over 6 months. Capsule adherence measured over 6 months.
ANOVA of differences between groups[a]. 95 % CI of ANOVA [b]
ANCOVA of differences between groups[c]. 95 % CI of ANCOVA [d]
*Significant difference for B12 supplement group at $p \leq 0.025$ (group identified from post hoc Tukey HSD or planned simple contrasts)
**Significant difference for B12 supplement group at $p \leq 0.001$ (group identified from post hoc Tukey HSD or planned simple contrasts)

Table 19
*ANOVA and ANCOVA of Change in HoloTC over 6 Months using Intention to Treat and Missing Values Datasets*

| | ITT dataset | MV dataset |
|---|---|---|
| [a] HoloTC | 7.4 (*2, 57*) | 7.2 (*2, 49*) |
| *p*(95% CI)[b], $R^2$ | <0.001**[3.1, 12.0], 0.18 | 0.002* [2.8, 12], 0.20 |
| [c] HoloTC with age as CV | 6.3 (*2, 57*) | 5.9 (*2, 49*) |
| (*p*[95% CI]), $R^2$ | 0.003* [2.3, 11]), 0.17 | 0.005* [1.9,10], 0.18 |
| Influence of CV age | 0.06 (*2, 57*) | 0.32 (*2, 49)* |
| (*p*[95% CI]) | 0.48 [-0.1, 0.20] | 0.57[-0.08,1.5] |
| [c] HoloTC with dietary B12 as CV | 6.9 (*2, 57*) | 7.4 (*2, 49*) |
| (*p*[95% CI]), $R^2$ | 0.002* [2.6,11.2], 0.22 | 0.002*[2.8,12], 0.25 |
| Influence of CV B12 dietary intake | 4.2 (*2, 57*) | 4.9 (*2, 49*) |
| (*p*[95% CI]) | 0.04 [0.1,8.4] | 0.03 [0.44, 9.3] |
| [c] HoloTC with capsule adherence as CV | 11.5 (*1, 37)* | *11.8 (1, 33)* |
| (*p*[95% CI], $R^2$ | 0.002* [4.5, 18.5], 0.22 | 0.002* [4.6, 19.0], 0.24 |
| Influence of CV capsule adherence | 1.2 (*1, 37)* | 0.8 (*1,33*) 0.78 |
| (*p* [95% CI]) | 0.29 [-1.1, 3.5] | 0.38 [-1.0, 2.6] |

*Note.* ANOVA /ANCOVA on log transformed change in holoTC over 6 months with post hoc Tukey's honestly significant difference or simple contrasts. Bonferonni adjustment applied so significant at $p \leq 0.025$ ITT = intention to treat dataset with last observation carried forward for missing values. MV =missing values dataset with missing values excluded pairwise from analysis, CV = covariate. ANOVA/ANCOVA reported as F (degrees of freedom), (significance), [95% CI], $R^2$ = adjusted coefficient of determination. Dietary B12 intake analysed as change in intake over 6 months. Capsule adherence measured over 6 months.
ANOVA of differences between groups[a]. 95 % CI of ANOVA [b]
ANCOVA of differences between groups[c]. 95 % CI of ANCOVA [d]
*Significant difference for B12 supplement group at $p \leq 0.025$ (group identified from post hoc Tukey HSD or planned simple contrasts)
**Significant difference for B12 supplement group at $p \leq 0.001$ (group identified from post hoc Tukey HSD or planned simple contrasts)

**The percentage increase or decrease in B12 biomarkers as a measure of effect size.**

The serum B12 and holoTC variables were log-transformed prior to analysis of the change in biomarkers from baseline to six months, and then back transformed for reporting of the geometric mean (GM) percentage change, with 95% confidence intervals. The B12 Supplement Group demonstrated a 30% (95% CI [11%, 48%]) increase in serum B12 over the study duration, while the Placebo Group demonstrated an 8% [-18, 3] and the Dietary Advice Group a 3% [-15, 10] decrease respectively. The most significant change occurred in the first two months of the study (between baseline and two month measurements) when serum B12 for the B12 Supplement Group increased by 24% (95% CI [9, 36]). However, the magnitude of increase plateaued and there was a 6% (95% CI [-4, 17]) increase only for the B12 Supplement Group between

two month and six month measurements. The Placebo and Dietary Advice Groups both had a decrease in serum B12 at each of the measurement points (Table 20).

The effect size was even larger for the B12 Supplement Groups' holoTC results, with a 42% (95% CI [12, 72]) increase in holoTC from baseline to six months. Following the trend for serum B12 increases, the most significant increase occurred in the first two months of the study, with a 33% (95% CI [8, 58]) increase in holoTC. The magnitude of increase for the B12 supplement group plateaued between two and six months with an additional 12% (95% CI [8, 33]) increase only in holoTC. The Placebo and B12 Dietary Advice Groups each recorded a decrease in holoTC over the study. (Table 20)

Table 20
*Percentage Change in B12 Biomarkers by Treatment Group*

| | | Serum B12 | | | HoloTC | | |
|---|---|---|---|---|---|---|---|
| | | B12 Supplement | Placebo | Diet advice | B12 Supplement | Placebo | Diet advice |
| % change 0 | GM | 24 | -0.4 | -0.4 | 33 | -1.4 | -1 |
| - 2 months | 95%CI | 9, 36 | -9, 8 | -8, 7 | 8, 58 | -15, 12 | -8, 7 |
| % change 2 | GM | 6 | -7 | -2 | 12 | -2 | 1 |
| - 6 months | 95%CI | -4, 17 | -15, 2 | -10, 7 | -8, 33 | -17, 13 | -7, 8 |
| % change 0 | GM | 30 | -8 | -3 | 42 | -7 | 1 |
| - 6 months | 95%CI | 11, 48 | -18, 3 | -15, 10 | 12, 72 | -23,8 | -15, 10 |

*Note*. Geometric mean: expressed as a relative % of the exponential of log transformed change scores.
95% Confidence intervals (95 % CI) of geometric mean written as lower bound, upper bound. Expressed as a relative percentage.
GM = geometric mean

**Associations between capsule adherence and the change in B12 biomarkers.**

Between baseline and two months mean capsule adherence for the B12 Supplement Group was 76% (95% CI [65, 87]), which then decreased to 59% ([47, 72]) between two and six months. Similarly, for the Placebo Group, capsule adherence was 85% (95% CI [78, 93]) by two months, and then decreased to 59% [47, 71] by six months. Capsule adherence accounted for 27% ($R^2$) of the change in serum B12 (log) at two months for the B12 supplement group (r = 0.52, $p$ = 0.02, (95% CI [0.11, 0.78]), but only 15% of the change at six months (r = 0.39, $p$ = 0.08, [-0.05, 0.7]) (Figure 10).

Similarly between baseline and two months, capsule adherence accounted for 22% of the change in holoTC (log), with a significant association (r = 0.47, *p* = 0.03, 95% CI [0.05, 0.70]), but between two to six months, the association was small and not significant (r = -0.04, *p* = 0.76, [-0.40, 0.46]) (Figure 10). Participants with greater than 60% adherence with capsules over six months achieved a positive increase in serum B12, with increasing adherence generally associated with a higher increase in serum B12 (Figure 10). Similar results were received for holoTC and adherence (Figure 10).

There was no significant association between capsule adherence and change in serum B12 for the Placebo Group by the two month (r = 0.12, *p* = 0.62, 95% CI [-0.37, 0.56]), or the six month (r = -0.03, *p* = 0.91, [-0.49, 0.44]) measurement points (Figure 11). Associations were also non-significant for the Placebo Group between capsule adherence and change in holoTC by two (r = 0.042, *p* = 0.87, 95 % CI [-0.43, 0.50]) and six months (r = -0.06, *p* = 0.81, [-0.51, 0.42]) (Figure 11).

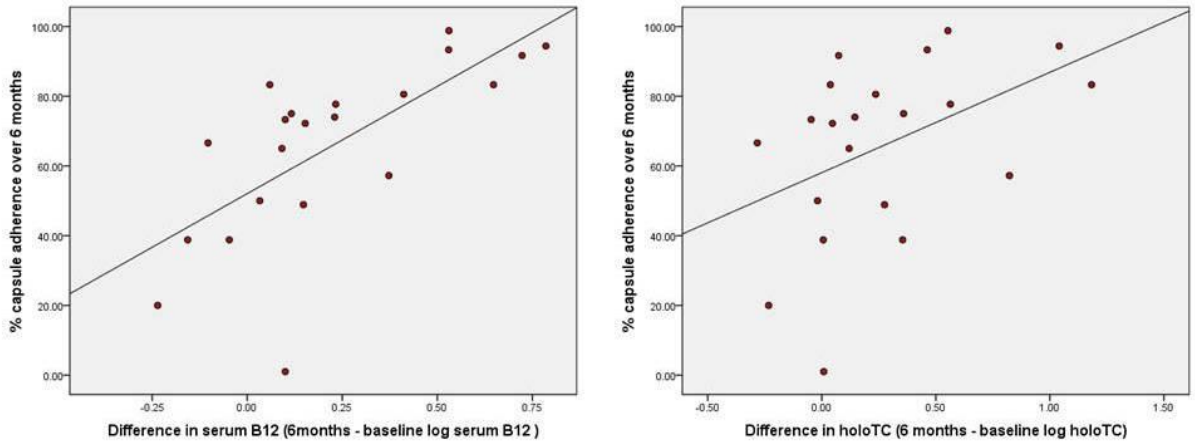



*Figure 10*. Associations between capsule adherence and change in B12 biomarkers over six months for the B12 supplement group

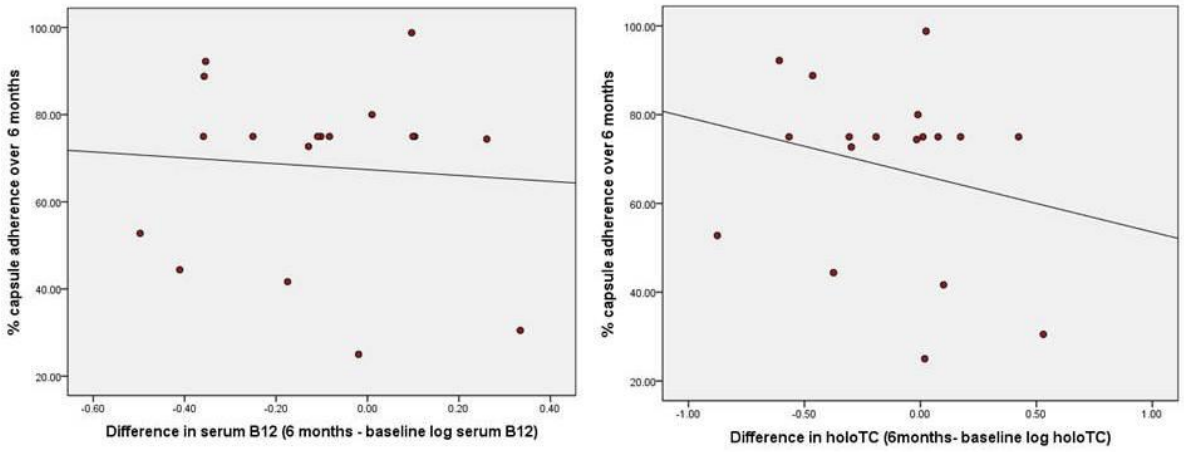



*Figure 11*. Associations between capsule adherence and change in B12 biomarkers over six months for the placebo group

**Associations between change in B12 dietary intake and B12 biomarkers.**

The magnitude of change in dietary B12 intake within groups is reported as the geometric mean (GM) percentage change, with 95% confidence intervals. From the B12FFQ, the Dietary Advice Group recorded an increase of 35%, (95% CI [2, 68]) in dietary B12 intake from baseline to six months. All of this increase occurred over the first two months of the study 35% (95% CI [8, 61]), with only a 1% [-11, 13] increase over the latter four months of the study (Table 21)

There was a significant moderate positive association between the change in dietary B12 intake and change in serum B12 from baseline to six months ($r$ = 0.63, p = 0.004, 95 % CI [0.25, 0.84]), and change in holoTC for the same period ($r$ = 0.55, $p$ = 0.015, 95 % CI [0.13, 0.80]) (Figure 12). The median dietary B12 intake for the Dietary Advice Group increased from 3.2 pmol/L to 4.1 pmol/L over the six-month study period (Table 21). However, this increase in dietary B12 intake was not sufficient for the previously described ANOVA and ANCOVA comparing group treatments on B12 biomarkers, to identify the Dietary Advice Group treatment as having a significant effect on either serum B12 or holoTC (Table 18 and Table 19). Change in dietary B12 intake was still influential because when added as a covariate with serum B12 in

ANCOVA comparing between group treatments, change in dietary B12 intake was significant for change ($F(2,57) = 12.9$, $p < 0.0001$, 95% CI [6.4, 20.3]) (Table 18).

The Placebo Group demonstrated a significant positive association between change in dietary B12 intake and the change in serum B12 from baseline to six months ($r = 0.622$, $p = 0.004$, 95 % CI [0.23, 0.84]). There was however, a non-significant association between the change in holoTC and change in dietary B12 ($r = 0.22$, p = 0.37, 95 % CI [-0.26, 0.61) (Figure 14). The Placebo Group reported a decrease of 22 %, (95% CI [-4, -40]) in dietary B12 intake over the six month study period and this corresponded with a decrease of 8% [-18, 3] in serum B12 and a 7% [-23, 8] decrease in holoTC (Table 21).

The B12 Supplement Group reported an increase of 14% (95% CI [-9, 36]) in dietary B12 intake over six months (Table 21). There were no significant associations between change in dietary B12 intake, and change in serum B12 ($r = 0.10$, $p = 0.66$, 95 % CI [-0.35, 0.91) and holoTC respectively ($r = 0.21$, $p = 0.35$, 95 % CI [-0.24, 0.59) (Figure 13).

Table 21
*Median B12 Dietary B12 Intake and Geometric Mean Change in Dietary B12 Intake as Reported on B12 FFQ*

| | | Placebo | Diet advice | B12 supplement |
|---|---|---|---|---|
| Baseline | Median[a] | 2.8 | 3.2 | 1.8 |
| | 25th /75th | 1.3/5.3 | 1.3/6.1 | 0.9/4.4 |
| 2 months | Median[a] | 2.3 | 3.8 | 2.1 |
| | 25th /75th | 1.5/5.5 | 2.5/6.1 | 0.9/3.0 |
| 6 months | Median[a] | 2.2 | 4.1 | 2.1 |
| | 25th /75th | 1.0/2.7 | 3.0/6.7 | 1.0/2.8 |
| % change 0-2 months | GM[b] | 1 | 35 | 14 |
| | 95% CI[c] | -18, 21 | 8, 61 | -9, 36 |
| % change 2-6 months | GM[b] | -18 | 1 | 6 |
| | 95% CI[c] | -3, -34 | -11, 13 | -11, 22 |
| % change 0-6 months | GM[b] | -22 | 35 | 14 |
| | 95% CI[c] | -4, -40 | 2, 68 | -9, 37 |

Data skewed so reported as median, 25th and 75th percentiles. [a] Dietary B12 intake measured in µg/day. [b]Geometric mean percentage change in reported B12 dietary intake. [b]95% confidence intervals of geometric mean. 25th /75th = 25th/75th percentiles. GM = geometric mean.

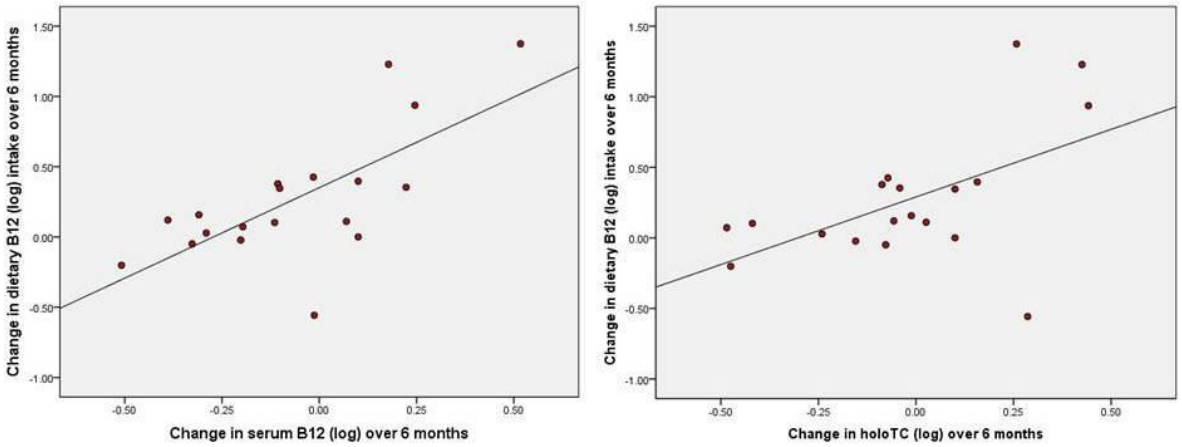



*Figure 12.* Associations between change in dietary B12 intake and B12 biomarkers for Dietary Advice Group

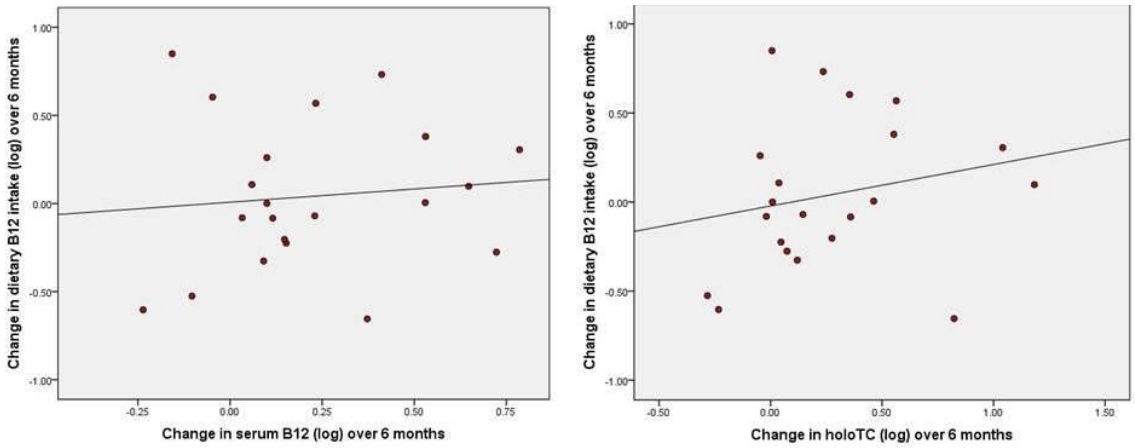



*Figure 13.* Associations between change in dietary intake of B12 and B12 biomarkers for B12 Supplement Group

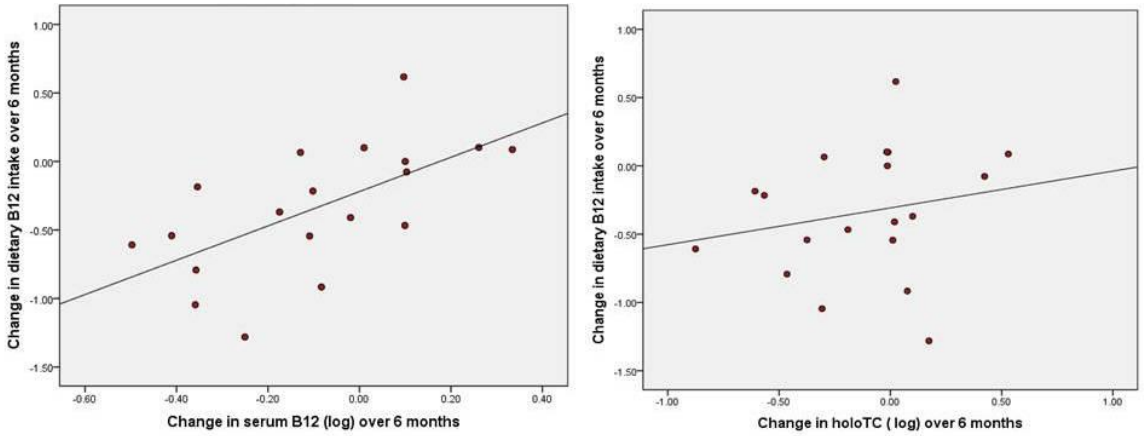



*Figure 14.* Associations between change in dietary B12 intake and B12 biomarkers for Placebo Group

**Relationships between Anthropometry, Insulin Resistance and B12 Biomarkers**

The VitB12 study population were relatively homogenous for body anthropometry, and although the mean BMI (25 kg/m2, 95% CI [18, 37]) was just within recommended guidelines for European standards (≤ 25 kg/m2), it could classify the population as overweight (≤ 23 kg/m2) by Asian Indian standards (Snehalatha, Viswanathan, & Ramachandran, 2003; World Health Organization, 2004). All of the

women recorded a high BF % (mean 51%, 95% CI [42, 61]), even in those with a BMI in the lowest quartile of normal range (Figure 15).

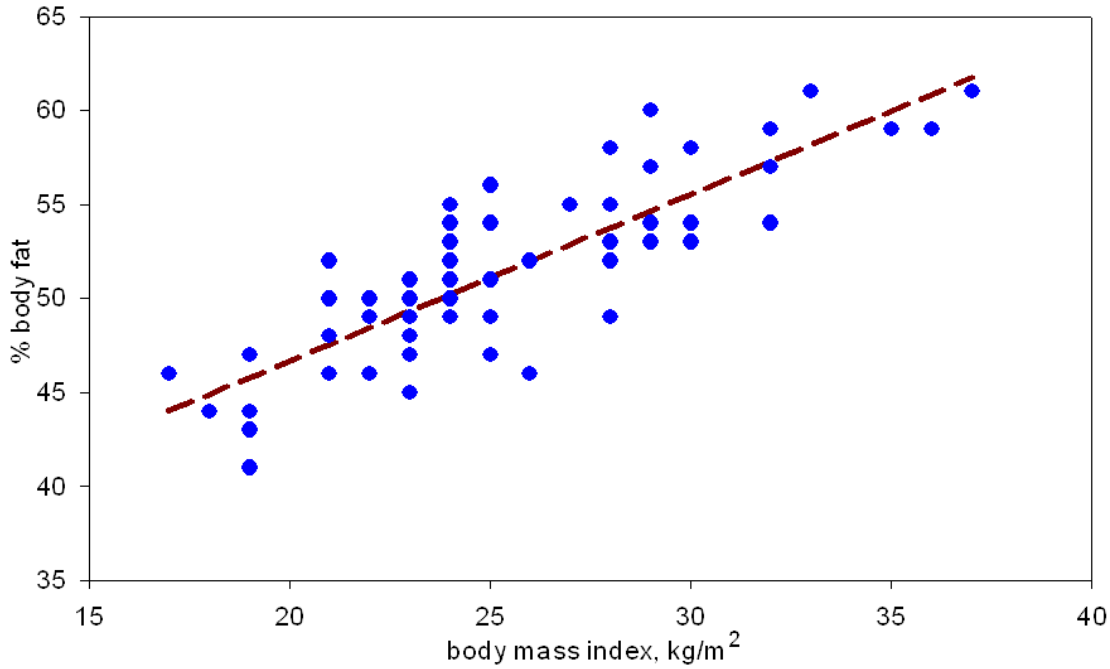



*Figure 15*. Associations between body fat percentage and body mass index

The women were short in stature (Table 13) and measures for central obesity, both the mean waist to hip (WHR) ratio (0.8, 95% CI [0.7, 0.9]), and mean waist to height (WHtR) ratio (0.5, [0.4, 0.6]) (Table 22), were on the recommended upper limit of normal for women (Ashwell & Shiun Dong, 2005; Dobbelsteyn, Joffres, MacLean, & Flowerdew, 2001). Grip strength was below limits for normal range, suggesting a low percentage of muscle mass. Grip strength was not significantly related to BF% ($r$ = 0.05, $p$ = 0.7, 95% CI [-0.21, 0.3]), but there was a significant positive association between fat-free mass (FFM) and grip strength ($r$ = 0.45, $p$ < 0.001, [0.22, 0.63]). For each of the variables body fat percentage (BF%), WHtR, and FFM, there were no significant associations with serum B12 or holoTC (Table 22). A small, but significant relationship was identified between left hand grip strength and holoTC; a relationship

that was not identified between right hand grip strength and holoTC, or between right or left hand grip strength, and serum B12 (Table 22). A higher BF% in the participants was significantly associated with higher concentrations for all lipid measurements (Table 23). Higher lipid concentrations were similarly associated with higher WHtR and higher WTH ratios (with the exception of TG/HDL, which was not significant) (Table 23). There was a small, still significant positive relationship between WHtR, and HOMA2-IR but a non-significant relationship between HOMA 2-IR and both BF% and WHR.

Table 22
*Correlations among Measures of Body Composition and B12 Biomarkers*

| | †Serum B12 | †HoloTC |
|---|---|---|
| BF% | -0.10 | -0.47 |
| | (0.4) | (0.6) |
| | [-0.56, 0.40] | [-0.53, 0.42] |
| FFM | 0.05 | 0.19 |
| | (0.72) | 0.14) |
| | [-0.02,0.30] | [-0.17, 0.42] |
| WHR | -0.05 | 0.03 |
| | (0.69) | (0.80) |
| | [-0.30, 0.21] | [-0.23, 0.28] |
| WHtR | -0.09 | 0.03 |
| | (0.50) | (0.80) |
| | [-0.55, 0.04] | [-0.51, 0.45] |
| GS -R | -0.03 | 0.14 |
| | (0.81) | (0.30) |
| | [-0.28, 0.23] | [0.05, 0.50] |
| GS -L | 0.07 | 0.30 |
| | (0.55) | (0.02)* |
| | [-0.19, 0.32] | [0.05, 0.51] |

†Positive skewed distribution so all results reported as Spearman's correlation, (significance), [95% CI]. BF% =body fat percentage, FFM = fat free mass, WHR = waist to hip ratio, WHtR = waist to height ratio, GS-R = grip strength right hand, GS-L = grip strength left hand. * significant at $p \leq 0.05$.

Table 23
*Correlations between Measures of Body Composition, Lipids and Insulin Resistance*

| | TC | LDL-C | HDL-C | †TG | C/HDL-C ratio | †TG/HDL-C ratio | †HOMA2 IR |
|---|---|---|---|---|---|---|---|
| BF% | 0.27 | 0.42 | 0.33 | 0.34 | 0.50 | †0.41 | †0.18 |
| | (0.04)* | 0.002)* | (0.01)* | (0.009)* | (<0.001)** | (0.001)** | (0.17) |
| | [0.02,0.49] | [0.19,0.61] | [-0.54, -0.08] | [0.09, 0.55] | [0.28, 0.67] | [0.17, 0.60] | [-0.08, 0.41] |
| WHtR | 0.30 | 0.46 | 0.36 | 0.32 | 0.56 | †0.47 | †0.25 |
| | (0.02)* | (<0.001)* | (0.005)* | (<0.001)** | (<0.001)** | (<0.001)** | (0.05)* |
| | [0.18, 0.59] | [0.23, 0.64] | [-0.56, -0.12] | [0.18, 0.59] | [0.36, 0.71] | [0.25, 0.65] | [0, 0.47] |
| WHR | 0.28 | 0.38 | 0.16 | 0.25 | 0.36 | †0.24 | †0.19 |
| | (0.03)* | (0.003)* | (0.08) | (0.05)* | (0.005)* | (0.07) | (0.14) |
| | [0.03, 0.5] | [0.14, 0.58] | [-0.4, 0.1] | [0, 0.57] | [0.11, 0.56] | [-0.01, 0.47] | [-0.07, 0.42 |

Reported as Pearson's correlation, (*p*) [95% CI], unless stated otherwise. †Positive skewed distribution for one variable so reported as Spearman's correlation. TC =total cholesterol mmol/L, LDL =low-density lipoprotein mmol/L, HDL= high-density lipoprotein mmol/L, TG =triglycerides mmol/L, C/LDL-C =cholesterol/high-density lipoprotein ratio, TG/ HDL-C ratio =triglyceride/high-density lipoprotein ratio, WHtR = waist to height ratio, WHR = waist to hip ratio.*Correlation between variables significant at $p \leq 0.05$.
**Correlation between variables significant at $p \leq 0.001$.

**Lipids, insulin resistance, associations with B12 biomarkers and body anthropometry.**

The TG, TG/HDL-C ratio and HOMA2-IR variables were positively skewed, so lipid results and HOMA2-IR are reported as medians, 25$^{th}$ and 75$^{th}$ percentiles and range (Table 24). Almost 75% of the women exceeded the ideal concentrations recommended by the New Zealand Heart Foundation for TC and LDL-C (NHFA/CSANZ, 2005). Median HOMA2 IR, TG, TG/HDL-C and C/HDL-C ratio were normal with approximately 75 % of the women within recommended guidelines for these variables (NHFA/CSANZ, 2005). Eight of the sixty women (13%) were identified as insulin resistant if defined by HOMA2-IR > 1.8 (Esteghamati et al., 2010; Geloneze et al., 2009). HOMA2- IR was unrelated to serum B12 ($r^s$ = 0.01, $p$ = 0.95, 95 % CI [-0.24, 0.26]) or holoTC B12 ($r^s$ = 0.02, $p$ = 0.83, [-0.24, 0.27]) (Figure 13). None of the lipid variables reported in Table 24 were significantly associated with HOMA2- IR.

Table 24
*Lipids and HOMA2 IR Results for the Study Population*

| | TC | LDL-C | HDL-C | †TG | C/HDL | †TG/HDL | †HOMA IR |
|---|---|---|---|---|---|---|---|
| Median | 4.6 | 2.6 | 1.4 | 0.9 | 3.3 | 0.65 | 0.70 |
| $25^{th}$, $75^{th}$ percentiles | 4.2, 5.0 | 1.9, 3.0 | 1.1, 1.6 | 0.7, 1.2 | 2.5, 3.8 | 0.46, 0.90 | 0.40,1.10 |
| Range [UL, LL] | 2.5, 7.0 | 1.1, 4.1 | 0.8, 2.6 | 0.4, 3.9 | 1.7, 5.8 | 0.15, 3.25 | 0.30, 2.30 |

†Skewed distribution
TC (total cholesterol mmol/L), LDL (low-density lipoprotein mmol/L), HDL (high-density lipoprotein mmol/L), TG (triglycerides mmol/L), C-LDL (cholesterol/high-density lipoprotein ratio), Trig. HDL ratio (triglyceride/high density lipoprotein ratio), HOMA IR (homeostatic model of insulin resistance)

**Chapter summary**

The aim of this B12 mixed methods research was to investigate methods to reduce B12 deficiency in South Asian women of childbearing age. In this RCT, 60 South Asian women, aged 18 to 50 years, participated to test the hypothesis that advice to increase dietary B12 intake would be more effective over six months than 6 µg B12 supplement, which in turn, would be more effective than placebo for increasing serum B12 and holoTC status in the women. The B12 Supplement Group was the only treatment group where the percentage of women, who were deficient or insufficient in B12 biomarkers changed (halved), thus supporting the research aim to reduce B12 deficiency among South Asian women. Although the reported dietary B12 intake for the Dietary Advice treatment group increased over the study, there was actually an increase in the percentage of women B12 biomarker deficiency or insufficiency. The Placebo Group similarly demonstrated an increase in the percentage of women with B12 biomarker deficiency or insufficiency, so the dietary advice and placebo treatments were not conducive to meeting the research aim. The hypothesis that dietary advice would be more effective than 6µg B12 supplement was rejected, but the hypothesis that 6µg B12 supplement would be more effective than placebo was supported.

Although the B12 supplement group recorded a marked decrease in the percentage of women who were B12 deficient or insufficient, effect size for the increase

in B12 biomarkers was only small for serum B12 (a 30 % increase on baseline serum B12 over six months) and moderate for holoTC (a 40 % increase). Effect size was significantly associated with capsule adherence, particularly in the first two months of the study when capsule adherence was high (76 %) and there was a 24 % increase on baseline serum B12 and a 33 % increase on baseline holoTC. However, the effect of B12 supplementation plateaued in the latter four months of the study, when capsule adherence dropped to 59 % and there was a mere 6 % increase in serum B12 and 12 % increase in holoTC. Another influential covariate on the change in B12 biomarkers was reported B12 dietary intake.

Secondary analysis of the relationships between body anthropometry and B12 biomarkers, and the relationships between HOMA2 IR and B12 biomarkers found no significant associations. However, all participants in the study recorded a BF % above the recommended healthy range, even those participants whose BMI was within recommended ranges. A higher BF%, WHtR and WTH ratio were each associated with higher concentrations of all lipid measurement (except TG/HDL ratio relationship WTH). Three-quarters of the participants had TC and LDL measurements that exceeded the New Zealand National Heart Foundation recommended upper limit. The implications of the findings from this chapter will be discussed in Chapter 8, with recommendations for action and future research.

# Chapter 8: Discussion, Implications and Conclusions

This community based participatory mixed methods research highlights that in 2010 in Auckland, New Zealand, inadequate vitamin B12 (B12) status occurred in at least one of two South Asian women of childbearing age. In the VitB12 study of 60 South Asian women aged 18 to 50 years, at baseline 48% were deficient or insufficient in serum B12 (< 222 pmol/L), and 50% in holotranscobalamin (holoTC) (< 45 pmol/L). Vitamin B12 insufficiency or deficiency was positively associated with dietary practices, in particular a problem for those who did not eat red meat regularly.

Convincing evidence from this three-group treatment, randomised controlled trial (RCT), found that when 21 South Asian women were randomised to a group that received a daily supplement of 6 µg cyanocobalamin over six months, concentrations of circulating holoTC increased by 42%, and serum B12 by 30%. The percentage of women sufficient in serum B12 improved from 33% (n = 7) to 76% (n = 16) while the percentage sufficient in holoTC improved from 29% (n = 6) to 43% (n = 9). No one suffered adverse effects from the supplementation. This was a clinically and statistically significant shift in both concentrations of B12 markers, and the prevalence of B12 deficiency and insufficiency. Given the goal of shifting all B12 deficient women to sufficiency before pregnancy and breastfeeding, this study provides support for the B12 supplementation treatment. Participants who consumed greater than 60% of the prescribed supplement over six months received the most benefit. A comparison of these results with two other treatment groups (Placebo and Dietary Advice) over the six-month study period highlights that the B12 supplementation treatment group was the only group to achieve an increase in B12 biomarker sufficiency.

It is important to consider the context of these findings, in particular how South Asian women feel about managing B12 deficiency and the treatments currently offered. A key finding of the focus group interview analyses was that women wanted more knowledge and understanding so they could make their own informed choice about the prevention and management of their B12 deficiency.

The following discussion will consider the points summarised above in relation to other evidence, local and international, on causes and treatment of B12 deficiency. The medical, cultural, social, and political environments influencing B12 deficiency due to inadequate intake are included in this analysis. The discussion will then focus on how to translate the findings into positive action, particularly with reference to the contribution that community leaders, nurses, midwives, general practitioners, dieticians and nutritionists can make to improve the health of women and the life course development of their future children. A description of strengths and limitations of the VitB12 study follows, with recommendations for future research in the area of B12 deficiency in women of childbearing age. The chapter concludes with a summary of the main findings and recommendations of this body of work.

**Vitamin B12 deficiency in Auckland based South Asian women of child bearing age**

South Asian women with vegetarian dietary practices were more likely to be low in B12 biomarkers (holoTC and serum B12), than those with non-vegetarian practices. However, eating meat did not preclude low B12 biomarkers or an inadequate dietary B12 intake for participants in the VitB12 study. The median serum B12 for women who did not eat meat was 199 pmol/L ($25^{th}$/ $75^{th}$ percentiles [154, 263]), and for those that ate meat 313pmol/L [209, 432] (Chapter 6, Table 11 and Table 12). Of the 35 participants with vegetarian practices, 63% (n = 22) were low in serum B12 (< 222

pmol/L) and 69% (n = 24) were low in holoTC (< 45 pmol/L). Of the 25 participants with non-vegetarian practices, 28% (*n* = 7) were low in serum B12 and 24% (n = 6) were low in holoTC (Chapter 6, Table 8 and Table 9). None of the participants in the VitB12 study was low in folate.

These findings are consistent with those from previous research undertaken at AUT University, where South Asian preadolescent girls and women of childbearing age with low or no red meat consumption were more likely to be low in B12 biomarkers: (Chhichhia, 2007; Rush, Chhichhia, et al., 2009; Xin, 2008). It is possible that the prevalence of B12 deficiency in the wider population of South Asian women is under estimated from the VitB12 study because participants with a history of chronic disease, hyperglycaemia, recent B12 supplementation or on B12 absorption inhibiting medications such as metformin (Reinstatler et al., 2012) were excluded.

The percentage of women who were low in serum B12 in the VitB12 study is similar to that reported in the Surya study conducted through Massey University, Auckland (Gammon, von Hurst, Coad, Kruger, & Stonehouse, 2012). In the Surya study of 124 women aged > 20years (90 non-vegetarian and 34 vegetarian), 59 % of those with vegetarian dietary practices were low in serum B12 (<222 pmol/L), and 38% of those with non-vegetarian dietary practices. However, the measures of central tendency and spread were lower in the Surya study, than in the VitB12 study. There was a significant difference in serum B12 between those with vegetarian and those with non-vegetarian dietary practices in the Surya study. For women who were vegetarian, the geometric mean serum B12 was 181 pmol/L, 95% CI [159 – 207], while for the non-vegetarian group geometric mean serum B12 was 257 pmol/L [235-281]. In the Surya study, two out of 124 women were low in folate.

The evidence presented in this thesis and from the literature confirms that South Asian women, in particular those with vegetarian or non-red meat eating dietary practices are at risk for B12 deficiency. The problem may be exacerbated because the lifetime and intergenerational history of low or non-meat eating habits of South Asian women, increase the likelihood of inadequate B12 stores and the intergenerational passing on of B12 deficiency (Jayanthi, 2001; Sharma et al., 2003; Yajnik, 2009b). Following an observation that an increase in holoTC after a small dose of vitamin B12 could be a biomarker of B12 absorption, Bhat et al (2009) investigated the response to three doses of either a 2 µg or 10 µg B12 (cyanocobalamin) supplement in a B12 deficient Indian population [109 children, 96 fathers and 108 mothers]). Findings supported that B12 absorption was intact in this population and supported the most likely cause of deficiency as inadequate dietary intake (Bhat et al., 2009).

There is a lack of information on B12 status of the wider New Zealand population of childbearing age women to compare with and determine if the risk is greater for South Asian women. Since the traditional Western meat eating diet provides between 3 to 30 µg of B12 per day, B12 deficiency from inadequate B12 intake is considered rare; a problem only in those with strict vegetarian or vegan dietary practices (Carmel, 2000; Hudson, 2010). Historically in countries such as New Zealand, the most common cause of B12 deficiency was malabsorption and this was primarily a problem in older adults (T. J. Green, Venn, Skeaff, & Williams, 2004; Hudson, 2010).

A proposal for folic acid food fortification from Food Standards Australia New Zealand reflects the history that B12 deficiency was only a problem in older adults as there is no mention of problems with B12 deficiency in younger adults (Food Standards Australia New Zealand, 2006, p. v). "*The expected average increase in folic acid intake arising from mandatory folic fortification is unlikely to pose any increased risk of*

*masking the diagnosis of vitamin B12 deficiency in* ***older*** *people or in the zinc status of the population."* Minutes from the New Zealand Pharmaceutical Management Agency (PHARMAC) consultation on folic acid supplementation in pregnancy also illustrates the tradition that B12 deficiency is very rare in women of childbearing age (PHARMAC, 2009, p. 27). *"The Committee considered that women would be on a folic acid supplement for a short period of time, and although this could mask the diagnosis of vitamin B12 deficiency, this was very rare and is a treatable condition in women of child-bearing age."*

There is evidence that B12 deficiency may be more common in young people, particularly women, than previously assumed (Baer & Peter, 2011; Tucker et al., 2000). A United States (US) RCT of B12 supplementation screened 300 women aged 26 to 50 years for B12 deficiency (Baer & Peter, 2011). Based on the serum B12 results, 137 women (46%) had either borderline low (< 185 pmol/L) or low serum B12 (< 148 pmol/L) results. The percentage of deficiency in the target population was possibly even larger because this US study excluded women who did not eat red meat, or with a history of gastric bypass surgery. Unlike the VitB12 study, B12 deficiency in the US study was not associated with inadequate dietary intake as the mean reported dietary B12 intake was 8.6 (± 1.4) µg/day. Up to 40% of those identified with deficiency, consumed medications known to inhibit B12 absorption such as metformin, calcium supplements, histamine 2 antagonists, proton pump inhibitors and oral contraceptives; these were inferred to contribute to the high rates of B12 deficiency (Baer & Peter, 2011). For Indian women, however, prior research confirmed that B12 deficiency is a matter of inadequate intake rather than absorption (Bhat et al., 2009; Refsum et al., 2001).

The US based Framingham Offspring Study (FOS) of 2999 men and women aged 26 to 83 years, reported that serum B12 deficiency was just as common in younger as older age groups, with a significantly higher prevalence in women (Tucker et al., 2000). For the age group 26 to 49 years, 17% were low in serum B12 (< 185 pmol/l). A further 22 % were in the indeterminate range (defined by the FOS) of 185 to 258 pmol/l (Tucker et al., 2000); concentrations at which metabolic changes indicative of cellular deficiency can occur (Herbert, 1994; Lindenbaum, Rosenberg, Wilson, Stabler, & Allen, 1994; Tucker et al., 2000). The mean dietary B12 intake was 8.7 (±0.3) µg/day, and evenly matched across all age ranges, with males reporting a slightly higher intake than females. There was a positive association with serum B12 concentrations across all levels of dietary B12 intake.

The findings of the two studies cited above, and this VitB12 study, suggest that prevention of B12 nutritional deficiency in younger adults, particularly women, needs more focus and publicity than currently given. It is not clear whether general practitioners routinely test women of South Asian origin, or women with low or non-meat eating dietary habits, for B12 deficiency. The majority of women in the RCT were not aware of their B12 deficiency, but that may have been because those with a history on B12 supplementation were excluded. Four women with B12 deficiency, who, during the course of the study were prescribed intramuscular (IM) B12 supplements by their General Practitioner (GP), only came to the attention of their GP when other concurrent, abnormal VitB12 study blood results were referred back to the GP. However, in the focus groups, women were more aware of B12 deficiency, and had previously been tested, or had family members tested (and some treated), for B12 deficiency by their GP.

*I have not been checked for [B12 deficiency] myself yet but my husband, my brother, my mom, yeah everyone under our roof, but I haven't been checked myself no. They all go for the injections and stuff, once a year or twice a year but I haven't done for myself yet.* (Participant 1[P1], Focus group 2[FG2])

*Are you vegetarian?* (Researcher)

*Yes but I haven't been checked for myself yet.* (P1, FG2)

In the past, B12 deficiency was diagnosed if there were neurological signs and symptoms, or macrocytic anaemia; however, production of metabolites such as homocysteine (Hcy) and methylmalonic acid (MMA), can occur long before overt symptoms of deficiency present (Čabarkapa et al., 2007; Herbert, 1994; Herrmann et al., 2003). In the VitB12 study, none of the women presented with macrocytic anaemia. For some of the participants, microcytosis induced by concurrent thallasemeia or iron deficiency may have inhibited macrocytic changes from B12 deficiency; this finding has been documented in previous studies investigating macrocytic anaemia (Chan et al., 2007; Oosterhuis, 2000).

The serum B12 concentrations in the VitB12 study were in the low or borderline low range, with the lowest recorded at 119 pmol/L. This is consistent with the literature on B12 deficiency, where intact absorption and efficient enterohepatic recycling of existing B12 stores means that B12 deficiency due to inadequate B12 intake is not usually as profound as it is in people with malabsorption of B12 (Herbert, 1994; Herrmann et al., 2003). Even in mild deficiency however, metabolites from tissue deprivation accumulate, increasing risk factors for neurological deficits and possibly noncommunicable disease risk (Krishnaveni et al., 2009; Lindenbaum et al., 1995; Pac et al., 2005; Sadeghian et al., 2006; Yajnik et al., 2006).

The direct associations between B12 deficiency and risk of NCD (independent of the effects mediated through raised Hcy), need further investigation because research into these associations has provided conflicting evidence to date. A systematic review of seven studies linking the direct effects of low blood B12 and increased risk of CVD and T2DM, found insufficient support for an association once factors such as lipid concentrations, folate/B12 balance and Hcy were controlled for (Rafnsson, Saravanan, Bhopal, & Yajnik, 2011). In this systematic review, only one study found a significant link; low B12 was associated with increased risk of cerebral ischemia (Weikert et al., 2007). This association was less significant with Hcy controlled for, suggesting that the effects of low B12 on disease susceptibility are mediated thorough hyperhomocysteinaemia (Rafnsson et al., 2011; Weikert et al., 2007).

The most significant influence between low B12 concentrations and risk of diabetes and CVD appears to be those discussed in Chapter 2 on the effects of early intrauterine life (Yajnik et al., 2008). These exacerbated risks from B12 deficiency are additive to the known risks of poor pregnancy outcomes, NTD, and increased risk of offspring neurocognitive deficits from B12 deficiency (Bak et al., 2009; Bhate et al., 2008; Pepper & Black, 2011; Refsum, 2001; Stabler & Allen, 2004). These considerations, plus the increasing trend towards B12 deficiency in those previously not recognised at risk, indicate that testing for B12 deficiency needs undertaking more often in women of childbearing age (Baer & Peter, 2011; Bryan, 2010).

**Dietary intake of B12 and association with B12 biomarkers**

The finding from the VitB12 study that there was a moderate positive association between dietary B12 intake determined from the 30 question B12 food frequency questionnaire (B12FFQ) and B12 biomarkers, is consistent with the report from the FOS (Tucker et al., 2000). In the FOS, if the dietary intake of B12 assessed

from a 126 item FFQ doubled, then plasma B12 increased by 45 pmol/L. While the FOS report meat (not poultry or fish) as making the largest contribution to the B12 intake of the population, findings also showed that plasma B12 concentrations were most closely associated with the quantity of fortified cereals, oral supplements and milk consumed. In a more detailed analysis of the FOS, the third of the study population who reported consuming the most meat, including poultry and fish, had up to three times the intake of B12 compared to the lowest tertiles of dietary B12 intake. This did not translate to differences in B12 plasma concentrations though, as across all the tertiles for plasma concentration, the difference for meat eaters was lower than for cereal and dairy sources of B12. The authors questioned whether B12 in meat is absorbed as efficiently as B12 from supplements, fortified foods and milk, or whether the B12 in meat undergoes degradation during cooking. The FOS does not report the proportion of participants that were vegetarian or non-red-meat eating, and although almost 40% had B12 concentrations less than 258 pmol/l, the mean dietary B12 intake was above 8 µg per day and was not different by age or gender. In contrast to the high dietary B12 intake in the FOS, the median reported dietary intake in the VitB12 study was less than 2.4 µg per day, except for those women who ate red meat (median intake 5.5 µg /day). Using the slightly lower cut–off point of 150 pmol/L to define B12 deficiency (De Benoist, 2008; Herbert, 1994; World Health Organization, 2008) and 222 pmol/L to define insufficiency (L. H. Allen, 2009; Molloy et al., 2009), approximately half of the women in the VitB12 study were insufficient or deficient (Table 8, Chapter 6)

The FFQ tools used to assess intake may account for some of this difference between median B12 dietary intake in the VitB12 study and FOS. The FOS used a detailed FFQ inclusive of a wide variety of foods, and in addition to the food sources high in B12, it may have captured multiple small sources of B12 in miscellaneous food

items. In contrast, the B12FFQ in the VitB12 study focused only on selected food items known to be moderate or high in B12 content. In agreement with the FOS, the highest reported dietary source of B12 in the VitB12 study was red meat, and for those with vegetarian dietary practices, milk and yoghurt. Cereals in New Zealand are not B12 fortified, so these were not a source of dietary B12 (Lesperance, 2009; New Zealand Food Safety Authority, 2010). Some of the difference may be errors in calculation of dietary B12 intake using food composition tables. Food composition tables are a pooled average of the nutrient content from different sources for that food. There may be variability between the nutrient (B12) content in food consumed by study participants and the nutrient (B12) content listed in a food composition table. (Lesperance, 2009)

**Inadequate B12 intake**

There are indications that inadequate dietary B12 intake may be an issue among young women in the New Zealand population. In the 2008/2009 New Zealand Food and Nutrition Survey (2008/9 NZFANS), 23% of women aged 19 to 30 years, and 16% of women aged 30 to 50 years, reported an inadequate dietary B12 intake (University of Otago & New Zealand Ministry of Health, 2011). Women of childbearing age were the only groups to report an inadequate dietary B12 intake. The results for B12 biomarkers are not available yet from the 2008/9 NZFANS to determine whether the reported inadequate intake was associated with B12 deficiency. Vegetarian dietary practices were not directly reported in the 2008/9 NZFANS, but red meat had not been eaten by 8.4% of women aged 15 to 18 years and by 7.7% of women aged 19 to 30 years in the four weeks prior to the survey. Furthermore, the women aged 15 to 18 years and 19 to 30 years were the two groups in the 2008/9 NZFANS who reported the lowest meat, chicken and fish consumption (University of Otago & New Zealand Ministry of Health, 2011).

The dietary pattern in young women needs further investigation to see if low or non-meat-eating dietary practices are becoming more common as indicated by the 2008/9 NZFANS, and if these practices are associated with an increase in the prevalence of B12 deficiency, defined by biomarker status.(Walker, 1995). The trend towards less meat eating may be for economic reasons. The widening income gap between low and high-income households in New Zealand and other OECD countries affects food security, making some foods unaffordable for those at the lower end of the income scale (Ricciuto & Tarasuk, 2007). New Zealand has a widening gap between low and high-income earners; based in the Gini coefficient of income inequality (coefficient of zero is perfect equality, 100 total inequality) New Zealand ranked 33$^{rd}$ to 34$^{th}$ out of OECD countries, with a greater inequality than the median score of 31 (Ministry of Social Development, 2010). In the New Zealand Social Survey 2010, 16% of people reported not having enough income to meet the basic necessities of life such as food and shelter, while 32% reported having just enough (Statistics New Zealand, 2011b). The 2008/2009 NZFANS found that 8.8% of females and 5.6% of males reported low food security: they did not have access to nutritional and safe food and/or the ability to purchase acceptable nutritious food (University of Otago & New Zealand Ministry of Health, 2011).

Both natural and fortified sources of B12 are becoming increasingly expensive, particularly the dairy products that are important as a B12 source for those who do not consume meat. Between May 2007 and May 2008, the price of fresh milk increased by 22% and the price of cheese by 59 % (Statistics New Zealand, 2008a). From June 2008 until June 2011, the price increase was 13% for milk, 12% for cheese and eggs, 12% for meat and poultry and 8% for fish (Statistics New Zealand, 2011a). Price rises such as this, when wages or social benefits remain static or for some decrease, may make it a

challenge for households to afford B12 containing foods (Statistics New Zealand, 2011b).

A simple, non-invasive screen of risk for low B12 consumption would have utility for health professionals working with women of childbearing age. This VitB12 study has highlighted that reported dietary practices (vegetarian versus non-vegetarian) alone, are not sufficient to screen for inadequate dietary B12 intake. The B12FFQ was a useful tool to identify risk for inadequate B12 intake, so a questionnaire like this would be relevant for clinical practice. Using the criterion of recommended daily intake (RDI) dietary B12 intake < 2.4 µg/day (Ministry of Health, 2003), the 30 question B12FFQ which only takes 10 minutes to administer or may be self-completed, accurately identified 9 out of 9 women with serum B12 deficiency and 9 out of 20 with serum B12 insufficiency (Chapter 6, Figure 3). Using the B12FFQ as a screening tool, 8 out of 31 women were false positives, identified with inadequate dietary B12 intake when they actually were sufficient in serum B12. Application of the criterion of 4.0µg RDI for B12 per day to the B12FFQ to detect risk for deficiency identified the risk for 26 out of the 29 women in the VitB12 study who were serum B12 deficient or insufficient. This is relevant for a later discussion where the RDI of B12 is suggested to increase to 4.0µg per day (Bor et al., 2010). The B12FFQ showed similar screening results with holoTC deficiency and dietary B12 intake from the B12 FFQ (Chapter 6, Figure 4).

Of the four women excluded during the VitB12 study due to their general practitioner prescribing a course of IM 1000µg B12 injections, three of these women recorded a dietary B12 intake of less than 2.0µg per day on the B12FFQ, and their serum B12 results prior to supplementation ranged from 119 to 180 pmol/l. This supports that insufficient dietary B12 intake was the probable contributor to B12 deficiency for at least three of them. A readily administered screening tool such as the

B12FFQ may have assisted with the identification of reasons for B12 deficiency. If developed into a screening tool, the B12FFQ could be used by health professionals to screen for inadequate dietary B12 intake or the B12 FFQ plus accompanying calculator could be made available online and used by women to calculate their average daily B12 intake. Users could experiment with increasing their dietary B12 intake by hypothetically increasing quantities of B12 foods entered in the B12FFQ, and calculating the difference it would make to their estimated daily intake. Alternatively, the B12 FFQ and an accompanying calculator could be developed into application software for electronic devices.

There is debate about the validity and reliability of food frequency questionnaires (FFQs) as tools for measuring nutrient intake (Bingham et al., 1997; Cade et al., 2004). Although the quantity and frequency of consumption for a particular nutrient is just an estimate, FFQs still provide a useful snapshot into habitual nutrient intake (Rimm et al., 1992; Willett et al., 1985). The B12FFQ in the VitB12 study was a valid measure of dietary B12 intake; this was supported by moderate positive associations with serum B12 ($r$=0.50, $p$ <0.001, 95 % CI [0.28, 0.67]) and holoTC ($r$=0.55, $p$ <0.001, 95 % CI [0.34, 0.71]) (refer to Chapter 6). However, the B12FFQ has currently only been validated on a small sample of South Asian women living in Auckland, so it would be useful to test the questionnaire on a more diverse population of women to research it's utility for estimating dietary B12 intake in other female populations of childbearing age. The reliability of the B12FFQ has not been established, so this would also need to be measured prior to use in further research.

**Low dose oral B12 supplements associated with a reduction in B12 deficiency**

Daily dietary supplementation with an oral B12 supplement capsule (6mcg) was associated with a statistically significant and clinically meaningful improvement in both

serum B12 (by 30%) and holoTC (by 48%) for women allocated to the B12 supplement group. This has pragmatic clinical importance as it supports the principle that low dose oral B12 supplementation does work, and in addition is a non-invasive, low-cost intervention that women could self-administer to prevent or treat vitamin B12 deficiency. However, more research is needed to identify an appropriate physiological dose and dosing interval that can overcome the problem of lapses in adherence. Although supplementation was effective in increasing B12 status for the B12 supplement group in the VitB12 study, the median [$25^{th}$/$75^{th}$ percentile] increase in serum B12 (31 pmol/L [0.01, 98.5]) was less than half of the mean change in serum B12 in a study with children in Kenya (66 ± 71pmol/L) (Siekmann et al., 2003). It was also less than the reported serum B12 increase in a study undertaken in Pune, India comparing both a 2µg and 10µg of oral cyanocobalamin supplement (Deshmukh et al., 2010). In that study, the reported increase in mean serum B12 over six months was 99 ± 73 pmol/L for the 2 µg dose and167 ± 75pmol/L for the 10 µg dose, with mean supplement adherence above 80% throughout the study. In the VitB12 study however, median adherence decreased over six months and once it dropped from over 80% at two months, to around 60% at six months, there was no further increase in serum B12 or holoTC.

While the 6µg supplement dose selected for this study was physiological, the actual amount of B12 consumed may have been lower if the women overstated their adherence to the supplement or the remaining capsule count was not correct. In the Pune study (Deshmukh et al., 2010) there was an initial improvement in B12 biomarkers in the first four months for both supplement dose groups, but the improvement was static for the remaining eight months of the study even though apparent adherence remained at over 80%. This evidence informs the need to answer research questions such as: “Is a

higher oral dose, still within the physiological range, effective if taken less frequently (e.g., 50µg dose taken once to twice a week)?" and " Would this regime be associated with improved adherence" and "Is this 50 µg dose high enough to increase B12 stores?". Low adherence to daily medications over the longer term is well-documented (Cramer et al., 2008), so a higher dose of B12 supplement may produce an increase in B12 biomarkers, yet still accommodate lapses in adherence. Systematic reviews on biphosphonate medicine adherence, and research on iron supplementation all found that weekly dosing schedules, with flexibility on what to do about missed doses, were associated with higher medicine adherence rates than daily dosing schedules (Beard, 2000; Cramer, Gold, Silverman, & Lewiecki, 2007; de Souza, Batista Filho, Bresani, Ferreira, & Figueiroa, 2009; Silverman & Gold, 2008).

Higher doses may not be proportionally absorbed because B12 carrier proteins in the gastrointestinal (GI) tract saturate at a B12 dose cited as somewhere between 2 µg to 5 µg (Herrmann & Obeid, 2011; Herrmann et al., 2001; Scott, 1997). While 1% to 3% of any dose is still absorbed systemically by passive diffusion, the total percentage of B12 dose absorbed decreases for a few hours until carrier proteins become available again for further transport and absorption (Herrmann & Obeid, 2011; Heyssel, Bozian, Darby, & Bell, 1966). In the FOS, a subgroup of participants who consumed B12 supplements were analysed for relationships between supplement dose and plasma B12, and those who consumed around 6 µg of B12 supplement had similar plasma B12 concentrations to those who consumed 10 to 30 µg (Tucker et al., 2000). However, as described earlier, in the Pune supplementation study (Deshmukh et al., 2010), there were much higher increases in plasma concentrations with the 10 µg, compared with the 2 µg dose. Evidence from these studies suggests a dose somewhere between 2 µg and 10 µg is optimally absorbed (Deshmukh et al., 2010; Tucker et al., 2000). As well as

focusing on dose and dosing interval, the follow on research suggested from the VitB12 study could also focus on behavioural factors and health literacy that may be associated with higher adherence with B12 supplements for South Asian women (Schaefer, 2008).

**Food based approaches to increasing dietary B12 intake and B12 Biomarkers.**

Women in the focus groups expressed frustration at the lack of choices for treating B12 deficiency and with B12 injections as the dominant treatment prescribed. They wanted choices about how to manage their B12 deficiency, in particular about how to increase the amount of B12 in food consumed so they could prevent B12 deficiency. However, this desire did not translate into improved B12 status for the Dietary Advice Group in the VitB12 RCT (Chapter 7, Table 20) or a significant improvement in the percentage of women who were B12 sufficient. Although there was a geometric mean increase of 35% (95% CI 2, 68]) in the daily intake of dietary B12 over the study, this was not sufficient to produce an increase in B12 biomarkers even though the median dietary B12 intake increased from 3.2 µg/day at baseline up to 4.1µg/day at 6 months. It is not clear whether the lack of biomarker response was because the dietary advice group treatment did not translate into increased consumption of B12 foods, or whether the increased B12 foods consumed were ineffective in increasing B12 biomarkers. FFQs can be unreliable (Xinying, 2004), so there may have been inaccuracies in reported B12 dietary intake in the two and six month B12 FFQs. The lack of biomarker response may be because 70% of this group (14 out of 20 women) were already sufficient in B12 at baseline; B12 transport proteins would not up-regulate to increase B12 absorption in the same way they would if there were deficiency (Herrmann et al., 2001; Scott, 1997).

A Florida study of 299 men and women aged 18 to 50 years identified significant correlations between all biomarkers of B12 status and reported B12 dietary

history in the preceding 12 months (Bor et al., 2010). When divided into quintiles of dietary B12 intake, there were corresponding increases in serum B12, holoTC, and decreases in Hcy and MMA until these reached a plateau at a B12 dietary intake of 4 to 7 µg per day. This illustrated that an increase in B12 biomarkers plateaued when B12 status was sufficient, and supported the findings of other studies that between 4 to 7 µg per day was the dietary intake needed to maintain B12 sufficiency (Bor et al., 2006; Bor et al., 2010; Tucker et al., 2000; Vogiatzoglou et al., 2009). The RDI in the VitB12 study of at least 2.4 µg of B12 per day may have been inadequate to increase B12 biomarkers (Bor et al., 2010). Low B12 bioavailability from food may also have been an issue in the VitB12 study. Eggs were one of the main recommended sources for B12 in participants with lacto-ovovegetarian dietary preferences, but the bioavailability of B12 in eggs is low and affected by cooking methods (Levine & Doscherholmen, 1983).

The small increase in dietary B12 intake over the six-month VitB12 study highlights the challenges involved in translating dietary advice into a change in dietary patterns with meaningful improvements in biomarker status. A New Zealand (Dunedin) study on improving folate biomarkers through food-based approaches reports similar challenges with dietary counselling, ongoing support, guidelines, and sample food plans implemented to increase consumption of natural folate rich foods (Riddell, Chisholm, Williams, & Mann, 2000). Although there was a reported increase in dietary folate intake, this did not translate into improved folate biomarkers, with low bioavailability from the folate food sources cited as a possible reason for lack of success (Riddell et al., 2000; Venn et al., 2002).

Studies of successful translation of B12 dietary advice into improved B12 biomarkers were either implemented under controlled research conditions (Siekmann et al., 2003) or relied extensively on foods fortified with B12 (Baer & Peter, 2011; Tucker

et al., 2004). In a Boston study of 189 participants aged 50 to 85 years (random selection so not necessarily B12 deficient) allocated to either a fortified cereal (containing B6, folic acid and B12) or placebo cereal group, the fortified cereal group significantly increased serum B12 and decreased plasma Hcy compared with the control group over 14 weeks (Tucker et al., 2004). The percentage of those serum B12 deficient (B12 <185pmol/l) in the fortified cereal group decreased from 9.7% at baseline to 3.3% at 14 weeks, while the percentage deficient in the control group was static at 11.5% ($p < 0.005$) (Tucker et al., 2004). These findings are similar to the US based study of 137 women low or deficient in B12, where 52 elected to consume cereal fortified with 100% of RDI of B12 daily for three months (Baer & Peter, 2011). Consumption of fortified cereal was sufficient to increase serum B12 in those with a baseline serum B12 of greater than 185 pmol/l, but not enough to reverse deficiency in those with a baseline serum B12 of less than 185pmol/l (Baer & Peter, 2011). In the VitB12 study, increasing B12 intake using foods was not as achievable as in the two previously cited studies. The variety of B12 foods was decidedly limited as cereals and tofu are not fortified in New Zealand, so for those with lactovegetarian dietary preferences, milk, yoghurt and cheese (if eaten), were the only B12 foods consumed. Fortified rice milk, soya milk and marmite were expensive food items or consumed infrequently by South Asian women.

Preventing B12 deficiency through improved dietary B12 intake was a preferred strategy of the community (focus group women) to prevent vitamin B12 deficiency. It is therefore important to follow through on the negative findings of the dietary advice treatment in the VitB12 study. This should involve collaborative research with the South Asian community to establish effective health literacy programs that increase consumption of B12 foods with translation into improved B12 biomarkers. The importance of adhering with traditional foods is documented in literature (Jayanthi,

2001; Sharma et al., 2003) and focus group findings provided insight into some of the challenges South Asian women experience when changing their diet. In particular, the traditional way that food is prepared, and the occasions at which it is consumed, emerged as an integral aspect of culture; there were situations where it was not negotiable to change, and traditional recipes should be adhered to.

> *The only time I would ever use the silver top one [milk]is if you are going to make the shrikhand, the sweet yoghurt that we do at the weddings and then, then it is proper that you need silver top for that...*(Participant 3 [P3], Focus group 2 [FG2])

> *... if you make darselli the sweet, sweet dish we can get away with it, the green one [milk]*. (P4, FG2)

Continuing family traditions with dietary practices and consuming traditional (inside) food was essential, even when practices such as vegetarian food preferences contributed to personal health problems such as iron and B12 deficiency. Focus group discussions included a collective approach to modifying traditional recipes, or traditional techniques for food preparation, so that they still fitted the perception of food that defined each woman's culture. These foods were consistent with traditional methods for food preparation, but were lower in saturated fats and included a higher B12 content.

> *...an option to look at because all of us know how to make it* [yoghurt] *though eh. So the light blue milk or the trim milk is going to take a little bit of the fat out. But remember it has to be heated in hot water, then otherwise it won't set because it is thinner and we have to put more starter into it to stabilize it. But an option we can look at because we need our families to eat the milk but we don't want them to cholesterol, and can make it at home and can make raita... We are getting the calcium intake, we are getting the B12 intake and we are also getting the vegetable intake.*(P2, FG2).

These discussions legitimised changes to traditional recipes and illustrated the collective nature, in particular of the Indian culture, as portrayed in the literature (Mehta & Belk, 1991; Vaidyanathan, 1989). A person's identity is not so much one of self, but

rather the collective identity of the group to which the person belongs. The customs and traditions of the group, the person's relationship within the group, and the obligations created by that relationship, make up the person's identity. Individual identity, needs, or achievements are less salient; respect for greater authority of the group and maintaining the collective identity of the societal group are the priority (Mehta & Belk, 1991; Vaidyanathan, 1989).Two New Zealand studies of migrant Indian Hindu women highlighted the continuity of this collective culture identity, even in the everyday interactions of working and living in New Zealand (Nayar, 2005, 2010).

Based on this concept of collective identity for South Asian (and particularly Indian Hindu) women, the provision of dietary advice to increase B12 intake may have been more successful if the Dietary Advice Group treatment used homogeneous cultural support groups. These groups could have followed similar processes to the focus groups discussions, with women collaborating on how to alter dietary patterns and traditional recipes to provide healthy, B12 containing foods that were still culturally acceptable. It would be useful to include mutual support groups in follow-up research that also assesses readiness of the women for change and dietary counselling (Kumanyika et al., 2000; Molaison, 2002). However, the logistics of implementing research using support groups may be a challenge given the busy family lives that South Asian women have, the diversity of cultural groups, and reservations about research participation within the South Asian community (Rooney et al., 2011).

Research assessing readiness for change could include discussions with participants about the problems associated with B12 deficiency, reasons and motivation to change, and integration of high B12 food consumption into their daily lives (Molaison, 2002). In the VitB12 study, the women in the Dietary Advice Group struggled to plan the increased B12 food consumption patterns around fasting periods or

days, celebration events, or days when they only ate only fruit and vegetables. In future research, as well as providing dietary advice, a collaborative approach for assessing barriers to increasing consumption of B12 foods and then collectively planning how to minimise those barriers is advised (Kumanyika et al., 2000).

Closer attention to health literacy may also improve the success of dietary counselling to increase B12 food consumption. The dietary intervention in the VitB12 study involved a one on one session on how to increase consumption of B12 foods followed by written suggestions (in English) of foods high in B12. This was accompanied by follow-up telephone, email or SMS support from the researcher when required. This may not have been sufficient to improve nutritional health literacy. Delivery of the advice by a South Asian community member, plus culture-specific resource materials in preferred languages may achieve better success (Schaefer, 2008). Advice must be inclusive of the philosophical and cultural underpinnings of dietary practices, and consider the implications of this on the types of resources used to compliment the dietary advice. Focus group findings highlighted the desire for vegetarian-only food resources for nutrients such as B12. These were not available for the VitB12 research, but a peer-reviewed information pamphlet specifically for those on vegetarian diets would address the need for information when the exclusivity of being vegetarian was philosophically fundamental.

It is feasible that for women in the dietary advice group, as well as inadequate B12 intake, there was some underlying impairment of food-bound B12 absorption. B12 is normally bound to food proteins and requires acid hydrolysis in order to unbind and attach to R binder transport proteins. This process is pre-requisite for further B12 transport into the ileum, attachment with intrinsic factor, and systemic absorption. In contrast, the B12 supplement (cyanocobalamin) does not require acid hydrolysis for

absorption as it already exists unbound, so in the situations of low gastric acid release, a cyanocobalamin supplement or food additive is more readily absorbed than naturally food-bound B12 (Andrès et al., 2001; Andrès & Mecili, 2011). High rates of *Helicobacter pylori* and associated gastric atrophy in South Asian populations may contribute to reduced gastric acid hydrolysis with impaired food-bound B12 malabsorption (L. H. Allen, 2008; Khubchandani, Kulkarni, Teckchandani, & Chitale, 2011; Serin et al., 2002). The potential for food-bound B12 malabsorption needs follow up with further research, because if that is the case, then dietary advice needs to promote fortified foods that contain cyanocobalamin for ready absorption without gastric acid hydrolysis (Baik & Russell, 1999; T. J. Green et al., 2004). Systemic absorption of B12 in Asian Indian people was investigated by Bhat et al. (2009) and provided support for intact gastrointestinal B12 absorption, but this was tested using cyanocobalamin supplements. Further research is needed to investigate absorption specifically of food bound B12.

**Body anthropometry and B12 deficiency**

In the VitB12 study, there were no significant relationships between B12 biomarkers and anthropometric measures of body fat percentage (BF%) by bioelectrical impedance analysis (BIA), waist to hip ratio (WHR) or waist to height ratio (WHtR). This differs from Holdsworth Memorial Hospital (HMH) study findings from 519 pregnant women in Mysore, South India, where serum B12 deficiency (<150 pmol/L) was associated with a higher body mass index (BMI), larger sum of skin fold thickness and higher BF% than those without deficiency (Krishnaveni et al., 2009). Deficiency was more prevalent in the HMH study than in the VitB12 study and this may account for some differences in the findings; 42.6% of participants in the HMH study had a serum B12< 150 pmol/L, compared with 15% in the VitB12 study. The low serum B12

results in the HMH study may also be influenced by the physiological decrease in serum B12 that occurs during pregnancy (Morkbak, Hvas, Milman, & Nexo, 2007).

Women in the VitB12 study were relatively homogenous for both body anthropometry characteristics and B12 biomarkers. They clustered at low normal to borderline-low concentrations for B12 biomarkers (Appendix 14, Figure 18*)*, were short in stature with a high BF%, with borderline high WHR and WHtR (Appendix 14, Figure 19).

The lowest body fat recorded in the VitB12 study was 41%, and the highest 61%, so all of the study participants were within the obesity range based on body fat estimated using BIA (Gallagher et al., 2000; Rush, Chandu, et al., 2006; World Health Organization Expert Consultation, 2004). The corresponding BMI measures did not reflect this range of obesity with the lowest BMI recorded at 18 $kg/m^2$ and the highest at 37 $kg/m^2$.

The disparity between BMI and BF% is consistent with other research studies that identified ethnic differences in the BMI and BF% relationship, particularly in South Asian or Asian Indian, who had 8 to10% higher BF% for the same BMI when compared with European populations (Rush, Chandu, et al., 2006; Rush, Freitas, et al., 2009; Rush, Goedecke, et al., 2007). In an Auckland study of 211 adults of Asian Indian ethnicity (110 men and 101 women), designed to develop a prediction equation of fat free mass, the mean BF % of women was 43 %±6.8, while mean BMI was 26.3 ±4.5 $kg/m^2$ (Rush, Chandu, et al., 2006). The disparity between BMI and BF % is higher for women in the VitB12 study with mean body fat percentage of 52 % ± 4.7and mean BMI of 26.4 ±4.5 $kg/m^2$.

For the South Asian population, this study reaffirms that BMI is not a reliable indicator of BF%, or obesity risk for NCD, because it does not represent the central

obesity typical of this population (Sniderman, Bhopal, Prabhakaran, Sarrafzadegan, & Tchernof, 2007; Yajnik & Ganpule-Rao, 2010; Yajnik & Yudkin, 2004). It is this central obesity, rather than total obesity that is the biggest risk for NCD (Despres & Lemieux, 2006). While the World Health Organization (WHO) acknowledges that some ethnic groups have different BMI cut-offs, the BMI cut-offs to indicate risk for overweight (> 25 $kg/m^2$), obese (> 30 $kg/m^2$) or morbidly obese (> 40 $kg/m^2$), have been intentionally kept the same to promote a standardised approach to public health measures for managing these risks (World Health Organization, 2004). This may hinder appropriate health promotion advice for South Asian if BMI is the measure used to reflect BF%. The recommended BMI cut-off for people of South Asian origin to indicate risk for overweight is greater than 23 $kg/m^2$ and for obese, greater than 25 $kg/m^2$ .(Snehalatha et al., 2003). The application of these cut-off points more accurately identifies risk factors for future disease for participants in the VitB12 study, based on body fat percentage as estimated by BIA.

WHR is a suggested measure that has a high sensitivity for detecting central obesity, with the ratio for women recommended as under 0.8 to reduce risk for NCD (de Koning, Merchant, Pogue, & Anand, 2007). Another measurement, the WHtR is recommended as 0.5 or less for reduced risk of central obesity (Ashwell & Shiun Dong, 2005; Garnett, Baur, & Cowell, 2008). The women in the VitB12 study were short in stature and with high normal WHR and WHtR measurements, but these still did not reflect the high BF% results recorded in the study. Instrument error may have contributed to the high BF% results, or error in the waist, hip and height measures although measurements were repeated to within specified tolerance levels to reduce error. In addition, all anthropometry measurements were recorded on three separate occasions over the study, with agreement between measurements. Bioimpedance

measurements were taken early in the morning after overnight fasting to coincide with concurrent fasting blood tests, and since a state of under hydration may alter the doubly indirect fat-free mass calculation, this may have inflated the BF% calculations (Kyle et al., 2004).

### Lipids, insulin resistance and associations with anthropometry and B12 biomarkers

It is surprising in a population with a high percentage of vegetarian and low meat eating dietary practices to find that nearly 75% of the sample population had total cholesterol (TC) and 50 % had low density lipoprotein cholesterol (LDL-C) concentrations greater than recommended by the New Zealand Heart Foundation (New Zealand Guidelines Group, 2009; NHFA/CSANZ, 2005). Traditionally, vegetarian dietary practices are associated with lower concentrations of TC and LDL-C, therefore decreased CVD risk (Panebianco, 2007; Rajaram & Sabaté, 2000). The raised TC and LDL-C results in a predominantly young female population, and the positive associations with high BF% and WHtR, raise concerns about this age and gender population, particularly the younger women who are not traditionally identified as an at risk group. However, the TC/high-density lipoprotein (HDL-C) ratio was still within recommended guidelines. There was no statistically significant association between B12 deficiency and lipid concentrations so the population risks associated with raised TC and LDL-C appeared independent of any population risks associated with B12 deficiency.

Raised triglyceride concentrations and triglyceride/HDL-C ratios are associated with an increased risk of insulin resistance and CVD (Bittner et al., 2009; Cordero et al., 2009; Hellerstein, 2002). Fortunately, in this VitB12 study, at least 75% of the participants were below the recommended limit of 1.7mmol/l for triglycerides (New

Zealand Heart Foundation, 2012), and less than the 1.67 to 1.8 range identified as the triglyceride /HDL-C ratio above which risk for CVD and T2DM increases respectively (Bittner et al., 2009). HDL-C concentrations were also well above the recommended HDL-C concentration of greater than 1 mmol/l for 75% of the participants (New Zealand Heart Foundation, 2012; NHFA/CSANZ, 2005). These at least provide some protection against insulin resistance and risk of T2DM (Bittner et al., 2009). There were no significant associations between B12 concentrations, and triglycerides, triglyceride /HDL-C ratio or HDL-C. Only 13% of the women met the literature cut-off values for insulin resistance (HOMA 2 IR) (Esteghamati et al., 2010; Geloneze et al., 2009; Wallace et al., 2004).

**Life course noncommunicable disease risk for South Asian population**

The women in this VitB12 study had increased risk factors for NCD, but unlike the PMNS and the HMH studies previously cited (Krishnaveni et al., 2009; Yajnik et al., 2008) , the risks from high BF% and B12 deficiency were independent of each other. However, BF% was associated with TC and LDL-C, and close to 75% of the women in the study had increased risk factors for all three of these, thereby increasing risks of CVD in later life (Krishnaveni et al., 2007; NHFA/CSANZ, 2005; Tziomalos, Weerasinghe, Mikhailidis, & Seifalian, 2008).

If the VitB12 study population is representative of South Asian women of childbearing age living in New Zealand, then 50% of the population are insufficient or deficient in B12 and may pass on life-course risk factors for NCD to any children they have. This highlights the importance of preventing B12 deficiency in South Asian women with a long-term view of reducing the intergenerational population risks of noncommunicable disease (NCD)(Yajnik, 2009b).

The public health issue of increased NCD risk is not the only concern. B12 concentrations around 185pmol/l to 222 pmol/l are the cut-offs below which there is increased risk of neural tube defect (NTD) in pregnancy (Groenen et al., 2004; Molloy et al., 2009). The high prevalence of B12 deficiency may increase the population risk for NTD (Ray et al., 2007), and is consistent with the high reported rates of NTD in the South Asian population (Cherian, Siju, Bullock, & Antony, 2005). None of the study sample was low in serum folate, the micronutrient usually the focus of NTD prevention strategies (Krishnaswamy & Nair, 2001; Pitkin, 2007). This suggests that for South Asian women, folate deficiency is less of a concern for early pregnancy compared with B12 deficiency. This finding is consistent with the PMNS, where only one woman out of 797 was low in folate, but 380 (60%) had serum B12 concentrations of less than 150pmol/l (Yajnik et al., 2008).

**Translation of research findings**

This study has shown that it is possible to improve B12 status of South Asian women by providing a daily low dose B12 supplement. However, the feasibility and practicality of daily low dose B12 supplementation requires refinement and a better understanding of the factors influencing adherence.

Based on the research findings of this VitB12 study (both focus group and RCT findings), translation of VitB12 study findings into practice should raise awareness of the problem of B12 deficiency for South Asian women of childbearing age. This involves increased focus on consuming a diet high in B12 containing foods, or taking B12 supplements if dietary practices limit consumption of B12 foods. Translation includes access to suitable vegetarian or vegan only resources for dietary B12 information.

Translation of findings to the practice of the health professionals caring for South Asian women of childbearing age would mean screening and close monitoring for B12 deficiency before women get pregnant. Health professionals would prevent B12 deficiency in those identified at risk using dietary advice or B12 supplementation, and consider the cause of B12 deficiency before selecting evidence based treatment options. Successful translation would have women with deficiency due to inadequate dietary intake offered a choice of treatments appropriate for their cause of deficiency. The VitB12 study only supports that physiological dose oral B12 supplements are associated with an increase in B12 biomarkers in women whose deficiency is due to inadequate intake, but proposed future research will aim to provide evidence for optimal dose and dose frequency. Future research will also provide evidence on models for provision of resources and support to increase dietary B12 including working with the food supply chain. The scope of this thesis was for South Asian women but the principles and findings have applicability to all women of childbearing age who consume low quantities of foods containing vitamin B12.

The challenge is how to effectively translate the VitB12 research findings. There is a wealth of literature highlighting the challenges of translating research evidence into clinical practice (Gabbay & May, 2004; Straus & Haynes, 2009). Research translation moves beyond just dissemination or diffusion of research, to effectively facilitate its use in evidence based practice (Straus, Tetroe, & Graham, 2009). In the case of B12 treatments, research regarding the effectiveness of oral supplementation has been translated into evidence based practices and clinical management guidelines in Sweden and Canada only. (Guidelines and Protocols Advisory Committee, 2012; Lederle, 1998; Loökk et al., 2001; Nilsson et al., 2005; Nyholm et al., 2003).

The documented difficulties in translating research findings into evidence based practice poses challenges when translating the findings of the VitB12 research into current clinical practice and guidelines or into policies such as the government funding of B12 supplements. In New Zealand, pharmacological doses of B12 injections still appear to be the preferred B12 supplement (Mearns, Rush, Koziol-McLain, & Rowan, 2011). The only government funded B12 supplement is hydroxycobalamin, the intramuscular (IM) B12 supplement. There are no government funded oral B12 supplements and the publicly funded oral multivitamin or B vitamin complex do not contain B12 (PHARMAC, 2012). It is difficult to determine reasons for this non-translation of evidence for oral B12 supplementation. It may relate to perceived problems with compliance with oral supplements, particularly with the concern about irreversible neurological manifestations (Carmel, 1998, 2008), or to the perceived low cost and high therapeutic efficacy for increasing B12 stores (Elia, 1998).

Research suggests it is more likely that clinicians are not updating their knowledge about evidence around treatments for B12 deficiency (da Silva, 2003; Graham et al., 2007; Lederle, 1998; Loökk et al., 2001; Nyholm et al., 2003). Studies of research translation identified that healthcare provided by health professionals was based on usual management, or tacit knowledge developed from colleagues' practice experiences, rather than research evidence-based. Time constraints on accessing the large volume of evidence were a major barrier to translation of evidence into practice (Dawes & Sampson, 2003; Gabbay & Le May, 2011; Gabbay & May, 2004; Graham et al., 2006; Straus & Haynes, 2009). Experts in translation of research recommend a planned approach that assesses existing knowledge about treatments; identifies key stakeholders for translation of the evidence and effective platforms for communication of findings to those key stakeholders (such as in conferences, published papers,

community forums). This includes the removal of environmental, social and political barriers to application of the research evidence (Gabbay & Le May, 2011; Graham et al., 2006).

### Assessing existing knowledge about B12 deficiency and treatments

The VitB12 study focus group findings provide insights into South Asian women's knowledge, preferences and assumptions about B12 deficiency treatments. However, the focus group findings, and the number of exclusions from the study due to prior or during-the -study B12 injections, provided only a snapshot into health professional knowledge of B12 deficiency and decision making about treatments.

> *I get tested. I took tablets and they worked and bought it up again so my doctor stopped them. Then my B12 dropped again so my doctor gave me the injection because he said the tablets won't work.* (P5, FG3)

> *They [patients] ask for injections [B12].The results aren't done, but they feel tired and the injections stop them being tired.* (P2, FG6)

Further research investigating health professional's knowledge and attitudes, as well as management of B12 deficiency is the next logical step in following up on the findings of the VitB12 research. This research will be modelled on research by Loökk et al. in Sweden and (2001) and Lederle (1998) in Minneapolis surveying B12 knowledge and treatment practices of physicians and internists. As well as GPs, follow on research from the VitB12 study will include other key health professionals such as practice nurses, health promoters, midwives dieticians and nutritionists.

### Communication of findings to the community, health professionals and policy makers

The previously mentioned health professionals and the South Asian community are target groups for translation of key findings into evidence-based practice for

preventing, detecting and managing B12 deficiency. The findings of the VitB12 study need publishing in peer refereed publications in order to add to the body of knowledge about preventing or managing B12 deficiency. Local dissemination of research findings will include presentations at health professional conferences, plus community forums, workshops, and meetings with key contacts within organisations. Local dissemination is important as studies highlight that doctors and nurses more frequently use tacit knowledge obtained by consultation with colleagues, follow existing practice, read brief summaries of research, attend seminars and conferences or talk with pharmaceutical representatives, rather than referring to research evidence or even practice guidelines to inform the decisions made in clinical practice (Dawes & Sampson, 2003; Gabbay & May, 2004).

The Community Consultation Research Group (CCRG) who advised on, and supported the VitB12 research will assist with continued dissemination of information to the South Asian community. Meetings are underway again with the CCRG to plan this dissemination of the VitB12 research findings. The partnership with the CCRG is important, as focus groups' findings highlighted relationships with trusted key people within the community as integral to being able to work with the community to heighten awareness about detecting, preventing, or managing B12 deficiency. The Asian community in Auckland also has a network of support organisations. One of these, The Asian Network Incorporated (TANI) publishes community letters and organises community forums to discuss health related issues relevant for community members. These resources and forums provide a platform for dissemination of information to the South Asian community, and to health professionals working within these communities. Further research on how best to provide dietary advice and the use of support groups

may provide models for translation of research findings into dietary changes with increased consumption of B12 containing foods.

**Political, social and health context for B12 deficiency**

Environmental, social and political barriers need to be recognised and if possible removed, for example, cost and availability of oral supplements, in order for research evidence to translate successfully into practice. The only NZ government funded B12 supplement for the treatment of B12 deficiency is high-dose, IM B12 injection (PHARMAC, 2012). The lack of a low-dose funded oral supplement does not offer women with low dietary B12 intake, the opportunity for non-invasive, affordable prevention of B12 deficiency. A publicly funded, low dose oral B12 formulation would provide affordable supplementation when risk of deficiency due to inadequate dietary B12 intake exists. It can be justified that prescription of a low dose B12 supplement to a woman at risk from B12 deficiency, or with low borderline B12 status, will prevent progression to deficiency, and avoid the higher health related costs and lower work productivity associated with deficiency. It is not justifiable to prescribe a series of high pharmacological doses of IM supplements to a person without a clear diagnosis of deficiency.

There are documented cases of severe hypersensitivity reactions to IM hydroxycobalamin; however, these are also documented, albeit less severely, to oral cyanocobalamin (Denis, Amin, & Cummins, 1996; Snowden, Chan-Lam, Thomas, & Ng, 1999). Although there are few documented adverse effects from IM hydroxycobalamin or from increasing serum B12 concentration to two to three times the upper recommended normal limit, a lack of evidence of adverse effects does not mean there are not any, or condone prescribing of a drug, and at a dose or route of administration when it is not clinically justified (Mearns et al., 2011; Woodcock, 2011).

In addition, there are clearly documented risks as well as personal discomforts associated with IM injections. Sciatic injury from gluteal injection sites is the biggest risk (Mishra & Stringer, 2010), but excess bruising, bleeding, infection, or nerve injury are documented risks from any anatomical injection site (Greenblatt & Allen, 1978; Velissaris et al., 2009). It begs the question "why inject if it is not necessary?" The research team are proposing a submission to the New Zealand Pharmaceuticals Technical Advisory Committee recommending the funding of an oral vitamin B12 supplement. The IM B12 injection (hydroxycobalamin) preparation (manufacturer price of $6.15 per three ampoules of 1000 µg) is cheaper than current oral B12 supplements available on the consumer market (Blackmores $15.50 per 75 tablets of 50µg, Thompsons $16.95 per 100 tablets of 50µg) (PHARMAC, 2012; Pharmacy Direct, 2012). The total cost of oral supplementation depends on the dosing interval; if the prescribed dose were 50 µg per week, then the previously mentioned oral formulations would last two to three months and cost $5 to $6 per month. Intramuscular injections are more expensive to administer as they carry the additional costs of subsidized practice nurse consultations, injection equipment costs, plus the time, travel and health professional costs to the person receiving the injections. The significant workload requirements of vitamin B12 injections are another consideration when comparing the benefits of government funded IM versus oral B12 supplements (Middleton & Wells, 1985; Nyholm et al., 2003).

Another barrier to translation of research findings is the medicalization of B12 deficiency when it is primarily a nutritional deficiency in South Asian women. In the focus group discussions, women recounted how their doctors [erroneously] explained they were unable to absorb B12 so had to have B12 injections. This information was provided in response to women asking about the reason for deficiency, about B12

containing foods, and whether B12 was available in tablets. A disease-focused approach was evident in the focus group data and the reported frequency of B12 injection administration. This highlights the need for improved health literacy for South Asian women so they have a clear understanding of the reasons and risks for B12 deficiency and other nutrient deficiencies such as iron and options for preventing and managing these.

The medicalisation of B12 deficiency is also a problem for midwifery practice in New Zealand. Registered midwives in New Zealand are authorised to prescribe medicines and supplements for women within their scope of practice (for pregnancy related conditions in the preconception, ante, intra, and post partum periods) (New Zealand College of Midwives, 2009). Midwives currently prescribe iron supplements for iron deficiency, and folic acid supplements for folate deficiency as well as pre-conceptually to decrease the risk of neural tube defects due to insufficient folate. However, there is no similar emphasis on preconception B12 status, and if diagnosed with B12 deficiency, then women are referred to their GP for treatment, even if the probable cause of the deficiency is inadequate B12 intake. Folate and B12 are coenzymes in metabolic pathways, and deficiency of either increases NTD risk and poorer pregnancy outcomes. This highlights an anomaly, where midwives prescribe to prevent the risk of neural tube defect due to folate deficiency, but not the risk due to B12 deficiency. Midwives prescribe supplements for the management of iron and folate deficiency, but not for the management of B12 deficiency.

Folate and iron are included in the routine antenatal blood screen, and given the level of B12 deficiency identified in this VitB12 study and in the previously cited studies, monitoring of B12 status should be added. A small systematic review highlighted the lack of a gold standard test for B12 deficiency (Čabarkapa et al., 2007),

reinforcing the limitations of serum B12 in diagnosing B12 clinical deficiency, particularly at borderline concentrations. HoloTC is a more reliable test to screen for B12 deficiency in pregnancy because there is a physiological decrease in total serum B12 in pregnancy (due to a decrease in haptocorrin), yielding unpredictable test results (Morkbak et al., 2007). At approximately $14.00 plus GST per test, holoTC is an affordable screening test (personal email communication Counties Manukau District Health Board Laboratories), in contrast to MMA at $94.00 plus GST ( personal email communication Canterbury District Health Board) and plasma Hcy at $52.00 plus GST (personal email communication with LabPLUS Auckland District Health Board).

### B12 deficiency and folate fortification

The high percentage of B12 deficiency among South Asian women has implications for policy decisions such as folic acid food fortification. A proposed mandatory folic acid bread fortification policy, scheduled for a May 2012 enactment in New Zealand, was changed to voluntary fortification due to public pressure about fortification. This included concerns about high folate concentrations augmenting or masking the effects of B12 deficiency. The increased risks of undetected cognitive impairment and masking of B12 deficiency by folic acid fortification (and consequent trapping of folate) are considered rare in the general population, as the prevalence of B12 deficiency has historically been low (Food Standards Australia New Zealand, 2006; Morris, Jacques, Rosenberg, & Selhub, 2007; PHARMAC, 2009; Selhub, Morris, Jacques, & Rosenberg, 2009). The high prevalence of B12 deficiency in South Asian women however, makes them at risk from masking of B12 deficiency and exacerbation of CNS effects if accompanied by high folate concentrations. In addition, there may be increased risk of insulin resistance for offspring if these women are low in B12 and high in folate in pregnancy (Yajnik et al., 2008).

There are assurances that the quantity of folic acid fortification is too low to mask B12 deficiency (Food Standards Australia New Zealand, 2006), but there is no guarantee over quantity as it depends on the amount of folic acid fortified food consumed. Furthermore, assurances of the safety of folic acid food fortification relate to the risk of masking B12, and do not account for research associations of offspring risk from maternal B12 deficiency and high folate in pregnancy (Yajnik et al., 2008). The high prevalence of B12 deficiency in the VitB12 study highlights the need to reopen the debate on B12 fortification of food (R. Green, 2010), because for South Asian women, preventing low B12 and high folate may be a modifiable population risk factor for T2DM (Yajnik et al., 2008). Fortification of breads and cereals may not be as effective for South-Asian women who do not consume a traditional New Zealand diet, although lack of adherence to the latter reduces potential risks from folic acid bread fortification. For South Asian women, it may-be more effective to B12 fortify foods such as tofu or rice that are more commonly consumed in the South-Asian diet.

Fortification of affordable, commonly consumed foods with B12 has relevance for other populations of young women, as well as South Asian women. B12 food fortification may reduce B12 deficiency in low-income groups when food insecurity limits intake of foods containing B12. A Canadian study analysed the relationship between income disparities and the nutritional quality of food purchased, and in 2001, there was a 0.7% increase ($p < 0.007$) in the purchase of B12 food for each movement up into the next of eleven successive income brackets (Ricciuto & Tarasuk, 2007). Fortification of commonly consumed foods with B12 makes a difference to B12 status. The FOS found that serum B12 status was significantly higher in participants who ate B12 fortified cereals, than in those who did not eat the fortified cereal (Tucker et al., 2000). This was supported in the US study of 137 women (all non-vegetarian), where

sufficient B12 status was more likely in those who ate fortified cereals, than in those who ate meats and foods naturally high in B12 (Baer & Peter, 2011).

**Reflections on the success of approaches and methods used in the VitB12 study**

The CBPR process and mixed methods design that led to this body of work and its contribution to increased understanding about how to improve B12 status of South Asian women were accompanied by a steep learning curve. The inclusion of the CCRG to guide the research was successful in supporting the development of the research study, the initial recruitment of participants and critical guidance on preliminary conduct of the study. Once the RCT was underway, competing priorities for different CCRG members and for the researcher resulted in a lapse in active participation; however involvement from the CCRG has now reactivated in order to plan dissemination and translation of relevant research findings into the community. In future research, a more sustained approach to support the continued involvement of the CCRG will be promoted. Face to face meetings were used in the initial stages for the CCRG, but in the future, it may be more effective to use internet based resources such as Skype (voice-over-Internet-Protocol software)$^{TM}$ meetings to facilitate communication for busy group members. One of the difficulties for CCRG members was a lack of research funds to compensate them for their expertise and time. One of the team members in particular, was employed in different community based projects, so time spent on the VitB12 study, pulled her away from other paid activities. Another potential member of the CCRG was not able to assist with the VitB12 study because time spent on studies such as the VitB12 study was not part of her job description as a health promoter for the South Asian community. Funding for a CCRG needs to be built into future CBPR, although there is ongoing debate about the issue of payment for community member's participation in research involving their communities, in

particular ethical and fiscal issues such as appropriate honoraria versus volunteer time (Flicker, Travers, Guta, McDonald, & Meagher, 2007).

The methodology of post positivism was appropriate for the VitB12 study research questions. The interpretative philosophy that was used to understand the problem of B12 deficiency from the perspective of the South Asian community, placed the focus of the research onto those issues that were the most salient for community members. The embedded mixed methods approach was an effective approach to use for the research. The RCT results provide strong evidence on the success for oral supplementation to prevent B12 deficiency, with statistics able to support the benefits for those treated and to support estimates of future cost savings from changes in treatment practices. The focus group findings answer the research questions about the importance of dietary practices and their influences of vitamin B12 deficiency, and add depth to the interpretation of the RCT findings, such as analysing why the dietary intervention was not successful. This complexity of knowledge could not have been elicited from one method alone in this study and supports the benefit of a mixed methods approach to answering research questions involving changes in health related behaviours in communities. There were some conflicts though. The collective community philosophy of the South Asian participants in the VitB12 study meant they were keen to be actively involved in the research, to learn the results as they were received, to see the difference that the research was making, and to develop a community organisation that research participants could contribute to. This conflicted with the need for methods that controlled for extraneous influences that could bias the results of the RCT, for group assignment and treatment concealment processes, and for ethical requirements around anonymity and confidentiality. Although participants accepted that this was the way that the research was conducted, the tension between the

individualistic nature of research participation (in the RCT) and the collective philosophy of the South Asian community and of a CBPR approach, are acknowledged as areas that needs to addressed more effectively in future research.

## Strengths and Limitations of B12 Research Project

The identification of strengths and limitations of the research process described in more detail below underpin both the importance of participatory research and the need for a clear vision of what the research process can achieve.

### Strengths

Inclusion of key South Asian community members in the planning and implementation phases of the VitB12 research fostered excellent community interest and active involvement in the research. Focus group discussions with women, mostly of childbearing age, from the South-Asian community augmented that interest and involvement. Once a number of participants from the South Asian community had participated in the research, then there was a more widespread trust and acceptance towards the researcher and the research team and movement towards 'being on the inside'. Women who volunteered for the RCT were committed to participating, and most focused on adhering to the RCT treatments, as many of them had familial experience of the reality of B12 deficiency and were keen to find solutions to address this

The randomised clinical trial incorporated the following strategies to promote rigour and confidence that changes to the dependent variables were associated with the RCT treatments and were not just random or measurement error.

- This trial was registered with Australia New Zealand Clinical Trial Registry (ACTRN12610000262000) and the trial methods approved by expert reviewer(s) from this group before the trial started.
- A robust study design was applied to maximise experimental variance and minimize error variance; a RCT design with a placebo group for comparison and with double blinding of both participants and researcher as to group allocation for the placebo and B12 supplement groups.
- The supplement and placebo capsules were prepared by an independent pharmaceutical compounding company and avoided the bias associated with sponsorship from a recognised nutraceutical company. A registered health professional (dietician) was responsible for the allocation and labelling of treatments A and B for delivery by the researcher.
- The size of the study sample was calculated to power the RCT to detect a clinically and statistically meaningful change in serum B12. A midpoint interim data review provided a checkpoint for assessing that the trial was powered to detect a significant difference.
- Analysis included an 'intention to treat' approach in order to avoid statistical bias from a differential dropout rate between intervention groups, providing a more robust statistical analysis (Polit & Gillespie, 2009).
- The stratified by dietary pattern randomisation process was applied to reduce an uneven spread of meat versus non-meat eaters between the groups that could produce random error and affect the validity of results.
- Blood testing was undertaken by an IANZ registered laboratory (DML) to ensure that measurements were accurate and reliable.

- All anthropometric measurements were in duplicate within specified levels of tolerance using standardized equipment to reduce instrument and measurement bias.
- The supervisor entered the laboratory measurements so that the researcher was not exposed to information that would bias interaction with the participants. Any outliers were scrutinised to ensure the value entered was not due to recording error.
- All statistical analyses were checked and confirmed by a biostatistician (VO) to ensure tests met pre-requisite assumptions, that they were applied appropriately to the data, and reported honestly.
- The six month time frame for the study provided an opportunity to assess sustainability of the interventions as well as efficacy.

**Limitations**

Unlike a drug RCT, there were limitations because of the researcher undertaking multiple roles in the study and the relatively small, closely-knit and very communicative South Asian community.

Participants were a convenience sample recruited purposively through advertisements placed in Indian newspapers, community centres and medical practices, and by word of mouth. There may have been characteristics in this convenience population that differed from the general population of South-Asian women of childbearing age. For example, participants who self-select are more likely to be interested in B12 deficiency or in health related behaviours, so there was potential for selection-bias as these participants may be more likely than the study population to comply with the group treatments

Although participants were stratified and randomised into groups, the number was relatively small and with the best of processes, the stratification strategy was not totally successful as there was an uneven distribution of vegetarian versus non-vegetarian practices between treatment groups as well as unequal baseline distribution of holoTC. Compounding an imbalance in design is the statistical phenomenon of regression to the mean, where extreme scores or measurements will naturally gravitate towards the mean with bigger effects for extreme baseline results than for results closely clustered around the mean at baseline (Bewick, Cheek, & Ball, 2003; Twisk & de Vente, 2008).

Matching on meat and non-meat eating dietary preferences was attempted but became unbalanced, due to the timing of participants enrolling into the study. The non-blinded dietary advice was difficult to administer when other volunteers randomised to capsule treatment were in communication with that particpant, so there was the potential for contamination bias

Resources, particularly time and funding, limited the scope of this VitB12 study. The gold standard biomarkers serum Hcy and MMA are preferred when assessing B12 deficiency as these metabolites reflect alternative metabolic pathways occurring in response to cellular B12 deficiency (Carmel, 2000; Vogiatzoglou et al., 2009). These tests are very expensive in New Zealand, so samples for measuring these metabolites from the VitB12 study are frozen and stored at minus 80 degrees Celsius ready for testing if funding becomes available.

There are limitations to collecting B12 dietary intake information via a FFQ where the questions ask about frequency of food consumption. Under and over reporting of nutritional intake via FFQ is well documented (Kipnis et al., 2003; Rush et al., 2004b; Willett et al., 1985; Willett et al., 2001), and although the FFQ in this B12

study had a moderate association with B12 biomarkers, measurement error could have contributed to some variance in B12 biomarkers between treatment groups. In addition, food composition tables cannot guarantee B12 content documented for a given food, as there is so much variability of B12 content within a given food. The FFQ collected information on foods and quantities eaten, but for simplicity of completion did not include details such as food preparation and cooking, and these affect the bioavailability of B12 (Levine & Doscherholmen, 1983; R. M. Russell et al., 2001; Vogiatzoglou et al., 2009; Watanabe, 2007)

Supplement adherence influenced B12 biomarker response to the B12 supplement treatment. However "remaining capsule count method" was a very crude process for measuring adherence, so the actual relationship between therapeutic efficacy of the 6µg supplement and supplement adherence is not clear. Supplement adherence was a secondary outcome in this study and although there are more rigorous monitoring methods for supplement adherence, these are costly and may have changed the adherence of the participants with treatments so that it did not reflect every day practices (Ingersoll & Cohen, 2008).

The general practitioner prescription of high pharmacological doses of vitamin B12 injections to four participants while the study was in progress was a potential source of bias. These participants were withdrawn subsequent to the injections, but as these four participants had serum B12 results in the lower quartile of study results prior to the IM injections, their data were systematically different from the remaining participant results and therefore not missing at random (Salim, Mackinnon, Christensen, & Griffiths, 2008; Schafer & Graham, 2002).

*Limitations of statistical analysis model*

Correlations used to determine linear relationships between changes in B12 biomarkers and capsule adherence as well as relationships between the change in the B12 dietary intake and change in B12 biomarkers demonstrated positive associations. However, there are only two variables in the correlation models, and the strength of associations may have been derived by another related variable not included in the model. Thus although the correlation coefficients reported imply a positive linear relationship, they cannot do this with absolute certainty and cannot be interpreted as cause and effect (Bewick et al., 2003).

Academic debate exists as to the utility of change scores to distinguish between variance associated with treatment group and variance associated with random error (Lord, 1958; Maxwell & Howard, 1981; Norman, 1989; Peter, Churchill, & Brown, 1993).Although Repeated Measures ANOVA/ANCOVA is the preferred statistical test, and was the pre-planned statistical test proposed for this study, data did not meet pre-requisite assumptions for repeated measures ANOVA. Univariate ANOVA on change scores was the alternate approach supported by the literature for analysis of recurring measures over time (Allison, 1990; Maxwell & Howard, 1981; O'Brien & Kaiser, 1985; Tabachnick & Fidell, 2007; Vickers, 2005). Given the debate over the use of change scores, other methods of analysis may be associated with a slightly different interpretation. However inspection of the data (Appendix 13, Figure 16 & Figure 17) shows that there was an unequivocal change in the supplement group compared with the other two treatments.

**Future research**

Proposed future research studies discussed throughout this chapter, are summarised in Table 1.

Table 25
*Summary of future recommended research*

| Proposed research | Rationale for the research |
|---|---|
| **B12FFQ extended validation study**<br>1. Test the reliability and validity of the B12FFQ and associated Excel spreadsheet for estimating B12 intake of childbearing age women across different ethnicities.<br>2. Descriptive study of B12 biomarker concentrations across the populations above to measure if the prevalence of deficiency/insufficiency extends to the populations mentioned above. | 1. B12FFQ has potential as screening tool for risk of B12 deficiency/ insufficiency in clinical practice, but has only been tested in South Asian women aged 18 to 50 years.<br>2. To investigate if the problem of B12 deficiency/insufficiency identified in the VitB12 study in South Asian women, and international studies (Baer & Peter, 2011; Tucker et al., 2000), extends to other populations of New Zealand women. |
| **RCT of higher weekly dose of supplement**<br>3. A RCT of the efficacy and acceptability of an increased B12 supplement dose taken less frequently e.g. B12 50 µg, taken once per week.<br>4. Study to include analysis of factors influencing adherence. | 3. Adherence affects the efficacy of the low dose B12 supplement. A less frequent dosing schedule may encourage better adherence, with a higher dose of B12 supplied in each capsule, to compensate for the less frequent doses.<br>4. Better understanding of factors influencing adherence enables more effective planning of strategies to support adherence. |
| **2 x 2-crossover study of natural B12 food compared with B12 fortified food.**<br>5. A two by two cross over study to determine if lack of response to dietary advice is due to poor absorption of food bound B12.Two treatment groups: natural B12 foods versus fortified B12 foods, up to an average intake of 4µg per day. Cross over to compare natural food sources for B12 versus fortified food sources, to determine if either is better absorbed than the other.<br>6. Assessment of readiness to change dietary patterns to the B12 dietary treatment B12 (based only on foods eaten by the participants), with a focus on health literacy of B12 foods, delivered by a member of South Asian community and supported by appropriate supplemental resources.<br>7. The treatment groups to use homogenous support groups to discuss how to include recommendations within traditional dietary preferences and pattern. | 5. The lack of B12 biomarker response in the dietary advice group versus response in the B12 supplement group may be due to food bound B12 malabsorption. A higher recommended daily intake of B12 may be required. A 2 x 2-crossover design keeps participant numbers lower and allows comparison between absorption of natural versus fortified B12 food sources, in the same participant.<br>6. Closer attention to readiness to increase B12 in diet, culturally appropriate health literacy and dietary counselling may improve dietary B12 intake<br>7. Individual dietary advice and sample guidelines were not successful in producing an effective increase in B12 dietary intake. A consensus approach, commensurate with the collective identity of different South Asian ethnicities may be more effective. |

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|---|---|
| 8. Focus groups followed by an electronic survey on health professionals' understanding and attitudes around B12 deficiency, and the decision-making processes underpinning management of B12 deficiency. | 8. For effective translation of research findings, it is important to understand current mind-lines and decision-making practices that underpin management(Gabbay & May, 2004).Research based on the surveys undertaken in Sweden(Loökk et al., 2001) and Minneapolis(Lederle, 1998). |
| 9. A longitudinal study following South Asian women through pregnancy. Supplement or increase dietary B12 intake pre conception so that women are B12 sufficient prior to pregnancy. Monitor biomarker status of mother and growth and development of baby. | 9. Monitor for difference in B12 biomarkers and insulin resistance, in mother and child and growth and development of child when mother B12 sufficient. Use findings from PMNS(Yajnik et al., 2008) and HMH study (Krishnaveni et al., 2009) to compare with B12 deficient population of Indian women. |

**Conclusion**

There is an increasing body of evidence linking maternal B12 deficiency in pregnancy with phenotypic changes in the offspring. These changes are, from an early age, associated with the development of insulin resistance and increased central and total adiposity, with exacerbated susceptibility for developing T2DM in adult life. B12 deficiency is a significant problem in South Asian women of childbearing age; half of the women in the VitB12 RCT were low in B12, and 45% reported an inadequate intake of dietary B12 due to cultural preferences for low or no meat consumption. Women in the South Asian community focus groups were aware of the problem of B12 deficiency, but mainly in terms of their own, or their families' experience of a diagnosis of B12 deficiency and high dose B12 injection treatments. The findings of this VitB12 study, that a simple dosing regimen of supplementation can improve B12 status, may have implications for reducing the life course risk for T2DM, and related NCD for the South Asian population. The development of a simple dietary screening tool that allows identification of women most likely at risk, is a time and cost efficient way for a health professional to initiate a discussion about the importance of either including B12 rich

foods in the diet or taking a supplement. This has particular importance for women of childbearing age who may become pregnant or are breastfeeding.

The South Asian community were motivated to address the problem of B12 deficiency, but there was a clear need for improved health literacy around the health risks, causes, prevention and management of B12 deficiency for lay community members, health professionals, health funders, and policy makers. Vitamin B12 is referred to as the ‘exclusive’ vitamin because it is only naturally contained in foods of animal origin. Although a critical cofactor in the folate one-carbon metabolism pathways, B12 is overlooked, or addressed inadequately in the debate, policies and practice translation on screening for micronutrient deficiencies in pregnancy, reducing risk for neural tube defects, folic acid supplementation in pregnancy, proposals for food fortification and treatments for micronutrient deficiency. Instead of being the exclusive vitamin, B12 is the ‘excluded’ vitamin. In order to prevent the life-course risks associated with maternal B12 deficiency in pregnancy, it is time for that to change.

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# Appendices

## Appendix 1: AUTEC Ethics Approval

# M E M O R A N D U M

## Auckland University of Technology Ethics Committee (AUTEC)

| | |
|---|---|
| To: | Elaine Rush |
| From: | **Madeline Banda** Executive Secretary, AUTEC |
| Date: | 23 December 2008 |
| Subject: | Ethics Application Number 08/02 **Feasibility study of vitamin B12 supplementation in Indian women of child-bearing age.** |

Dear Elaine

Thank you for providing written evidence as requested. I am pleased to advise that it satisfies the points raised by a subcommittee of the Auckland University of Technology Ethics Committee (AUTEC) at their meeting on 21 January 2008 and that in consultation with Dr Bell I have approved your ethics application. This delegated approval is made in accordance with section 5.3.2.3 of AUTEC's *Applying for Ethics Approval: Guidelines and Procedures* and is subject to endorsement at AUTEC's meeting on 19 January 2009.

Your ethics application is approved for a period of three years until 23 December 2011.

I advise that as part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online through *http://www.aut.ac.nz/about/ethics*. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 23 December 2011;
- A brief report on the status of the project using form EA3, which is available online through *http://www.aut.ac.nz/about/ethics*. This report is to be submitted either when the approval expires on 23 December 2011 or on completion of the project, whichever comes sooner;

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are reminded that, as applicant, you are responsible for

ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.

Please note that AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to make the arrangements necessary to obtain this. Also, if your research is undertaken within a jurisdiction outside New Zealand, you will need to make the arrangements necessary to meet the legal and ethical requirements that apply within that jurisdiction.

When communicating with us about this application, we ask that you use the application number and study title to enable us to provide you with prompt service. Should you have any further enquiries regarding this matter, you are welcome to contact Charles Grinter, Ethics Coordinator, by email at charles.grinter@aut.ac.nz or by telephone on 921 9999 at extension 8860.

On behalf of the AUTEC and myself, I wish you success with your research and look forward to reading about it in your reports.

Yours sincerely

Madeline Banda

**Executive Secretary**

**Auckland University of Technology Ethics Committee**

# M E M O R A N D U M

## Auckland University of Technology Ethics Committee (AUTEC)

To: Elaine Rush
From: **Madeline Banda** Executive Secretary, AUTEC
Date: 8 October 2009
Subject: Ethics Application Number 08/02 **Feasibility study of vitamin B12 supplementation in Indian women of child-bearing age.**

Dear Elaine

I am pleased to advise that I have approved minor amendments to your ethics application, allowing changes to the recruitment flyers and allowing participant blood samples to be taken in their homes by an experienced phlebotomist, rather than at collection rooms. This delegated approval is made in accordance with section 5.3.2 of AUTEC's *Applying for Ethics Approval: Guidelines and Procedures* and is subject to endorsement at AUTEC's meeting on 9 November 2009.

I remind you that as part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online through *http://www.aut.ac.nz/research/research-ethics*. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 23 December 2011;
- A brief report on the status of the project using form EA3, which is available online through *http://www.aut.ac.nz/research/research-ethics*. This report is to be submitted either when the approval expires on 23 December 2011 or on completion of the project, whichever comes sooner;

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are reminded that, as applicant, you are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.

Please note that AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to make the arrangements necessary to obtain this. Also, if your research is undertaken within a jurisdiction outside New Zealand, you will need to make the arrangements necessary to meet the legal and ethical requirements that apply within that jurisdiction.

When communicating with us about this application, we ask that you use the application number and study title to enable us to provide you with prompt service. Should you have any further enquiries regarding this matter, you are welcome to contact Charles Grinter, Ethics Coordinator, by email at ethics@aut.ac.nz or by telephone on 921 9999 at extension 8860.

On behalf of the AUTEC and myself, I wish you success with your research and look forward to reading about it in your reports.

Yours sincerely

Madeline Banda
**Executive Secretary**
**Auckland University of Technology Ethics Committee**
Cc: Gael Mearns

# M E M O R A N D U M

## Auckland University of Technology Ethics Committee (AUTEC)

| | |
|---|---|
| To: | Elaine Rush |
| From: | **Madeline Banda** Executive Secretary, AUTEC |
| Date: | 20 April 2010 |
| Subject: | Ethics Application Number 08/02 **Feasibility study of vitamin B12 supplementation in Indian women of child-bearing age.** |

Dear Elaine

I am pleased to advise that the Chair of AUTEC has approved minor amendments to your ethics application, allowing changes to the recruitment criteria and, where possible and as requested, for the researcher or the research assistant to take participant blood samples. This delegated approval is made in accordance with section 5.3.2 of AUTEC's *Applying for Ethics Approval: Guidelines and Procedures* and is subject to endorsement at AUTEC's meeting on 10 May 2010.

I remind you that as part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online through *http://www.aut.ac.nz/research/research-ethics*. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 23 December 2011;
- A brief report on the status of the project using form EA3, which is available online through *http://www.aut.ac.nz/research/research-ethics*. This report is to be submitted either when the approval expires on 23 December 2011 or on completion of the project, whichever comes sooner;

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are reminded that, as applicant, you are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.

Please note that AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to make the arrangements necessary to obtain this. Also, if your research is undertaken within a jurisdiction outside New Zealand, you will need to make the arrangements necessary to meet the legal and ethical requirements that apply within that jurisdiction.

When communicating with us about this application, we ask that you use the application number and study title to enable us to provide you with prompt service. Should you have any further enquiries regarding this matter, you are welcome to contact Charles Grinter, Ethics Coordinator, by email at ethics@aut.ac.nz or by telephone on 921 9999 at extension 8860.

On behalf of the AUTEC and myself, I wish you success with your research and look forward to reading about it in your reports.

Yours sincerely

Madeline Banda
**Executive Secretary**
**Auckland University of Technology Ethics Committee**
Cc: Gael Mearns gael.mearns@aut.ac.nz,

**Appendix 2: Focus Group Information Sheet**

# Participant Information Sheet: Focus groups

Date Information Sheet Updated: 07/05/2009

**Project Title**

Feasibility study of vitamin B12 supplementation in South Asian women between 18 and 50 years of age

**An Invitation**

You are invited to participate in community focus groups that will discuss acceptable and affordable ways to increase vitamin B12 intake in Indian women pre-pregnancy. The research is being conducted as a project for a PhD degree at AUT University. My name is Gael Mearns, I am the PhD student and I will be conducting the research under the supervision of Professor Elaine Rush. Participation in the focus groups is voluntary. If you do decide to participate and then change your mind, you can withdraw at any time without any adverse consequences.

**What is the purpose of this research?**

The purpose of the focus groups is to establish discussion between local health professionals and community representatives in order to understand more about motivators and barriers to increasing dietary intake of vitamin B12. The discussion will also include suggestions for increasing compliance with interventions to increase vitamin B12 intake in Indian women. The findings from the focus group discussions will be used to develop strategies to increase vitamin B12 intake in Indian women. These will be implemented in the intervention stage of the feasibility study and will compare whether an increase in dietary intake of vitamin B12 or

vitamin B12 supplements work more effectively to increase body stores of vitamin B12. The results from the feasibility study will be published in relevant health professional publications and presented at conferences to inform health professionals about effective ways to partner with the community to increase vitamin B12 stores in Indian women pre-pregnancy.

**How was I chosen for this invitation?**

We are looking for local health professionals and community representatives living in the Mt Roskill, Mt Albert, and Blockhouse Bay area to participate in the focus groups. If you fit these criteria and you are interested in participating in the focus group discussions, then please contact the researcher or research supervisor.

**What will happen in this research?**

The researcher will contact you to organize a time for the focus groups meeting. There will be 6 participants in each focus group. Focus groups will be facilitated by the researcher. Notes will be taken and if everyone consents, then the focus group discussion will be tape recorded. You can also contribute written comment if you choose to. The researcher will compile a written summary of the focus group discussion and send it back to you to check that the summary of the discussion is correct.

**What are the discomforts and risks?**

The risks are negligible. Participants may be unable to voice their opinions in a group situation

**How will these discomforts and risks be alleviated?**

For those participants who are not able to voice their opinion in the focus group discussion, we will also accept written contributions

**What are the benefits?**

The benefit of this research is that in working with the community, the research team will be able to identify effective and appropriate strategies for increasing vitamin B12 intake in women. Previous studies undertaken with Indian women have identified a link between low vitamin B12 levels in pregnancy and an increased risk of cardiovascular disease and diabetes in the children in later adult life.

This highlights the need to increase vitamin B12 intake in pregnant women or women planning a pregnancy, who may be a risk of low vitamin B12 levels.

**What compensation is available for injury or negligence?**

In the unlikely event of a physical injury as a result of your participation in this study, rehabilitation and compensation for injury by accident may be available from the Accident Compensation Corporation, providing the incident details satisfy the requirements of the law and the Corporation's regulations.

**How will my privacy be protected?**

Your participation in the focus groups will be kept confidential. Any information contributed will not be personally linked back to you. The consent form that members of the focus group sign will acknowledge the need to keep the identity of other focus group members confidential and to maintain the privacy of any information contributed. At the start of the focus group meeting, everyone will be asked to verbally confirm that they will maintain the confidentiality of information contributed and the anonymity of forum group members. Signed consent forms will be kept in a locked filing cabinet in a secure office when they are not being used. All consent forms will be retained for a period of six years following completion of the research and then will be destroyed.

**What are the costs of participating in this research?**

There is no monetary cost to you for participating in the focus groups. Petrol vouchers will be provided to refund the cost of transport to the venue for the focus groups. There is a total time commitment for the focus groups of one hour.

**What opportunity do I have to consider this invitation?**

We are collecting this information over a 6 month period. You have until November 2009 to advise us that you are willing to participate in the focus group discussions

**How do I agree to participate in this research?**

Either email or telephone the researcher or the research supervisor (details given below). We will contact you by phone or email to explain the focus groups in more detail. If you decide to participate, we will forward a consent form for you to sign and return to us. We will then contact you about a suitable time for the focus group meeting.

**Will I receive feedback on the results of this research?**

Yes. You will receive a written summary of the focus group discussion. You will also be provided with a summary of the main research findings after completion of the feasibility study.

**What do I do if I have concerns about this research?**

Any concerns regarding the nature of this project should be notified in the first instance to the Project Supervisor, Professor Elaine Rush 9219999 ext 8091 or elaine.rush@aut.ac

Concerns regarding the conduct of the research should be notified to the Executive Secretary, AUTEC, Madeline Banda, *madeline.banda@aut.ac.nz* , 921 9999 ext 8044.

**Whom do I contact for further information about this research?**

**Researcher Contact Details**

Gael Mearns 9219999 ext 7108 or email gael.mearns@aut.ac.nz

**Project Supervisor Contact Details**

Professor Elaine Rush 9219999 ext or elaine.rush@aut.ac.

***Approved by the Auckland University of Technology Ethics Committee on 23rd December, 2008. AUTEC Reference 08/02***

**Appendix 3: Focus Group Consent Form**

# Consent Form

For use when focus groups are involved..


*Project title:* Feasibility study of vitamin B12 supplementation in Indian women between 18 and 50 years of age

*Project Supervisor:* ***Professor Elaine Rush***

*Researcher:* ***Gael Mearns***

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- I have read and understood the information provided about this research project in the Information Sheet dated 07/05/2009.
- I have had an opportunity to ask questions and to have them answered.
- I understand that identity of my fellow participants and our discussions in the focus group is confidential to the group and I agree to keep this information confidential.
- I understand that notes will be taken during the focus group and that it will also be audio-taped and transcribed.
- I understand that I may withdraw myself or any information that I have provided for this project at any time prior to completion of data collection, without being disadvantaged in any way.
- If I withdraw, I understand that while it may not be possible to destroy all records of the focus group discussion of which I was part, the relevant information about myself including tapes and transcripts, or parts thereof, will not be used.
- I agree to take part in this research.
- I wish to receive a copy of the report from the research (please tick one): Yes○ No○

Participant's signature:

.....……

…

Participant's name:

.....……
…

Participant's Contact Details (if appropriate):

……..

……..

……..

Date:

***Approved by the Auckland University of Technology Ethics Committee on 23rd December, 2008. AUTEC Reference 08/02***

*Note: The Participant should retain a copy of this form.*

## Appendix 4: Study Advertisement Flyers

# Invitation to participate in community focus groups

AUT
AUCKLAND UNIVERSITY OF TECHNOLOGY
TE WĀNANGA ARONUI O TĀMAKI MAKAU RAU

**Vitamin B12 Research**

**Low maternal vitamin B12 in pregnancy is associated with a number of health issues in the mother and baby. South Asian women have been identified as a population who frequently present with low vitamin B12 levels.**

You are invited to participate in community focus groups that will discuss ways to increase vitamin B12 intake in South Asian women. The research is being conducted by Gael Mearns as part of a PhD degree project at AUT University.

If you are a South Asian community member or a health professional working within the South Asian community and you are interested in participating or finding out more about the research, either email or telephone Gael for more details. She will contact you to explain the focus groups in more detail.

**Gael Mearns**
**(09) 921 9999 ext 7108**
**E-mail: gael.mearns@aut.ac.nz**

# Invitation to participate in a study

AUT
AUCKLAND UNIVERSITY OF TECHNOLOGY
TE WĀNANGA ARONUI O TĀMAKI MAKAU RAU

**Are you a South Asian woman, aged between 18 – 50 years?**
**We invite you to participate in a study on increasing vitamin B12 intake in South Asian women.**

**WHAT IS THE RESEARCH ABOUT?**

South Asian women have been identified as a population at risk of low vitamin B12 in their blood. We are looking at ways to increase intake of this essential nutrient for South Asian women by whole foods or supplement capsules.

Participation is voluntary and will not cost you anything. Blood tests will be taken and you will benefit with a free health screen for body composition, blood pressure, anaemia, glucose and lipids. The study involves a total time commitment of approximately 4 hours. Your details will be kept completely anonymous and confidential. You will be given the results of your health screen once the study is completed.

**If you would like more information or would like to take part in this study, please contact:**
**Gael Mearns:**
**(09) 921 9999 ext 7108**
**E-mail: gael.mearns@aut.ac.nz**

## Appendix 5: Focus Group Questions

## Format for focus group interview questions

*Questions:*

**Opening question** (1) Purpose: General question so that participants can introduce themselves.

1. Can you tell me a little about yourself; your name, background, your family and where you originate from ?

**Introductory question** (1) Purpose: To provide a general introduction to the topic of discussion and provide participants an opportunity to reflect on their experiences – definition, explanation or overview

2. Can you tell me what you know about vitamin B12?

**Transition questions** (2-3) Purpose: To move the conversation towards the key questions and help participants envision the topic on a wider scope. If necessary, elaborate on introductory question.

Vitamin B12 is contained in foods derived from living organisms- meat, fish, poultry eggs, milk, yoghurt and yoghurt products, and a form of vitamin B12 is added ( or fortified) to products such as soya milk, rice milk and marmite.

3. Can you tell what foods from these groups would be acceptable to people with vegan, lactovegetarian, lactoovovegetarian and meat eating dietary practices?
4. What are some of the issues around increasing intake of vitamin B12 for people from each of these groups?

**Key questions** (2-5) Purpose: To establish factors that would influence adherence with B12 supplementation or food augmentation. There are key questions that drive the study. May need 10 – 15 minutes for each question

Explain that we are looking at undertaking research to increase B12 levels in women of child bearing age. Research will probably compare a vitamin B12 supplement capsule with advice to increase B12 intake in diet, monitoring B12concentrations form blood test results.

5. Can you tell me some of the issues around taking supplement capsules?
6. Can you suggest ways to encourage women to remember to take the supplement capsule?
7. Are there any other comments or suggestions you would like to make about this type of research approach?
8. What strategies can we use to recruit women and retain them in the trial for the six months that they will be in the study?
9. What are some strategies that we can use to encourage women to increase the quantity of foods in their diet that contain B12?

**Ending question** (2). Purpose: To summarise and gain confirmation on the key messages in the focus group discussions.

10. What are important things that we need to remember when undertaking research with South Asian women?
11. What are effective ways for getting the message about adequate intake of vitamin B12 to future mothers in the community?

**Final question** (1)

12. Is there anything else you would like to add that you think would be useful for us to know?

**Appendix 6: Participant Information for the Randomised Controlled Trial**

# Participant Information Sheet: Intervention study

## Date Information Sheet Produced

8th April, 2010

## Project Title

Feasibility study of vitamin B12 supplementation in South Asian women between 18 and 50 years of age

## An Invitation

You are invited to take part in a research project that investigates ways to increase vitamin B12 levels in South Asian women. Participation in this research is voluntary. If you decide to participate and then change your mind, you can withdraw at any time without adverse consequences.

This research is being conducted as a PhD degree project at AUT University for the researcher Gael Mearns. The project will be conducted under the supervision of Professor Elaine Rush.

## What is the purpose of this research?

This study aims to identify ways to effectively increase vitamin B12 stores in women who may be at risk of low vitamin B12.

Low maternal vitamin B12 in pregnancy is associated with a number of health issues in the mother and baby including an increased risk that the baby may develop diabetes and cardiovascular disease in adult life. The study will compare whether increasing the intake of vitamin B12 containing foods in vegetarian and non-vegetarian diets works more effectively

to increase body stores of vitamin B12 in women than low dose vitamin B12 supplement or placebo capsules. Increasing maternal vitamin B12 stores decreases the health risks associated with low vitamin B12 for mother and baby.

## How was I chosen for this invitation?

If you are a South Asian woman, aged between 18 – 50 years and you live in the Auckland area, then you are invited to participate in the study. The study needs to exclude women who are pregnant, planning a pregnancy during the study period or currently breast feeding, taking the prescription medicine *metformin* or women with chronic disease or major health conditions. Women who have had vitamin B12 injections in the past 12 moths are also excluded.

## What will happen in this research?

We will visit you either in your home or at a local community centre. This research will involve three visits; 1) on study enrolment, 2) at six weeks and 3) at six months post enrolment. At the initial visit, we will measure your height and weight and take measurements to determine your body composition. We will also record your blood pressure. A food frequency diary will be completed where you need to recall foods commonly eaten as well as a diary of food intake in the previous 24 hours. These measurements will be repeated at each visit. In this study you will either be asked to increase the intake of vitamin B12 containing foods in your preferred diet, or take a low dose vitamin B12 supplement or placebo capsule for six months. Depending on the timing of your research visit, the researcher may take the blood tests at the research visit or you will be contacted by the phlebotomy nurse to take the blood samples at another time. Blood tests will be taken on three occasions. The blood tests will test for blood sugar, insulin, lipids, anaemia, vitamin

B12 and folate levels. The blood tests will not cost you anything. You will be informed of your blood results once the study is completed

## What are the discomforts and risks?

There is some minor discomfort associated with the blood tests.

## How will these discomforts and risks be alleviated?

There may be some discomfort when blood is sampled. However the samples will be collected by an experienced phlebotomist or registered nurse in the participant's home or a location of their choice.

## What are the benefits?

The research may help you become more aware of how to increase your vitamin B12 levels and the importance of this vitamin for your health. You will also benefit by being screened for the blood tests mentioned above, body composition and blood pressure. You will receive a report and an explanation of your results at the completion of the study.

The results of this research will be used to plan a larger follow-on study aimed at increasing vitamin B12 levels in South Asian women prior to and during pregnancy. The findings from these studies may identify ways to reduce a number of low-vitamin B12 induced health risks to mother and baby. The findings will be disseminated among South Asian community groups, at community health forums and to health professionals working within the community. The results will also be presented at conferences and published in relevant health professional publications.

## What compensation is available for injury or negligence?

In the unlikely event of a physical injury as a result of your participation in this study, rehabilitation and compensation for injury by accident may be available from the Accident

Compensation Corporation, providing the incident details satisfy the requirements of the law and the Corporation's regulations.

## How will my privacy be protected?

We will use a coding process to keep your identity anonymous. Only the researchers will be aware of your identity in the study. All information will be kept confidential and care will be taken to ensure that any information used in the study cannot personally identify you. Blood results, measurement results, questionnaires and consent forms will be kept in a locked filing cabinet in a secure office when they are not being used. All consent forms and questionnaires will be retained for a period of six years following completion of the research and then will be destroyed.

## What are the costs of participating in this research?

There is no monetary cost to you for participating in the research. Any vitamin B12 supplement or placebo capsules will be provided by us. A phlebotomy nurse will visit you at home or a location of your choice to collect the blood samples. The phlebotomy nurse will contact you to organise a suitable time to visit. There is a total time commitment for participation in the study of approximately four hours. This includes one hour for each of the study visits and twenty minutes for each of the blood test visits.

## What opportunity do I have to consider this invitation?

We are collecting this information over a 6-12 month period. You have until July 2010 to advise us that you wish to participate.

## How do I agree to participate in this research?

Either email or telephone Gael Mearns (details given below). Gael will contact you to arrange a time to speak with you and explain the research in more detail. If you then decide to

participate, you will need to sign a form giving your consent. Gael will bring this form to you at the information meeting or the first visit.

## Will I receive feedback on the results of this research?

Yes. At the end of the study, you will be provided with a copy of your blood results and a report outlining the results of your food questionnaire and your body composition and blood pressure measurements. You will also be provided with a summary of the main research findings after completion of the study.

## What do I do if I have concerns about this research?

Any concerns regarding the nature of this project should be notified in the first instance to the Project Supervisor, Professor Elaine Rush 9219999 ext 8091 or elaine.rush@aut.ac.nz

Concerns regarding the conduct of the research should be notified to the Executive Secretary, AUTEC, Madeline Banda, *madeline.banda@aut.ac.nz* , 921 9999 ext 8044.

## Whom do I contact for further information about this research?

### Researcher Contact Details

Gael Mearns 9219999 ext 7108 or email gael.mearns@aut.ac.nz

### Project Supervisor Contact Details

Professor Elaine Rush 9219999 ext 8091or elaine.rush@aut.ac.

Approved by the Auckland University of Technology Ethics Committee on 23rd December, 2008, AUTEC Reference number *08/02*

**Appendix 7: Participant Consent Form for the Randomised Controlled Trial**

# Consent Form

For use when laboratory or field testing is involved.

*Project title:* Feasibility study of vitamin B12 supplementation in South Asian women between 18 and 50 years of age

*Project Supervisor:* ***Professor Elaine Rush***

*Researcher:* ***Gael Mearns***

---

- I have read and understood the information provided about this research project in the Information Sheet dated 08/04/2010.
- I have had an opportunity to ask questions and to have them answered.
- I understand that I may withdraw myself or any information that I have provided for this project at any time prior to completion of data collection, without being disadvantaged in any way.
- I am not suffering from heart disease, diabetes mellitus, or any illness, injury or infection that impairs my physical performance.
- I agree to provide blood samples.
- I agree to take part in this research.
- I wish to receive a copy of the report from the research (please tick one): Yes○No○

Participant's signature:

.....

…

Participant's name:

.....

…

Participant's Contact Details (if appropriate):

……..

……..

Date: ***Approved by the Auckland University of Technology Ethics Committee on 23rd December, 2008. AUTEC Reference 08/02*** *Note: The Participant should retain a copy of this form.*

**Appendix 8. Process for Blood Sampling**

The process for blood sampling was as follows:

1. Blood vacutainers were labelled with participant ID prior to taking blood sample.
2. Universal precautions were employed throughout the procedure. The researcher or phlebotomist wore gloves when taking blood samples.
3. Researcher or phlebotomist hands were disinfected with hand gel prior to commencing procedure.
4. The procedure was explained and the participant was made comfortable with arm outstretched. Both arms were inspected to find the most suitable vein.
5. The choice of vein was in the antecubital fossa, although for some participants, an alternative vein in the forearm was selected due to difficult venous access.
6. The vacutainer needle device was inserted into the vacutainer holder with the participant end of the needle left capped.
7. The first vacutainer tube was placed in the vacutainer holder. The order of vacutainer tubes was gold, grey, mauve then green.
8. The tourniquet was applied 5-7 cm above the venepuncture site on the selected vein and the vein visualised.
9. If the vein was not easily visualised, then the participant was instructed to squeeze their fist several time until vein became more distended.
10. The researcher or phlebotomist applied gloves
11. The area around the venepuncture site was wiped in a circular motion with an alcohol swab, and then allowed to dry.
12. The needle cover was removed

13. Researcher or phlebotomist used thumb of left hand to stabilise the vein below the level of the venepuncture suite and to pull the skin taut over the venepuncture site.
14. The needle was inserted at a 15-30 degree angle, bevel side up, into the vein.
15. The vacutainer holder was stabilised with the left hand and using the right hand the vacutainer tube was advanced into the vacutainer unit the rubber bung pierced and blood flowed back into the vacutainer tube.
16. If blood did not immediately flow into the tube the needle was advanced slightly further into the vein.
17. Once blood flowed freely into the vacutainer tube, the tourniquet was released.
18. Once filled, each vacutainer tube was removed and the successive vacutainer tube was advanced into the vacutainer holder.
19. The order for filling vacutainers was SST, Fluoride, EDTA, then Heparin
20. Once all vacutainer tubes were filled and removed, the needle was withdrawn and a gauze swab placed over the venepuncture site and held firmly.
21. The participant was instructed to apply firm pressure over the site.
22. The needle and vacutainer were disposed of as a complete unit into a sharps disposal unit.
23. The vacutainer tubes were gently rotated to ensure sufficient mixing of blood with the tube additives.
24. The labelling on the tube was double checked, and then the vacutainer tubes were inserted into the plastic biohazard sample bag. The laboratory request form was inserted into the sleeve of the biohazard bag.
25. The Heparin and EDTA tube for centrifuging were inserted into separate biohazard bag with an icepack to keep the Heparin tube cold until centrifuging.

26. The venepuncture site was inspected to ensure no further bleeding from the site and then a plaster dressing was applied.
27. The participant was advised not to lift anything heavy with the arm the sample was drawn from and to avoid strenuous activity with the arm for the next two to three hours.
28. The participant was also advised to remove the plaster dressing later that same day.
29. If the venepuncture was unsuccessful either the phlebotomist would visit to take the blood sample or the participant was referred to DML blood collection rooms.
30. The samples for testing by Diagnostic Medlab were delivered within 1-3 hours of sampling to either that Diagnostic Medlab testing laboratory in Mount Wellington Auckland , or they were delivered to the Milford (North Shore City) Diagnostic Medlab community collection centre for delivery to Diagnostic Medlab testing laboratory by the DML courier.

**Appendix 9: Questionnaire and Data Collection Form**

| B12 STUDY SOUTH ASIAN WOMEN 18 - 50 YEARS<br>DATA COLLECTION SHEET | ID |
|---|---|
| Date | |
| Age: | |
| Date of Birth | |
| Children: age | |
| Marital status | |
| Occupation | |
| Ethnicity | |
| Religion | |
| Parents ethnicity | |
| Maternal grandparents ethnicity | |
| Paternal grandaprents ethnicity | |
| Years in NZ | |
| Diet preference | |
| List of food avoided | |
| Mother's diet preference | |
| Menstrual Cycle | |

| Medical and family history | | Self | | Family |
|---|---|---|---|---|
| Diabetes | | | | |
| Anaemia | | | | |
| Heart disease | | | | |
| Numbness, tingling or nerve damage | | | | |
| Stroke | | | | |
| High blood pressure | | | | |
| Blood clotting disorders | | | | |
| High cholesterol | | | | |
| Thyriod disease | | | | |
| Osteoporosis | | | | |
| Cancer | | | | |
| Respiratory disease | | | | |
| Pregnancy - any problems | | | | |
| Significant Injury | | | | |

| | | | | |
|---|---|---|---|---|
| **Medications:** | | | | |
| **Supplements** | | | | |
| | | | | |
| **Blood Pressure / Resting Pulse** | | | | |
| | Reading 1 | Reading 2 | Reading 3 | Average |
| Systolic | | | | |
| Diastolic | | | | |
| Pulse | | | | |
| **Height&Weight** | | | | |
| | Reading 1 | Reading 2 | Reading 3 | Average |
| Height | | | | |
| Weight | | | | |
| Body Mass Index | | | | |
| **Girths** | | | | |
| | Reading 1 | Reading 2 | Reading 3 | Average |
| Mid-Upper Arm | | | | |
| Waist | | | | |
| Hips | | | | |
| Waist/height ratio | | | | |
| Waist/hip ratio | | | | |
| **Grip Strength** | | | | |
| | Reading 1 | Reading 2 | Reading 3 | Highest |
| Hand (R) | | | | |
| Hand (L) | | | | |
| **Bioimpedance** | | | | |
| | Reading 1 | Reading 2 | Reading 3 | Average |
| Impedance | | | | |
| Phase | | | | |
| Resistance | | | | |
| Reactance | | | | |

**Appendix 10: Example of Vitamin B12 Dietary Recommendations**

**Participant:** **Date:**

**Recommended daily intake of vitamin B12 is 2.4 mcg per day**

In order to reach a dietary intake of 2.4 mcg vitamin B12 per day, you need to eat four servings from either dairy or fortified foods.

**DAIRY: Aim for two servings <u>per day</u> from the following foods**

| **Servings** | **Vitamin B12 (mcg)** | |
|---|---|---|
| Yoghurt drink e.g lassi or smoothie | 1 cup | 0.3 |
| Yoghurt x 150 ml pottle | per 150ml | 0.5 |
| Cottage cheese or curd x 1/2 cup | per 1/2 cup | 0.7 |
| Milk or flavoured milk x 1 cup (preferably green or light blue top) | 1 cup | 0.8 |
| Cheese x 25 g (maximum of 2-3 times per week) | per 25g | 0.2 |
| Ice cream (maximum of 2-3 times per week) | 1 cup | 0.8 |

**FORTIFIED PRODUCTS: Aim for two servings <u>per day</u> from the following foods**

| **Servings** | | **Vitamin B12 (mcg)** | |
|---|---|---|---|
| Marmite x 1 teaspoon | | 1 tsp | 0.7 |
| Imagine ENRICHED Soy milk | | 1 cup | 1.2 |
| Sanitarium essential UHT So Good Soy Milk | | 1 cup | 1.0 |
| Complan made with water | (per one scoop) | one scoop | 0.5 |
| Complan made with milk | (per one scoop plus 250ml milk) | one scoop | 1.3 |
| Up and go cereal drink | | 250ml | 0.9 |

* Source for vitamin B12 content and recommendations:
Foodworks © 2007 version 5
MOH and Plant & Food Research Concise NZ Food Composition Tables 8th edition © 2009

## EXAMPLES OF DAILY INTAKE OF B12

| Sample daily B12 intake (vegetarian) | |
|---|---|
| One 250ml cup trim milk | 0.8mcg |
| One 250ml cup of soy milk | 1.2mcg |
| One 150ml pot of yoghurt | 0.5mcg |
| **TOTAL B12 intake for the day** | **2.5 mcg** |

| Sample daily B12 intake (vegetarian) | |
|---|---|
| Complan 1 scoop with milk | 1.3 mcg |
| Marmite 1 tsp | 0.7mcg |
| One 150ml pot yoghurt | 0.5mcg |
| **TOTAL B12 intake for the day** | **2.5 mcg** |

| Sample daily B12 intake (vegetarian) | |
|---|---|
| 25 gm cheese | 0.2mcg |
| One 250ml cup trim milk | 0.8mcg |
| Up and go cereal drink | 0.9mcg |
| One 150 ml pot yoghurt | 0.5mcg |
| **TOTAL B12 intake for the day** | **2.4 mcg** |

| Sample daily B12 intake (vegetarian) | |
|---|---|
| 25 gm cheese | 0.2mcg |
| One cup of lassi or smoothie | 0.3mcg |
| One cup of soy milk | 1.2mcg |
| One small cheese muffin | 0.2mcg |
| One 250ml cup ice cream | 0.6mcg |
| **TOTAL B12 intake for the day** | **2.5 mcg** |

**Appendix 11: Vitamin B12 Food Frequency Questionnaire (B12FFQ)**

# *Vitamin B12 study*
# *Vitamin B12 Foods Questionnaire*

***GENERAL INSTRUCTIONS***

- **Answer each question as best you can. Estimate if you are not sure. A guess is better than leaving a blank.**
- **Put an X in the box next to your answer.**
- **If you make any changes, cross out the incorrect answer and put an X in the box next to the correct answer. Also draw a circle around the correct answer.**
- **If you mark NEVER, NO, or DON'T KNOW for a question, please follow any arrows or instructions that direct you to the next question.**

*Questions appear on the next page*

## DAIRY

**Over the <u>past 3 months</u>…**

1. How often did eat **yoghurt (Please note curd included separately in question below)**?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month
c☐ 2–3 times per month
d☐ 1 -2 times per week
e☐ 3–4 times per week
f☐ 5–6 times per week
g☐ 1 time per day
h☐ 2 times per day
i☐ 3 times per day
j☐ 4 or more times per day

1a. Each time you ate **yoghurt** how much did you usually consume?

a ☐ Less than ½ cup
b ☐ ½ to 1 cup
c ☐ 1 to 2 cups
d ☐ More than 2 cups

1b Full cream or reduced fat yoghurt?

a ☐ Full cream
b ☐ Reduced fat

2. How often did you drink **yoghurt drinks (e.g.lassi, smoothies, raiti) ?**

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month or less
c☐ 2–3 times per month
d☐ 1–2 times per week
e☐ 3–4 times per week
f☐ 5–6 times per week
g☐ 1 time per day
h☐ 2–3 times per day
i☐ 4–5 times per day
j☐ 6 or more times per day

2a.Which type of **yoghurt drink** did you most commonly drink?

Please specify below

……..

2b. Each time you drank **yoghurt drinks**, how much did you usually drink?

a☐ Less than ¾ cup (200mL)
b☐ ¾ to 1¼ cups (200 to 300mL)
c☐ More than 1¼ cups (300mL)

3. How often did eat **curd, paneer, cottage cheese or quarg** (full or reduced fat)?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month
c☐ 2–3 times per month
d☐ 1 -2 times per week
e☐ 3–4 times per week
f☐ 5–6 times per week
g☐ 1 time per day
h☐ 2 times per day
i☐ 3 times per day
j☐ 4 or more times per day

3a. Each time you ate **curd, cottage cheese or quarg** how much did you usually consume?

a ☐ Less than ½ cup
b ☐ ½ to 1 cup
c ☐ 1 to 2 cups
d ☐ More than 2 cups

3b Full cream or reduced fat **curd, cottage cheese or quarg**?

a ☐ Full cream
b ☐ Reduced fat

4. How often did eat **cheese** (full or reduced fat)?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month
c☐ 2–3 times per month
d☐ 1 -2 times per week
e☐ 3–4 times per week
f☐ 5–6 times per week
g☐ 1 time per day
h☐ 2 times per day
i☐ 3 times per day
j☐ 4 or more times per day

4a. Each time you ate **cheese** how much did you usually consume?

a ☐ Less than 10g
b ☐11-20g
c ☐ 21-30gm
d ☐ 31-40gm

5. How often did you eat **eggs**?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month
c☐ 2–3 times per month
d☐ 1 -2 times per week
e☐ 3–4 times per week
f☐ 5–6 times per week
g☐ 1 time per day
h☐ 2 times per day
i☐ 3 times per day
j☐ 4 or more times per day

5a. Each time you ate **eggs**, how much did you usually consume?

a☐ Less than 1
b☐ 1 egg
c☐ 2 eggs
d☐ More than 2 eggs

6. How often did eat **cream cheese or sour cream** (full or reduced fat)?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month
c☐ 2–3 times per month
d☐ 1 -2 times per week
e☐ 3–4 times per week
f☐ 5–6 times per week
g☐ 1 time per day
h☐ 2 times per day
i☐ 3 times per day
j☐ 4 or more times per day

6a. Each time you ate **cream cheese or sour cream** how much did you usually consume?

A ☐ Less than ½ cup
b ☐ ½ to 1 cup
c ☐ 1 to 2 cups
d ☐ More than 2 cups

6b. Full cream or reduced fat **cream cheese or sour cream**?

a ☐ Full cream
b ☐ Reduced fat

7. How often did eat **cream**?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month
c☐ 2–3 times per month
d☐ 1 -2 times per week
e☐ 3–4 times per week
f☐ 5–6 times per week
g☐ 1 time per day
h☐ 2 times per day
i☐ 3 times per day
j☐ 4 or more times per day

7a. Each time you ate **cream ( non whipped)** how much did you usually consume?

A ☐ Less than ¼ cup
b ☐ ¼ to ½ cup
c ☐ ½ to 1 cup
d ☐ More than 1 cup

8. How often did eat **ice cream** (full or reduced fat)?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month
c☐ 2–3 times per month
d☐ 1 -2 times per week
e☐ 3–4 times per week
f☐ 5–6 times per week
g☐ 1 time per day
h☐ 2 times per day
i☐ 3 times per day
j☐ 4 or more times per day

8a. Each time you ate **icecream** how much did you usually consume?

A ☐ Less than ¼ cup
b ☐ ¼ to ½ cup
c ☐ ½ to 1 cup
d ☐ More than 1 cup

8b. Full cream or reduced **ice cream**?

a ☐ Full cream
b ☐ Reduced fat

9. Over the <u>past 3 months</u>, how often did you drink **milk or flavoured milk** (full, reduced or low fat)?

☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month or less
c☐ 2–3 times per month
d☐ 1–2 times per week
e☐ 3–4 times per week
f☐ 5–6 times per week
g☐ 1 time per day
h☐ 2–3 times per day
i☐ 4–5 times per day
j☐ 6 or more times per day

9a. Each time how much did you usually drink?

A ☐ Less than ¼ cup
b ☐ ¼ to ½ cup
c ☐ ½ to 1 cup
d ☐ More than 1 cup

9b. Full, reduced or low fat **milk**?

a ☐ Full cream
b ☐ Reduced fat
c ☐ Low fat

10. Over the <u>past 3 months</u>, how often did you drink **meal replacement drinks** e.g Up & Go?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month or less
c☐ 2–3 times per month
d☐ 1–2 times per week
e☐ 3–4 times per week
f☐ 5–6 times per week
g☐ 1 time per day
h☐ 2–3 times per day
i☐ 4–5 times per day
j☐ 6 or more times per day

10a. Which type of **meal replacement drinks** did you most commonly drink?
Please specify below

……

10b. Each time you drank **meal replacement drinks**, how much did you usually drink?

a☐ Less than one 250 ml carton
b☐ One 250 ml carton
c☐ Two 250 ml cartons

**MEATS**
**Over the <u>past 3 months</u>…**

11. How often did you eat **beef**?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1–6 times per year
c☐ 7–11 times per year
d☐ 1 time per month
e☐ 2–3 times per month
g☐ 2 times per week
h☐ 3–4 times per week
i☐ 5–6 times per week
j☐ 1 time per day

f ☐ 1 time per week
k ☐ 2 or more times per day

11a. Each time you ate **beef**, how much did you usually eat?

a ☐ Half a 50g steak or less than 1/2 cup
b ☐ One 50g steak or less than 1 cup
c ☐ One 100g steak or 1 to 2 cups
d ☐ One 200g steak or more than 2 cups

12. How often did you eat **lamb**?

a ☐ NEVER (GO TO NEXT QUESTION)

b ☐ 1–6 times per year
c ☐ 7–11 times per year
d ☐ 1 time per month
e ☐ 2–3 times per month
f ☐ 1 time per week
g ☐ 2 times per week
h ☐ 3–4 times per week
i ☐ 5–6 times per week
j ☐ 1 time per day
k ☐ 2 or more times per day

12a. Each time you ate **lamb**, how much did you usually eat?

a ☐ One small chop or less than 1/2 cup
b ☐ One medium chops or less than 1 cup
c ☐ Two medium chops or 1 to 2 cups
d ☐ More than two medium chops or 2 cups

13. How often did you eat **goat**?

a ☐ NEVER (GO TO NEXT QUESTION)

b ☐ 1–6 times per year
c ☐ 7–11 times per year
d ☐ 1 time per month
e ☐ 2–3 times per month
f ☐ 1 time per week
g ☐ 2 times per week
h ☐ 3–4 times per week
i ☐ 5–6 times per week
j ☐ 1 time per day
k ☐ 2 or more times per day

13a. Each time you ate **goat**, how much did you usually eat?

a ☐ Less than 1/2 cup
b ☐ Less than 1 cup
c ☐ 1 to 2 cups
d ☐ More than 2 cups

14.How often did you eat **pork**?

a ☐ NEVER (GO TO NEXT QUESTION)

b ☐ 1–6 times per year
c ☐ 7–11 times per year
d ☐ 1 time per month
e ☐ 2–3 times per month
f ☐ 1 time per week
g ☐ 2 times per week
h ☐ 3–4 times per week
i ☐ 5–6 times per week
j ☐ 1 time per day
k ☐ 2 or more times per day

14a. Each time you ate **pork**, how much did you usually eat?

a ☐ Less than 1/2 cup
b ☐ Less than 1 cup
c ☐ 1 to 2 cups
d ☐ More than 2 cups

15 . How often did you eat **liver**?

a ☐ NEVER (GO TO NEXT QUESTION)

b ☐ 1–6 times per year
c ☐ 7–11 times per year
d ☐ 1 time per month
e ☐ 2–3 times per month
f ☐ 1 time per week
g ☐ 2 times per week
h ☐ 3–4 times per week
i ☐ 5–6 times per week
j ☐ 1 time per day
k ☐ 2 or more times per day

15a. Each time you ate **liver**, how much did you usually eat?

a ☐ Less than 1/4 cup
b ☐ Less than 1/2 cup
c ☐ 1 cup
d ☐ 1 to 2 cups

16. How often did you eat **kidney**?

a ☐ NEVER (GO TO NEXT QUESTION)

b ☐ 1–6 times per year
c ☐ 7–11 times per year
d ☐ 1 time per month
e ☐ 2–3 times per month
f ☐ 1 time per week
g ☐ 2 times per week
h ☐ 3–4 times per week
i ☐ 5–6 times per week
j ☐ 1 time per day
k ☐ 2 or more times per day

16a. Each time you ate **kidney**, how much did you usually eat?

a ☐ Less than 1/4 cup
b ☐ Less than 1/2 cup
c ☐ 1 cup
d ☐ 1 to 2 cups

17. How often did you eat **shellfish**?

a ☐ NEVER (GO TO NEXT QUESTION)

b ☐ 1–6 times per year
c ☐ 7–11 times per year
d ☐ 1 time per month
e ☐ 2–3 times per month
f ☐ 1 time per week
g ☐ 2 times per week
h ☐ 3–4 times per week
i ☐ 5–6 times per week
j ☐ 1 time per day
k ☐ 2 or more times per day

17a. Each time you ate **shellfish**, how much did you usually eat?

a ☐ 1-2 shellfish
b ☐ 3-4 shellfish
c ☐ 5-6 shellfish
d ☐ more than 7 shellfish

17b. What shellfish did you usually eat?

a ☐ Oysters
b ☐ Mussels
c ☐ Prawns
d ☐ Shrimps
d ☐ Scallops
e ☐ Paua

18. How often did you eat **salmon or tuna**?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1–6 times per year
c☐ 7–11 times per year
d☐ 1 time per month
e☐ 2–3 times per month
f☐ 1 time per week
g☐ 2 times per week
h☐ 3–4 times per week
i☐ 5–6 times per week
j☐ 1 time per day
k☐ 2 or more times per day

18a. Each time you ate **salmon or tuna**, how much did you usually eat?

a ☐ Less than 1/2 cup
b ☐ Less than 1 cup
c ☐ 1 to 2 cups
d ☐ More than 2 cups

19. How often did you eat **sardines**?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1–6 times per year
c☐ 7–11 times per year
d☐ 1 time per month
e☐ 2–3 times per month
f☐ 1 time per week
g☐ 2 times per week
h☐ 3–4 times per week
i☐ 5–6 times per week
j☐ 1 time per day
k☐ 2 or more times per day

19a. Each time you ate **sardines**, how much did you usually eat?

a ☐ 1 sardine
b ☐ 2 sardines
c ☐ 3 sardines
d ☐ 4 or more sardines

20. How often did you eat **white fish**?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1–6 times per year
c☐ 7–11 times per year
d☐ 1 time per month
e☐ 2–3 times per month
f☐ 1 time per week
g☐ 2 times per week
h☐ 3–4 times per week
i☐ 5–6 times per week
j☐ 1 time per day
k☐ 2 or more times per day

20a. Each time you ate **fish**, how much did you usually eat?

a ☐ ½ fillet
b ☐ 1 fillet
c ☐ 1½ fillets
d ☐ 2 fillets

21. How often did you eat **chicken**?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1–6 times per year
c☐ 7–11 times per year
d☐ 1 time per month
e☐ 2–3 times per month
f☐ 1 time per week
g☐ 2 times per week
h☐ 3–4 times per week
i☐ 5–6 times per week
j☐ 1 time per day
k☐ 2 or more times per day

21a. Each time you ate **chicken**, how much did you usually eat?

a ☐ One small tenderloin or less than 1/2 cup
b ☐ One breast fillet or less than 1 cup
c ☐ Two breast fillets or 1 to 2 cups
d ☐ More than two breast fillets or 2 cups

**(OTHER) Fortified products**

22. Over the <u>past 3 months</u>, how often did you drink **soy milk ?**

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month or less
c☐ 2–3 times per month
d☐ 1–2 times per week
e☐ 3–4 times per week
f☐ 5–6 times per week
g☐ 1 time per day
h☐ 2–3 times per day
i☐ 4–5 times per day
j☐ 6 or more times per day

22a. Which brand of **soy milk** did you most commonly drink?

b☐ Sanitarium So Good
c☐ Home brand
d☐ Vita Soy
e☐ Get Natural
f☐ Pams Soy Milk
g☐ Signature Range
h☐ Other ( please specify below)

22b. Each time you drink **soy milk**, how much did you usually drink?

a☐ Less than ¾ cup (200mL)
b☐ ¾ to 1¼ cups (200 to 300mL)
c☐ More than 1¼ cups (300mL)

23. Over the <u>past 3 months</u>, how often did you drink **rice milk**?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month or less
c☐ 2–3 times per month
d☐ 1–2 times per week
e☐ 3–4 times per week
f☐ 5–6 times per week
g☐ 1 time per day
h☐ 2–3 times per day
i☐ 4–5 times per day
j☐ 6 or more times per day

23a. Which brand of **rice milk** did you most commonly drink?

a☐ Imagine rice dream
b☐ Home brand rice milk
c☐ Vita soy rice milk
d☐ Signature range
e☐ Vita soy rice milk
f☐ Other

23b. Each time you drank **rice milk**, how much did you usually drink?

a☐ Less than ¾ cup (200mL)
b☐ ¾ to 1½ cups (200-300mL)
c☐ More than 1½ cups (300mL)

24. How often did eat **tofu** ?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month
c☐ 2–3 times per month
d☐ 1 time per week
e☐ 2 times per week
f☐ 3–4 times per week
g☐ 5–6 times per week
h☐ 1 time per day
i☐ 2 times per day
j☐ 3 times per day
k☐ 4 or more times per day

24a. Which brand of **tofu** did you most commonly consume?

a☐ bean supreme
b☐ morinaga
c☐ tonzu
d☐ Other (please specify below)

……

24b. Each time you ate **tofu** how much did you usually consume?

a ☐ Less than ¼ cup
b ☐ ¼ to ½ cup
c ☐ ½ to 1 cup
d ☐ More than 1 cup

25. How often did eat **marmite** ?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month
c☐ 2–3 times per month
d☐ 1 -2 times per week
e☐ 3–4 times per week
f☐ 5–6 times per week
g☐ 1 time per day
h☐ 2 times per day
i☐ 3 times per day
j☐ 4 or more times per day

25b. Each time you ate **marmite** how much did you usually consume?

a ☐ Less than ½ teaspooon
b ☐ ½ - 1 teaspoon
c ☐ 1 - 1½ teaspoons
d ☐ 2 teaspoons or more

26. How often did drink **energy drinks** ?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month
c☐ 2–3 times per month
d☐ 1 time per week
e☐ 2 times per week
f☐ 3–4 times per week
g☐ 5–6 times per week
h☐ 1 time per day
i☐ 2 times per day
j☐ 3 times per day
k☐ 4 or more times per day

26b. Each time you drank **energy drinks**, how much did you usually drink?

a ☐ Less than half a can or 125 ml
b ☐ One can or 250 ml
c ☐ One bottle or 375 ml
d ☐ More than one bottle (375 ml)
d ☐ Concentrated mini-can

26c. Which brand of energy drink did you most commonly drink?

a☐ V energy drink
b☐ Red bull
c☐ Powerade
d☐ Demon
e☐ Other (specify)
……..

**BAKED GOODS**
Over the <u>past 3 months</u>

27. How often did eat **sweet muffins** ?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month
c☐ 2–3 times per month
d☐ 1 time per week
e☐ 2 times per week
f☐ 3–4 times per week
g☐ 5–6 times per week
h☐ 1 time per day
i☐ 2 times per day
j☐ 3 times per day
k☐ 4 or more times per day

27a. Each time you ate **sweet muffins**, how much did you usually eat?

a☐ 1 small muffin
b ☐ 1 large muffin

28. How often did eat **cheese muffins** ?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month
c☐ 2–3 times per month
d☐ 1 time per week
e☐ 2 times per week
f☐ 3–4 times per week
g☐ 5–6 times per week
h☐ 1 time per day
i☐ 2 times per day
j☐ 3 times per day
k☐ 4 or more times per day

28a. Each time you ate **cheese muffins**, how much did you usually eat?

a☐ 1 small muffin
b ☐ 1 large muffin

29. How often did eat **cheese scones** ?

a☐ NEVER (GO TO NEXT QUESTION)

b☐ 1 time per month
c☐ 2–3 times per month
d☐ 1 time per week
e☐ 2 times per week
f☐ 3–4 times per week
g☐ 5–6 times per week
h☐ 1 time per day
i☐ 2 times per day
j☐ 3 times per day
k☐ 4 or more times per day

29a. Each time you ate **cheese scones**, how much did you usually eat?

a☐ 1 small scone
b ☐ 1 large scone

30. Did you take any **vitamin supplements?**

30a. Name of supplement

……

30 b. Amount of iron …….
30 c. Amount of folic acid…….
30d. Amount of vitamin B12……..

**Thank you *very much* for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you:**

- **Did not skip any pages and**
- **Crossed out the incorrect answer and circled the correct answer if you made any change**

## Appendix 12. ANCOVA Tables for change in Serum B12 and HoloTC

Table 26
*Influence of Covariates on ANCOVA of Change in Serum B12 between Groups*

| | ANOVA change in (log)serum B12 baseline to 6months | ANCOVA change in (log)serum B12 with age as covariate | Effect of covariate age | *ANCOVA change in (log)serum B12 with change in B12 dietary intake as covariate | *Effect of covariate change in B12 dietary intake | *ANCOVA change in capsule adherence (placebo & B12 supplement groups only) | *Effect of covariate change in capsule adherence (placebo & B12 supplement groups only) |
|---|---|---|---|---|---|---|---|
| ITT data set (n=60) | #*(df2,57)*8.93 *(<0.001[3.8,14]) †0.24(0.21) | #*(df2,57)*7.15 *(0.002[2.8, 11.5]) †0.29(0.25) | #*(df2,57)* 4.01 (0.05[0.0 , 8.0]) | #*(df2,57)*10.16 *(<0.001[4.3 ,16]) †0.40(0.36) | # *(df2,57)*12.88 *(<0.001[6.4, 20.3]) | #*(df1,37)*19.3 *(<0.001[ 8.2, 30.4]) †0.42(0.38) | #(*df1,37)*7.7 *(0.009[2.1,13.3]) |
| Missing data set (n=52) | #(*df2,49*)8.11 *(0.001[3.5, 12.7) †0.29(0.25) | #(*df2,49*)6.42 *(0.003[2.3, 11]) †0.29(0.24) | #(*df2,49*) 2.61 (0.113[-0.63,5.8] ) | #(*df2,49*)11.47 **(<0.001[4.9,18]) †0.43(0.40) | #(*df2,49*)15.50 **(<0.001[6.6,24]) | #(*df1,33)16.9* **(<0.001 [7.1, 26.5]) †0.44(0.40) | #(*df1,33)*7.6 **(<0.009[2.0 ,13.2]) |

Table 27
*Influence of Covariates on ANCOVA of Change in HoloTC between Groups*

| | ANOVA change in (ln)holoTC baseline to 6months | ANCOVA change in (ln)holoTC with age as covariate | Effect of covariate age | ANCOVA change in (ln)holoTC with change in (ln)B12 dietary intake as covariate | Effect of covariate change in (ln)B12 dietary intake | ANCOVA capsule adherence with change in (ln) holoTC(placebo & B12 supplement groups only) | Effect of capsule adherence (placebo & B12 supplement groups only) |
|---|---|---|---|---|---|---|---|
| ITT data set (n=60) | #(*df2,57*)7.36 *(<0.001[3.1,11.6]) †0.21(0.18) | #(*df2,57*)6.30 *(0.003[2.3,11]) †0.21(0.17) | #(*df2,57*)0.06 (0.48[-0.1,0.20]) | #(*df2,57*) 6.93 **(0.002[2.6,11.2]) †0.26(0.22) | #(*df2,57*)4.24 (<0.044[0.1,8.4]) | #(*df1,37)*11.47 (0.002[4.5, 18.5] †0.26(0.22) | #(*df1,37)*1.2 (0.29[-1.1,3.5]) |
| Missing data set (n=52) | #(*df2,49*)7.20 *(0.002[2.8,12]) †0.23(0.20) | #(*df2,49*)5.92 **(0.005[1.9,10]) †0.23(0.18) | #(*df2,49)* 0.32 (0.57[-0.08,1.5]) | #(*df2,49*)7.36 **(0.002[2.8,12]) †0.30(0.25) | #(*df2,49*)4.88 (0.032[0.44,9.3]) | *#(df1,33)*11.8 **(0.002[4.6, 19.0] †0.28(0.24) | #(df1,33) 0.78 (0.38[-1.0,2.6]) |

#Reported as F statistic (significance [95% confidence intervals])
† R2 ( adjusted R2)
*Significant effect on variance at 0.025
**Significant effect on covariance at 0.017

## Appendix 13. Percentage change in Serum B12 and HoloTC over Six Months

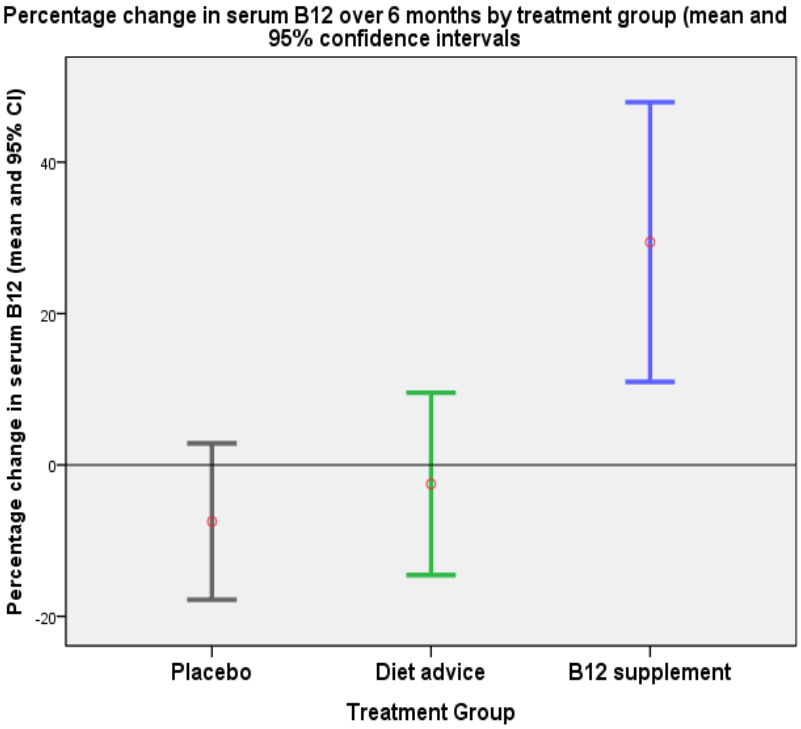


*Figure 16.* Percentage change in serum B12 by treatment groups, baseline to six months

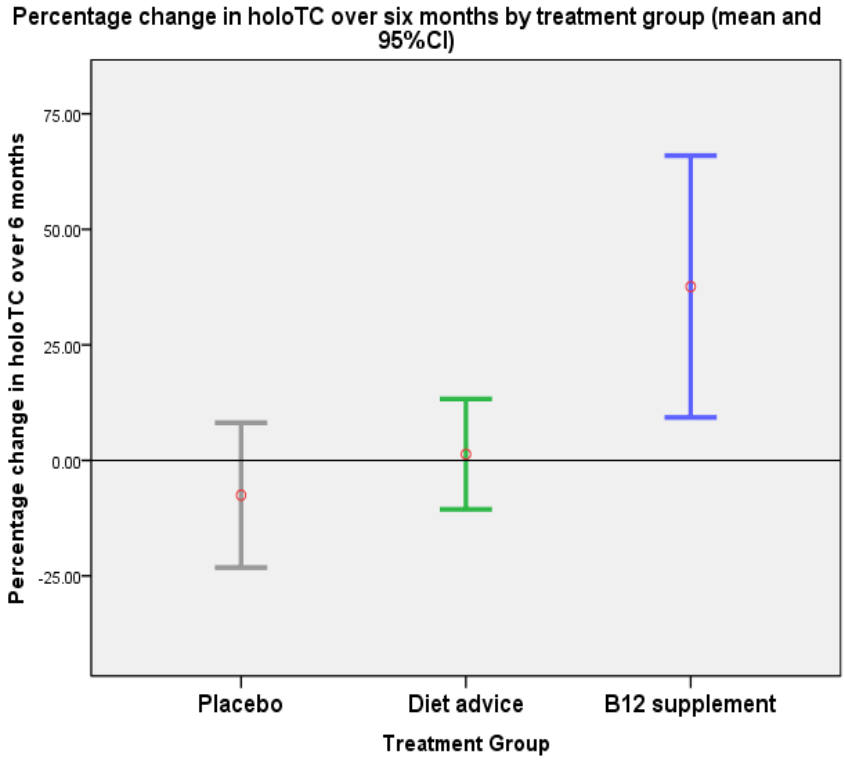


*Figure 17.* Percentage change in holoTC by treatment groups, baseline to six months

## Appendix 14: Distribution of Baseline Characteristics

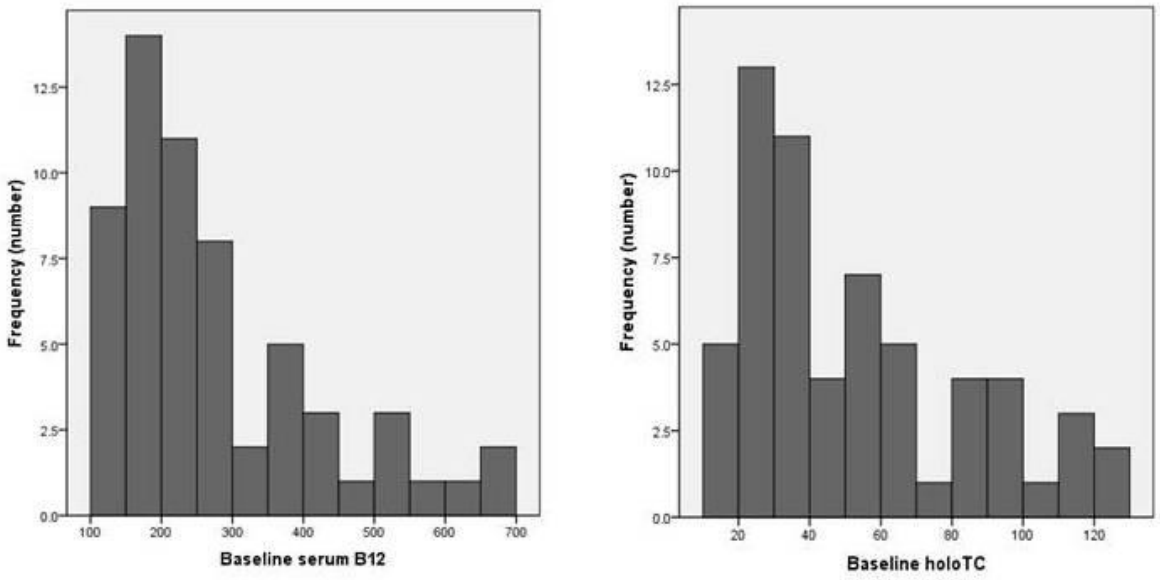


*Figure 18.* Distribution of baseline B12 biomarkers across study population

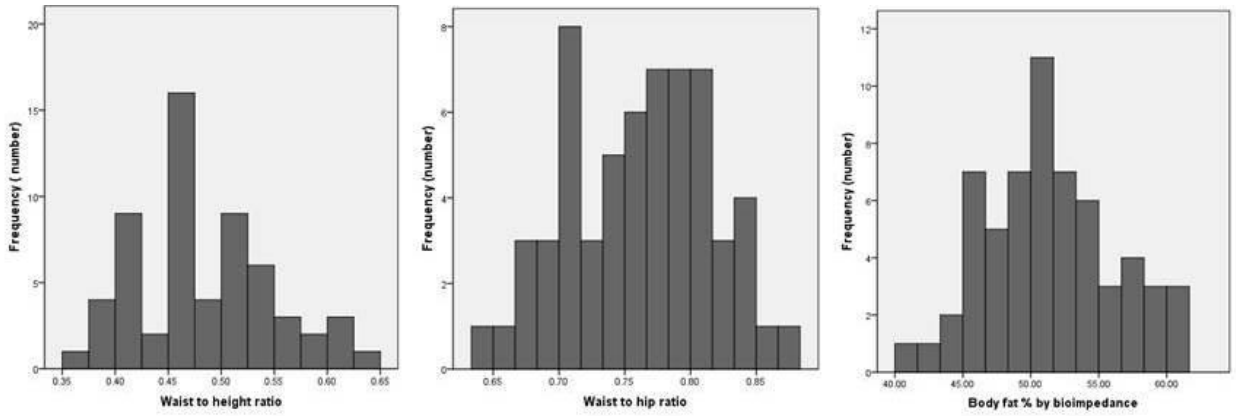


*Figure 19.* Distribution of selected anthropometry characteristics of study population