Women of childbearing age:

Dietary patterns and vitamin B_{12} status

A validation study

Liping (Sunnie) Xin

A thesis submitted to Auckland University of Technology in fulfilment of the degree of Master of Philosophy

2008

Division of Sport and Recreation

Primary Supervisor: Professor Elaine Rush (PhD)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

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 published or written 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 research institution of higher learning".

Signed……

Date…….

ACKNOWLEDGEMENTS

With the completion of this piece of work, I would like to thank everyone who was influential in helping me achieving this milestone in my life.

Firstly, I would like to thank **My Parents, Rocky and His Parents,** for helping me emotionally, financially and giving me this opportunity to come overseas and accomplish further studies.

Professor Elaine Rush (primary supervisor), Auckland University of Technology, for being my guide, mentor, in giving a sound direction to this work. I would like to thank her, for her patience and unrelenting support in helping me throughout the process.

Patricia Lucas (secondary supervisor), Auckland University of Technology, for her guidance and constant support as a supervisor and administrator.

Nicola Power (adviser), Auckland University of Technology, for her help with participant recruitment and thesis writing.

Nick Garrett, Auckland University of Technology, for his help and guidance in statistical analysis and understanding the NNS97.

Professor Lindsay Plank, University of Auckland for extending his support in allowing us to store the blood samples at the body composition unit.

Dr Janet Rowan, National Women's Health and Neonatal Unit, Auckland City Hospital, for her medical expertise in analysing the blood reports.

Health Clinic (Akoranga campus), Auckland University of Technology, for providing a quiet and safe place for data collection.

Diagnostic Medlab Ltd Auckland, Middlemore Hospital Auckland, for their help and assistance in analysing the blood samples.

The **38 participants (most of them are staff members and students of Auckland University of Technology),** without their enthusiasm and co-operation this research would not have been possible.

All my friends and colleagues, Purvi Chhichhia, Jewel Wen, Jennifer Crowley, Geoff Kira, Kate White, Yuting Zhu, Riitta Freese, Gael Mearns, and Neil Snowling.

ABSTRACT

From conception the dynamic balance between nutritional and activity factors play a role in the accumulation of risk for future disease. Maternal nutrient balance and the subsequent dietary pattern of the family set the path for the growth and development of the individual and therefore also for their offspring. There is strong evidence from studies in India that mothers who have a low vitamin B_{12} status, but high folate, will have children with higher adiposity and more cardiovascular risk factors than those with adequate B_{12}. The B_{12} status is closely linked to the dietary pattern particularly the consumption of red meat which has a high B_{12} content. In New Zealand there is an increasing number of Indian migrants. Vegetarianism is also practiced by an increasing number including young women. In addition, there is a high rate (up to 60%) of unplanned pregnancies in New Zealand.

In the 1997 New Zealand National Nutrition Survey (NNS97) report, vitamin B_{12} intake appeared adequate for the New Zealand population and breakfast cereals were reported as one major dietary source of B_{12}. Cereals in New Zealand however, were not fortified with B_{12} and there was an error in the FOODfile™ data entries for B_{12} in some cereals. The raw data of reported B_{12} intakes in the 24-hour diet recall (24HDR) of NNS97 was reanalysed at the individual level by subtracting the B_{12} derived from breakfast cereals and applying the 2005 revised estimated average requirement (EAR) value. The possible prevalence of B_{12} insufficiency was 2.4 times that originally reported by the NNS97, translating into a prevalence of up to 27% of the population sampled. This analysis was limited as it was not adjusted for day-to-day variance or to the New Zealand population. This apparently high prevalence of risk for inadequate B_{12} intake in the surveyed individuals required confirmation that the B_{12} intake from 24HDR and also a 7-day diet diary (7DDD) was a valid assessment of B_{12} status. The group of particular interest is women of childbearing age (18-50y) with a range of eating patterns.

Thirty eight women aged 19-48y; 12 non-red-meat-eaters (5 Indians vs 7 non-Indians) and 26 red-meat-eaters (1 Indian vs 25 non-Indians) participated in this validation study. Anthropometry and hand-to-foot bioelectrical impedance (BIA) were measured

on the same day as a 24HDR was recorded. Fasting serum lipids, glucose, haematological parameters, and serum B_{12}, holotranscobalamin II (holo-TC II, a specific B_{12} biomarker), and folate concentrations were measured. Foods eaten and time spent in physical activity during the following 7 days were extracted from 7DDD and 7-day physical activity diary (7DPAD).

There was no significant correlation between dietary intake (24HDR or 7DDD) and biomarkers for B_{12} status. Indians reported lower mean daily B_{12} intakes in 7DDD than non-Indians (1.6 vs 4.5 μg/day, $p<0.001$) and this was confirmed by Indians' significantly low serum B_{12} (203 vs 383 pmol/L, $p=0.04$) and holo-TC II (35 vs 72 pmol/L, $p=0.02$) concentrations compared to non-Indians. A similar pattern was found between non-red-meat-eaters and red-meat-eaters in daily B_{12} intake in 7DDD (2.3 vs 4.8 μg/day, $p<0.001$) and in B_{12} biomarkers (serum B_{12}, 263 vs 397 pmol/L, $p=0.01$; holo-TC II, 43 vs 77 pmol/L, $p<0.005$). Non-red-meat-eaters reported significantly higher daily folate intake in 7DDD (359 vs 260 μg/day, $p=0.01$) than red-meat-eaters but no significant difference was found in serum folate concentration between these groups (29 vs 24 pmol/L, $p=0.10$). Indians/non-red-meat-eaters also reported lower daily protein intake and higher percentage of total energy from carbohydrate in 7DDD compared to non-Indians/red-meat-eaters but total reported energy intake tended to be under-reported and physical activity over-reported when assessed against estimated basal metabolic rate (BMR).

Body composition varied by dietary pattern. Indians/non-red-meat-eaters had higher body fat percentage (BF%) and weaker grip strength than non-Indians/red-meat-eaters. In addition, Indians had a significantly higher waist-to-hip ratio (WHR) than non-Indians. Overall, the whole group reported that they were inactive. The median time spent in moderate, high and maximal intensity activities was only 19 minutes a day, which did not meet the NZ guideline for adults of 30 minutes a day.

In this small study nutrient analysis of diet by 24HDR or 7DDD, was not a reliable or accurate way to assess B_{12} insufficiency. Questions about dietary patterns such as "do you eat red meat", and taking ethnicity into account could more easily identify the at risk population. Supplementation and/or fortification of B_{12} should be considered before pregnancy.

ABBREVIATIONS

Throughout this thesis standard international units and standard abbreviations have been used. Wherever applicable units used for measurements are stated.

7DDD	7-day diet diary
7DPAD	7-day physical activity diary
24HDR	24-hour diet recall
B_{12}	vitamin B_{12}, cobalamin
BF%	body fat percentage
BIA	bioelectrical impedance analysis
BMI	body mass index
BMR	basal metabolic rate
CNS2002	2002 New Zealand National Children's Nutrition Survey
CoA	coenzyme A
CPT1	carnitine palmitoyl transferase - 1
DNA	deoxyribonucleic acid
EAR	estimated average requirement
ES	effect size
FFM	fat free mass
FM	fat mass
HDL cholesterol	high-density lipoprotein cholesterol
Holo-TC	holotranscobalamin
IDF	International Diabetes Federation
IF	intrinsic factor
LDL cholesterol	low-density lipoprotein cholesterol
MCH	mean cell haemoglobin
MCM	methlymalonyl CoA mutase
MCV	mean cell corpuscular volume
MMA	methylmalonic acid
MRI	magnetic resonance imaging technique
MUAC	mid upper arm circumference.
NFHS-2	National Family Health Survey-2
NNS97	1997 New Zealand National Nutrition Survey
NR-NCD	nutrition-related non-communicable disease
NTD	neural tube defect

PAL	physical activity level
PCV	packed cell volume
P:S ratio	polyunsaturated: saturated fat
RDI	recommended dietary intake
RNA	ribonucleic acid
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SES	socioeconomic status
tHcy	plasma total homocysteine
THF	tetrahydrofolate
WHO	World Health Organization
WHR	waist-to-hip ratio

GLOSSARY

Asian: refers to Southeast and East Asian and includes Chinese, Japanese, Indonesia, and Filipina.

Beta-oxidation (β-oxidation): The process by which long carbon chain of fatty acids are broken down to two carbon units of acetic acid (Acetyl CoA) in the mitochondria, the entry molecule for the Krebs Cycle.

Biomarkers: A substance used as an indicator of biological state. They must have a biochemical feature or facet that can be used to measure the progress of disease or the effect of treatment.

Body Mass Index (BMI): A useful index to assess overweight and obesity. It is measured by dividing weight by height squared (kg/m^2).

Breakfast cereals: All types cereals (muesli, porridge, puffed/flakes/extruded & bran cereals) – originating from rice, wheat, corn.

Deoxyribonucleic acid (DNA): Nucleic acid that contains genetic instructions for development and function of living organism. The principal role of DNA in the cell is long term storage of information. Parts of the DNA segments carry genetic information, while part if it has structural purposes like regulating the expression of genetic information.

Estimated average requirement (EAR): A daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group.

European: includes European, South African and American whites.

Indian: includes Asian Indians and Fijian Indians.

Non-red-meat-eaters: People who do not consume any red meat, including pork, lamb, and beef. They might be vegetarians or consume white meat, such as chicken.

Recommended dietary intake (RDI): It refers to the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97-98 percent) healthy individuals in a particular life stage and gender group.

Red-meat-eaters: People who consumes red meat in their habitual diets, including pork, lamb, and/or beef.

Sensitivity: the proportion of people correctly identified as having the condition i.e. true positives.

Specificity: the proportion of people identified correctly as not having the condition i.e. true negatives.

CHAPTER 1: INTRODUCTION

Malnutrition, either under-nutrition, over-nutrition or both, has become a global issue. It is an imbalance not only between energy intake and energy expenditure, but also among the status of various nutrients. The dynamic balance between nutritional and activity factors play a role in maintaining the health of an individual from conception to death. Individuals, who were undernourished during childhood, are more likely to develop obesity, especially abdominal obesity, and nutrition-related non-communicable diseases (NR-NCDs), such as type 2 diabetes mellitus, heart diseases and hypertension (Uauy & Solomons, 2006), in adulthood. The nutritional status of children is influenced by both the prenatal and postnatal environment in relation to maternal nutrient intake, and the subsequent dietary pattern of the family.

Nutrient intake refers to macronutrients: carbohydrate, proteins and fats, and micronutrients: vitamins, minerals, phytonutrients, and antioxidants. Risk associated with nutrition accumulates from conception, with pathophysiological changes (some irreversible) occurring throughout the life span (Ben-Shlomo & Kuh, 2002). It is important to examine and address health problems of women of childbearing age before they get pregnant and risk compromising the growth and development of their offspring. This is particularly important in New Zealand where there is an identified high rate of unplanned pregnancies, up to 60% of pregnancies (Ministry of Health, 2003b).

B_{12} and folate work together as part of the metabolic pathways leading to DNA synthesis and maintenance of a healthy nervous system. Elevated malonyl-CoA concentration, due to B_{12} deficiency, can also adversely affect protein synthesis, lipogenesis, and insulin resistance (Ruderman et al., 1999), particularly when accompanied by a high folate level. The New Zealand Ministry of Health (1997) recommends that periconceptional folic acid supplements be taken to reduce the incidence of Neural Tube Defect (NTD) and folic-acid fortified foods may be supplied to the whole population. The government is considering mandatory fortification of foods with folic acid to increase folic acid intake of most women throughout their childbearing years. A problem with fortification is that others in the

population might be sensitive to excess intake. In the case of folic acid fortification, the elderly and non-red-meat-eaters, including vegetarians, might be at risk of the adverse affects of high folate and low B_{12} levels. The imbalance of high folate and low B_{12} status are related to anaemia and cognitive impairment in seniors (Morris, M. S. et al., 2007), and the possibility of obesity and insulin resistance in children (Yajnik et al., 2008).

However, the New Zealand Ministry of Health suggested that there was a negligible risk of B_{12} deficiency among New Zealanders. Intake "appeared adequate" according to the reports of both the 1997 National Nutrition Survey (NNS97) and the 2002 National Children's Nutrition Survey (CNS2002). Therefore, there is no requirement for B_{12} fortification in New Zealand. However, B_{12} supplements are recommended for vegetarians and on some occasions; for example, gestational diabetes mellitus patients treated with the drug Metformin, which can reduce B_{12} absorption. An issue is that New Zealand breakfast cereals, which do not contain active B_{12}, were considered to be one of the B_{12} sources in these two surveys. Apparently this was due to the use of Australian food composition data where cereals are fortified with B_{12}.

This anomaly was first discovered in the pilot study of 12 Indian preadolescent girls in New Zealand (Chhichhia, 2007). Non-meat-eaters in this study with a high intake of breakfast cereals, showed a high B_{12} intake but low fasting serum level. This evidence gave rise to two questions:

1. What effect would subtracting B12 from cereals have on the interpretation of the NNS97?
2. What effect does not eating meat have on B12 status?

The first question is addressed in a separate chapter (chapter 2) while the second question, the life course model of health and the validity of dietary assessment are explored further in this introduction.

The following review of literature will explore these issues:

- The life course approach to good health
- The dual burden of malnutrition and the nutrition transition
- Introduction and functions of B_{12}.

- B_{12} deficiency and tests of B_{12} status
- Results of imbalance between B_{12} and folate
- Different dietary patterns

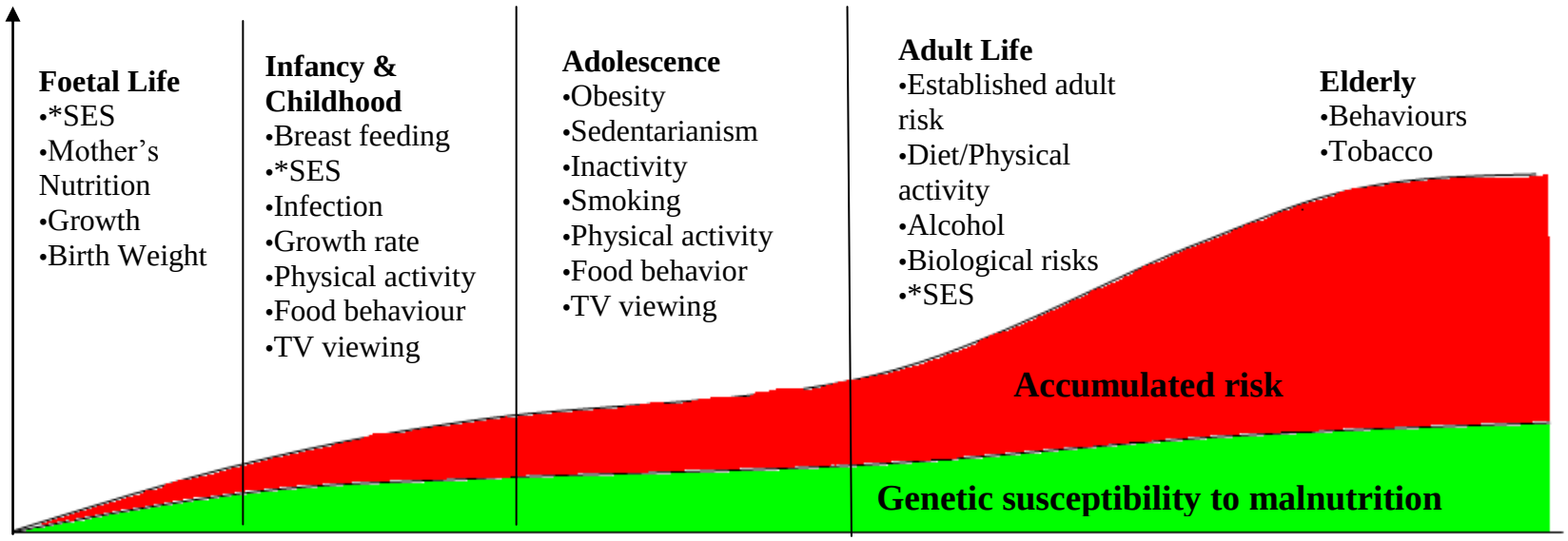
The explanation and exploration of the literature related to the above issues will inform the aim, hypothesis and significance of the study.

1.1 Review of literature

1.1.1 The life course approach to good health

Life can be broadly divided into four stages: foetal life; infancy and childhood; adolescence; adult life and old age (Figure 1.1). This model predicts the accumulation of chronic disease risk as the factors that operate within each particular stage of life influence health in relation to nutritional status of the individual (Uauy & Solomons, 2006). Many of these factors relate to nutritional status. The factors at each stage in relation to manifestation and accumulation of risk to the next stage deserve more attention for the development of prevention strategies. Results of exposure to a particular factor at a life stage not only manifest in that phase of life but are also irreversibly carried forward to the following life stages. “The manifestation of these factors is also affected by their interactions with the genetic constitution of the individual” (Uauy & Solomons, 2006). Ravelli et al. (1998) suggested that it was important to monitor female nutrition and health since birth, as the development of her foetus not only depended on the nutritional intake during pregnancy, but also her health and nutrition status throughout her life.

Figure 1.1: Life course approach to health



*SES socio economic status

Source World Health Organization (2004)

1.1.2 The dual burden of malnutrition

Uauy and Solomons (2006) identified six different forms of malnutrition to categorise an individual's nutritional status:

- Underweight: defined by a low weight-for-age.
- Wasting: low weight-for-height.
- Stunting: low height-for-age.
- Overweight: excess weight for one's length and stature, measured as weight-for-height or BMI centile for age.
- Micronutrient deficiencies: due to poor quality of diets and nutrient wasting caused by inflammation and infection. Can be just one or multiple micronutrient deficiencies.
- Nutrition-related chronic diseases: related to diet and physical activity.

Malnutrition, an imbalance in nutrients, was largely considered to be the problem of the poor after World War II. Although under-nutrition continues to be a problem of the poor among developing countries, there has been escalation of the incidence of nutrition-related chronic diseases, such as obesity, coronary heart disease, and diabetes mellitus, among relatively affluent adults. It used to believe that, developing countries in particular face the dual burden of under-nutrition among the poor and over-nutrition among the affluent adults (Gopalan, 2000), while obesity has been concentrated among the poor for decades in developed countries (Hawkes et al., 2004). However, since the co-existence of underweight and overweight within households was first identified in Brazil, China and Russia (Doak et al., 2000), more and more examples of the two types of malnutrition have been reported. Doak et al. (2005) analysed data of seven national surveys (Brazil, China, Indonesia, the Kyrgyz Republic, Russia, Vietnam and the United States), and found that, excluding Vietnam, 22-63% of households suffered the dual burden of malnutrition. Countries with the highest prevalence of dual burden households were those in the middle range of gross national product (GNP). In five of these countries (except Russia and the Kyrgyz Republic), dual burden households were more likely to be urban and high income.

The "Barker Hypothesis" suggests that nutrition-related non-communicable diseases (NR-NCDs) of adulthood could be the "late" effects of early foetal under-nutrition

(Barker, 1997). Similar evidence (Gopalan, 2000) has been provided that infant under-nutrition increases the likelihood of over-nutrition later in life. Childhood stunting may be a risk factor for the development of obesity, especially abdominal obesity (Popkin et al., 1996), and NR-NCDs, such as type 2 diabetes, heart disease and hypertension (Uauy & Solomons, 2006). Those born in poverty and then attain affluence in adult life may be at special risk of malnutrition. Bhargava et al. (2004) studied 1492 young Indian adults (26-32 years) from birth and demonstrated that those with impaired glucose tolerance or diabetes in adulthood were more likely to be thin up to two years of age, followed by an accelerated weight gain during childhood and adolescence.

Causes of malnutrition

Under-nutrition arises due the mutually synergistic attributes of low family income, large family size, poor education, poor living environment, poor access to health care, and inadequate access to food (Gopalan, 2000). Poverty contributes to the major causes of under-nutrition, especially in developing countries. Poverty at the level of individual households, rather than the lack of overall availability of food at the national level, is the major factor underlying under-nutrition. Poor education and ignorance are also factors associated with malnutrition. Between 1994 and 1998, the number of overweight women in Bolivia, one of Latin America's poorest countries, increased nine percentage points, with the greatest increases among women with less education, while more than one quarter of infants were reported as stunted (Hawkes et al., 2004). The correlation of female literacy rate with child mortality rate and nutritional status of children was observed in 14 Asian countries (Bellamy, 1999). The level of female education is definitely a major determinant of health and nutrition status of their families. Educated mothers, even in low-income families, prove more resourceful in ensuring maximal possible benefit to health and nutrition status. Poor living environment and lack of proper personal hygiene favour the development of infections. Even worse, lack of adequate access to prompt treatment prolongs the duration of illnesses and consequently worsen nutrition status (Gopalan, 2000).

Over-nutrition, usually observed in affluent adults of developing countries and the poor of developed countries, is due to the changes of dietary habits and lifestyles brought by development. Reduced dietary fibre intake (due to highly polished

varieties of wheat and rice), increased intake of edible fat (especially saturated fat) and relatively high intake of ghee and butter, increased intake of sugar, increased overall energy intake in relation to reduced energy expenditure, are major factors of over-nutrition (Gopalan, 2000). Furthermore, poor quality diet is one reason that overweight links with micronutrient deficiencies. For example, Hawkes et al. (2004) reported that the declining consumption of fruits and vegetables and increasing intake of high-cholesterol foods in rural Mexico from 1980 to 1998 was associated with 78% increase in obesity rate and 62% increase in diabetes rate with more than 20% of women with anaemia.

In New Zealand, over-nutrition has a higher prevalence than under-nutrition. In the 1997 National Nutrition Survey (NNS97) (Russell et al., 1999), 17% of adults (15% males, 19% females) were considered obese and an additional 35% were considered overweight (40% males, 30% females). NZ Maori (27.0% males, 27.9% females and Pacific people (26.2% males, 47.2% females) were more likely to be classified as obese than NZ European & Others (12.6% males, 16.7% females). Mean body weight (of mainly European) increased by 3.2kg between 1989 and 1997. About 22% of males and 18% of females had hypertension and 23% of adults had total cholesterol levels >6.5mmol/L. In the 2002 National Children's Nutrition Survey (CNS2002) (Parnell et al., 2003), 21.3% of children were overweight and 9.8% obese. Pacific children had the highest overweight and obesity rates, followed by Maori and NZ European & Others. Food security, a cause of malnutrition, is also a problem in New Zealand. For example, in NNS97 report (Russell et al., 1999), 47.5% of Maori and 49% of Pacific households reported that lack of money affected the variety of food consumed either sometimes or often; 31% of Maori and 49.5% of Pacific households reported they ran out of food sometimes or often; 20.5% of Maori and 29.5% of Pacific households sometimes or often relies on others for food.

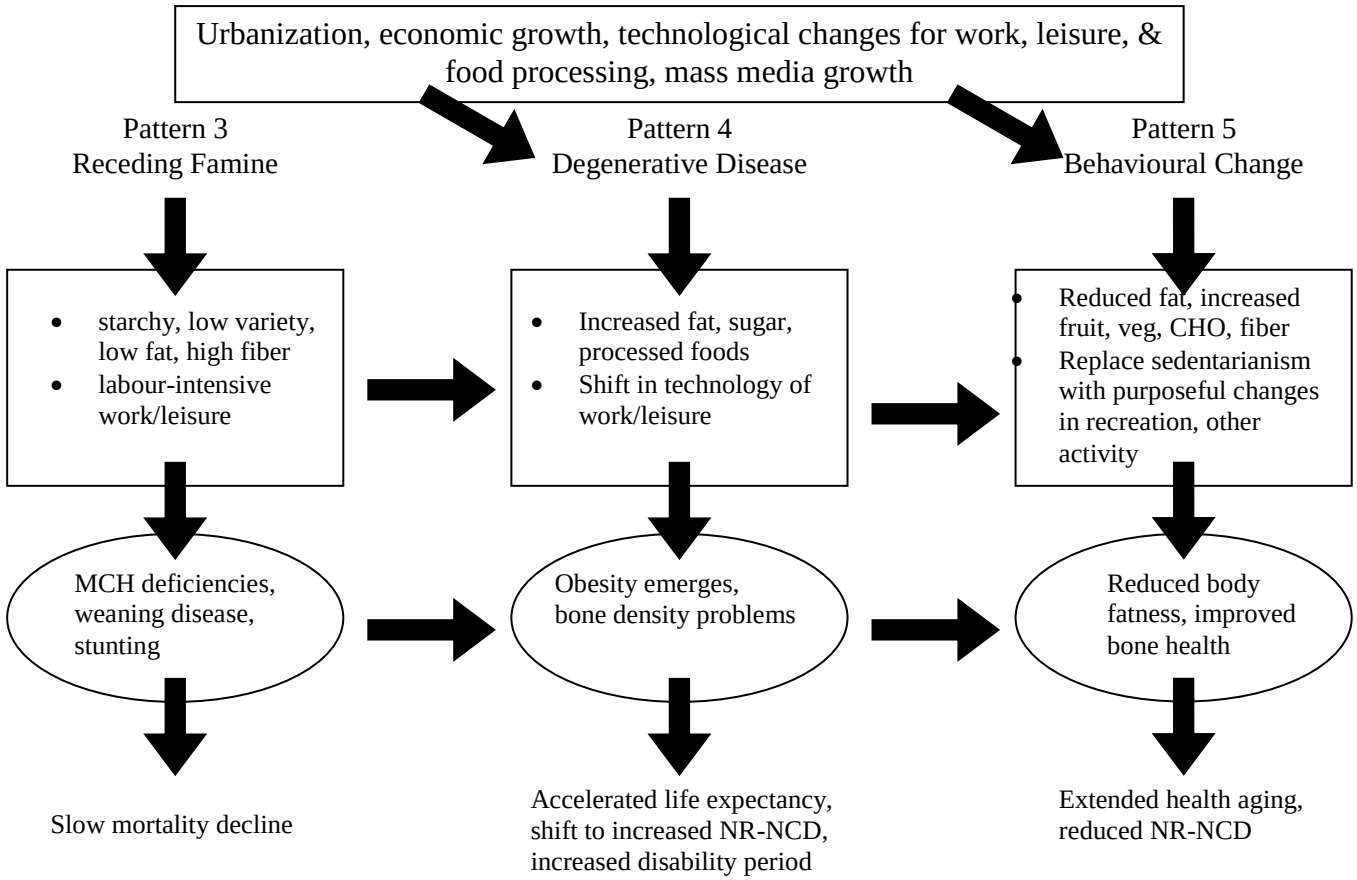
The nutrition transition

The term "nutrition transition" relates to the underlying shifts in economic, demographic, and related forces affecting fertility, mortality, disease patterns, diet structure, physical activity, and body composition trends (Popkin & Gordon-Larsen, 2004). It is closely related to the demographic and epidemiologic transitions. Large shifts in the structure of diet and in physical activity pattern are vital drivers of the

nutrition transition. The nutrition transition has followed five broad patterns (Popkin, 2002): 1) collecting food; 2) famine; 3) receding famine; 4) nutrition-related non-communicable disease (NR-NCD); and 5) behavioural change. The last three patterns are described in more detail in Figure 1.2. In pattern 3, famine recedes as income rises. In pattern 4, changes in diet and activity patterns trigger new disease problems and increase disability period. In pattern 5, behavioural changes reverse the negative tendencies and extend healthy aging (Popkin & Gordon-Larsen, 2004).

In the study by Doak et al. (2005), dual burden households were most prevalent in countries that were experiencing the chronic disease phase of the nutrition transition (pattern 4). Ideally pattern 5 - behavioural change should lead to improved health. There is a tendency however for the health messages to become confused. While preventing weight gains, there is the risk of avoidance of certain foods, e.g. animal products, to reduce fat intake but this dietary pattern may also result in inadequate intake of essential minerals or vitamins such as B_{12}.

Figure 1.2: The last three patterns of the nutrition transition



Adapted from Popkin & Gordon-Larsen (2004)

1.1.3 Introduction to B_{12} malnutrition

B_{12} is a member of the B complex vitamin family. It contains cobalt, which makes it a red colour, and is also called cobalamin in most biochemical and chemical texts. There are several cobalamin–dependent enzymes in bacteria, fungi, algae, and animals, but not in plants. B_{12} can be converted to either of the two cobalamin coenzymes that are active in humans: methylcobalamin, the main form in plasma, or 5-deoxyadenosylcobalamin, found in the liver, most body tissues, and foods. The synthetic form used in supplements and fortified foods is cyanocobalamin, which is again activated enzymatically in all cells to the two active forms (Scott, 1999). B_{12} is found almost exclusively in foods derived from animals, as the gastrointestinal fermentation of many animals supports the growth of B_{12} synthesizing microorganisms, and subsequently the vitamin is absorbed and incorporated into the animal tissues. In the human diet meat, fish, poultry, shellfish, milk, cheese, eggs, and fortified cereals are significant sources of B_{12}. Red meats, such as beef and lamb, are better sources of B_{12} compared to white meat, like chicken (Narayanan et al., 1991). In the Australian and New Zealand diet, about 25% of B_{12} comes from red meats; and for adults and children, about 30% and 50%, respectively, is from dairy products (Ministry of Health, 2005).

In nature, B_{12} is produced almost entirely by bacterial synthesis. In humans, this synthesis takes place in the small intestine and colon. However, only small quantities of B_{12} synthesized in the small intestine non of B_{12} synthesized in colon are absorbed (Albert et al., 1980). Highly specific absorption of cobalamin predominates in the ileum. After ingestion, the cobalamin in food is released by gastric pepsin, and then preferentially bound in stomach by a glycoprotein called haptocorrin (transcobalamin I, TC I). In the small intestine, haptocorrin is degraded by pancreatic enzymes, and B_{12} is transferred to intrinsic factor (IF), a protein synthesized in the parietal cells of the stomach. The IF-B_{12} complex is then absorbed via receptor, and B_{12} is subsequently bound to transcobalamin II (TC II) and exists into the bloodstream as holo-TC II. The time from oral ingestion to entry into the bloodstream takes up to 4 hours (Carmel, Ralph, 2006). The main storage site for B_{12} is the liver. Total body stores in healthy omnivorous subjects are about 2-3mg. A sizeable hepatic clearance of cobalamin from TC I is secreted into bile, estimated at 1.4μg/day (about 0.1% of

body stores), approximately 70% of which is reabsorbed for metabolic functions while the remainder is lost in faeces (Carmel, Ralph, 2006).

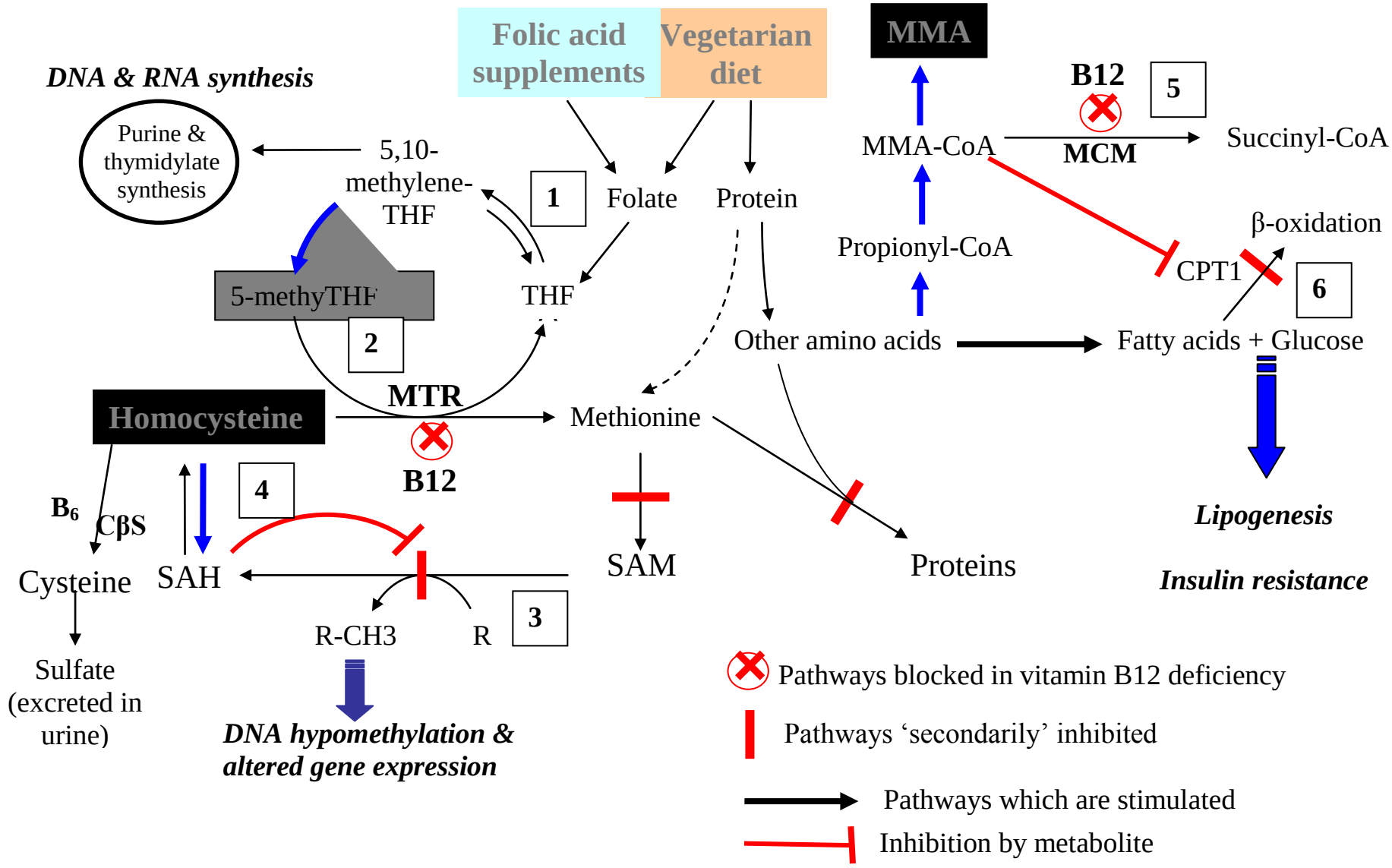
The biological half-life of body stores of B_{12} is estimated to be more than 480 days (Butler et al., 2006). Therefore, B_{12} deficiency/insufficiency would develop only in persons with long term low B_{12} intake, e.g. vegetarians; or those with limited intrinsic factor or malabsorption. The estimated average requirement (EAR) and recommended dietary intake (RDI) values for New Zealanders are listed in Table 1.1 for different age groups (Ministry of Health, 2005):

Table 1.1: EAR and RDI values of B_{12} for different age groups in NZ

*Age(yr)**	*EAR(µg/day)*	*RDI(µg/day)*
1-3	0.7	0.9
4-8	1.0	1.2
9-13	1.5	1.8
14+	2.0	2.4

EAR, Estimated average requirement; RDI, Recommended dietary intake; *EAR and RDI values for both sexes are the same in each age group.

Figure 1.3: Suggested metabolic mechanisms for adiposity, insulin resistance and altered gene expression in a situation of dietary vitamin B_{12} deficiency combined with adequate folate status (reference from CS Yajnik, 2006)



1.1.4 Functions of B_{12}

B_{12} is necessary for the rapid synthesis of DNA during cell division. This is especially important in tissues where cells are dividing rapidly, particularly the bone marrow tissues responsible for red blood cell formation. If B_{12} deficiency occurs, DNA production is disrupted and abnormal cells called "megaloblasts" occur, which result in anaemia. Moreover, low levels of B_{12} play a role in manifesting metabolic risk factors like fat accumulation, poor skeletal muscle and increasing insulin resistance. The details of the possible metabolic pathways are shown in Figure1.3 and explained in the following paragraphs.

B_{12} and folate are actively involved in "one-carbon metabolism", which is a network of interrelated biochemical reactions involving the transfer of one-carbon groups from one compound to another. Important coenzymes required for these reactions include B_{12}, folate, vitamin B_6 and vitamin B_2. Figure 1.3 represents the two critically important functions of one-carbon metabolism: biological methylation and the synthesis of nucleotides. Although modest dietary inadequacies of these nutrients may be insufficient to cause classical deficiency syndromes, they can still contribute to important diseases such as neural tube defects, cardiovascular disease and cancer (Mason, 2003).

The methylation pathway is an important portion of one-carbon metabolism, in which nutrients (folic acid, betaine, B_{12} and zinc) play an important role of affecting DNA methylation (Van den Veyver, 2002). As the present thesis is designed to explore the significance of B_{12} and folate with respect to the non-red-meat-eating vs red-meat-eating and Indian vs non-Indian dietary patterns, a systematic explanation of the processes involved in one-carbon metabolism is presented below in step 1 to 6.

Step 1

Folate is reduced by the enzyme dihydrofolate reductase to the dihydro- and tetrahydro- forms during passage through the intestinal mucosal cells. The tetrahydrofolate (THF) is further converted to 5,10-methylenetetrahydrofolate (5,10-MTHF), which then receives one carbon group from serine and glycine, and releases

methyl groups to be used in DNA and RNA synthesis, purine and thymidylate synthesis, and finally gets reduced to 5-methytetrahydrofolate (5-MTHF).

Step 2

5-MTHF is further reduced to THF by the enzyme methyl tetrahydrofolate reductase (MTR) which requires B_{12} as a co-factor. During this process a methyl group is transferred to homocysteine to form methionine (Van den Veyver, 2002). THF then is recycled in step 1 for methylation and nucleotide synthesis. However, the amount of regenerated THF is not enough for body requirement and replenishment through dietary intake is necessary (Scott, 1999).

Step 3

Methionine, an essential amino acid, is derived from homocysteine and also obtained from the diet (mainly in meat products). Excess methionine (approximately 60% of that from the diet) is degraded back to homocysteine via steps 3 and 4 (Figure 1.3). Methionine is converted to S-adenosylmethionine (SAM), which is a major donor of a methyl group for DNA and RNA biosynthesis during the process of further conversion to S-adenosylhomocysteine (SAH). Many methylation reactions including the methylation of myelin basic protein, which makes up about one-third of myelin, the insulation cover on nerves, are dependent on SAM (Weir et al., 1988). When this methylation reaction is interrupted, one of the clinical consequences is the demyelination of nerves, resulting in a neuropathy which leads to ataxia, paralysis, and even death. Methionine is required not only for methylation processes but also for protein synthesis (Van den Veyver, 2002). When levels of SAM are decreased, this results in increased levels of 5-MTHF and homocysteine, and a compensatory increase in methionine synthesis. In contrast, when levels of SAM are elevated, levels of 5-MTHF and homocysteine decrease, and a compensatory decrease occur in methionine synthesis (Allen et al., 1998).

Step 4

SAH is then hydrolysed to homocysteine, which enters the trans-sulphuration pathway and gets irreversibly converted to cysteine with the help of vitamin B_6, with the latter being used for energy. Alternatively homocysteine can be remethylated back to methionine, a reaction which in most tissues is catalysed by methionine synthase,

or is recycled to form methionine as explained in step 2. Whether homocysteine is degraded to cysteine or conserved back to methionine depends on how well the cycle is maintaining intracellular SAM levels (Scott, 1999). Hence, any discrepancy in the methylation pathway due to folate or B_{12} deficiency leads to:

- DNA hypomethylation and altered gene expression, as a result of absence of methyl groups. This results in decreased DNA biosynthesis and affects cell division, particularly rapidly dividing cells like erythrocytes, which may lead to anaemia (Van den Veyver, 2002).
- Increased levels of serum homocysteine, a recognized long-term risk factor for cardiovascular and neurogenerative diseases (Geisel et al., 2003).
- Functional folate deficiency, which is due to inadequate B_{12} status disabling the conversion of 5-MTHF to THF, resulting in accumulation of free folate, while decreasing the intracellular folate pool available for methylation reactions.

The role of B_{12} in steps 1-4

B_{12} is a co-factor in the reaction of step 2, which is the overlap of two cycles: the folate circulation and the methylation cycle. During B_{12} deficiency, 5-MTHF accumulates and the uptake of folate from serum is prevented, resulting in elevated homocysteine levels in plasma. In this case, similar biochemical effects of both folate and B_{12} deficiencies occur, including functional folate deficiency, hyperhomocysteinemia, and low methionine levels relative to the Hcy level (Refsum, Helga, 2001). Even worse, the pathways of methionine conversion to SAM and the whole range of related methyltransferases are secondarily inhibited. Herrmann et al. (2003a) studied B_{12} status in vegetarians and nonvegetarians, and found that omnivorous control subjects with tHcy concentrations <12μmol/L were with serum folate concentrations as low as 10.2nmol/L. In contrast, lacto-vegetarians or lacto-ovo-vegetarians (LV-LOV) subjects and vegans with hyperhomocysteinemia were observed with serum folate as high as 42.0nmol/L.

Step 5

Some amino acids obtained from diet are converted to propionyl-CoA, which is further converted to succinyl-CoA via methylmalonyl-CoA (MMA-CoA), by enzyme

methylmalonyl-CoA mutase (MCM) and co-factor B_{12} shown in Figure 1.3. In the case of B_{12} deficiency, MMA-CoA is hydrolysed to methylmalonic acid (MMA), increased serum levels of which are a classical marker of B_{12} deficiency and independent of folate. Moreover, B_{12} deficiency will also result in increased propionyl-CoA concentration (Allen et al., 1998).

Step 6:

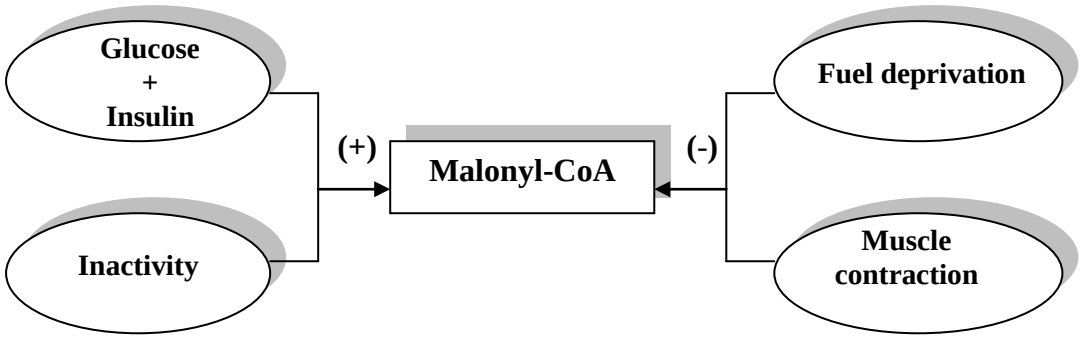
Increased propionyl-CoA can substitute for acetyl-CoA in fatty acid biosynthesis and lead to the formation of fatty acids containing an odd number of carbon atoms. Accumulated MMA-CoA due to B_{12} deficiency can also substitute for malonyl-CoA in fatty acid biosynthesis and lead to the formation of branch-chain fatty acids (Allen et al., 1998). It is postulated that the presence of odd carbon and branch-chain fatty acids might change the integrity of myelin structure and lead to demyelinisation (Metz, 1992).

In the liver, malonyl-CoA plays roles of both an intermediate in the de novo fatty acids synthesis (Wakil et al., 1983) and an allosteric inhibitor of carnitine palmitoyltransferase-1 (CPT1), the enzyme that controls the transfer of long-chain fatty acid into mitochondria for oxidation (McGarry, 1995). However, de novo fatty acids synthesis is minimal in tissues such as skeletal and cardiac muscle (Awan & Saggerson, 1993). Malonyl-CoA plays its dominant role of regulating CPT1 in muscle. Inhibition of CPT1 secondarily inhibits fatty acids oxidation and stimulates accumulation of fatty acids and glucose, thereby resulting in lipogenesis and insulin resistance (Figure 1.3). A high concentration of malonyl-CoA has been shown to elevate concentrations of triglyceride, diacylglycerol and fatty acids observed in insulin-resistant muscles (Pan et al., 1997), while acute decrease of malonyl-CoA level in skeletal muscle during exercise contributes to the increase in sensitivity and responsiveness of glucose transport and glycogen synthesis stimulated by insulin (Oakes et al., 1997).

Ruderman et al. (1999) explained that malonyl-CoA was a component of a fuel-sensing and signalling mechanism responding to changes in the fuel supply or energy expenditure of the muscle cell. They found that the concentration of malonyl-CoA increased two to sixfold within 20 mins when a rat soleus muscle was incubated with

glucose and insulin and within six hours when it was made inactive through denervation. Conversely, malonyl-CoA level acutely decreased during exercise or muscle contraction, or in a medium devoid of glucose (Ruderman et al., 1999). Figure 1.4 shows the effects of insulin, glucose, activity and fuel on malonyl-CoA level.

Figure 1.4: Regulatory factors for Malonyl CoA



Adapted from Ruderman et al. (1999)

1.1.5 B_{12} deficiency

The observations of Herbert (1994) suggested that, once someone stops eating B_{12}, he or she passes through the following four stages of negative balance: serum depletion (Ⅰ), cell depletion (Ⅱ), biochemical deficiency (Ⅲ), and finally clinical deficiency (Ⅳ). In stage Ⅰ, plasma stores become depleted and the levels of holotranscobalamin II (holoTC II) are reduced. In stage Ⅱ, holohaptocorrin decreases and the concentration of red cell B_{12} becomes low. Stage Ⅲ is characterized by functional imbalances indicated by elevated total homocysteine (tHcy) and methylmalonic acid (MMA) concentrations in plasma. In stage Ⅳ, clinical signs, such as anaemia, may become recognizable.

In humans, both B_{12} and folate deficiencies cause indistinguishable haematologic abnormalities, including anaemia, decreased white blood cell and platelet counts, and megaloblastic red blood cells (hypercellular bone marrow with abnormal maturation morphologic features). B_{12} deficiency, but not folate deficiency, also causes many neuropsychiatric abnormalities that involve demyelinisation of the nervous system,

including paresthesias, difficulty walking, loss of control of bowel and bladder, memory loss, dementia, depression and psychosis (Allen et al., 1998).

To be able to track the progression of B_{12} depletion or assess chronic insufficiency, different tests may be used.

1.1.6 Tests for B_{12} status

There are several methods of determining B_{12} status: serum B_{12} concentration, plasma total homocysteine (tHcy), methylmalonic acid (MMA), and holotranscobalamin II (holo-TC II). Their advantages and disadvantages are described below.

Serum B_{12}

Serum B_{12} reflects both intake and stores. It is transported in serum on two proteins: the circulating B_{12} delivery protein, transcobalamin II (TC II), and the circulating B_{12} storage protein, transcobalamin I (TC I), also called haptocorrin. The majority of serum B_{12} is bound to haptocorrin with a half-life of about six days in the blood (Herbert, V 1994), and only 6-20% is bound serum TC II with a half-life of only six minutes (Herbert, V. et al., 1990). Acceptable levels of serum B_{12} can be maintained for some time after deficiency occurs because of compensatory release of B_{12} from tissues. Lindenbaum et al. (1990) suggested that a proportion of people with normal serum B_{12} concentration were in fact B_{12} deficient. Low serum B_{12} concentrations would however represent long-term deficiency or chronic low intakes. The traditional clinical deficiency cut-point of serum B_{12} concentration is 150pmol/L, and concentration between 150pmol/L and 250pmol/L is defined as subclinical deficiency (Carmel, R. et al., 2003). This value might vary slightly among different laboratories. A cut-point of 170pmol/L was suggested by the Diagnostic Medlab Ltd who analysed the serum B_{12} concentration for the present validation study.

Plasma total Homocysteine (tHcy)

Homocysteine is normally present in the plasma at low concentrations (5-15μmol/L). Hence hyperhomocysteinemia is defined as a tHcy concentration >15.0μmol/L. Elevation of tHcy is a very sensitive indicator of clinical cobalamin deficiency (Lindenbaum et al., 1990). It has the major flaw however, of poor specificity, as tHcy

is also increased in folate and vitamin B_6 deficiencies, alcohol abuse (Carmel, R. & James, 2002), and renal insufficiency (Herrmann et al., 2001).

Methylmalonic acid (MMA)

Elevation of MMA, defined as a serum concentration >0.26μmol/L, is as sensitive a marker for clinical cobalamin deficiency as tHcy and is more specific. MMA rises when the supply of B_{12} is low or when absorption is affected (Joosten et al., 1996). However, the other causes for MMA elevation rather than cobalamin deficiency, are poorly understood, except renal insufficiency (Rasmussen et al., 1990) and concurrent use of antibiotics (Lindenbaum et al., 1990). The limitations of MMA include assay complexity, high cost, and limited availability (Carmel, R. et al., 2003).

Holotranscobalamin II (holo-TC II)

Most cobalamin in serum is carried on haptocorrin, which does not deliver its cobalamin to tissues. Holo-TC II is composed of B_{12} attached to transcobalamin, which represents the biologically active fraction that can be delivered into all DNA-synthesizing cells. Therefore, low holo-TC II is theoretically the earliest marker of cobalamin deficiency (Carmel, R. et al., 2003). However, the other influences on holo-TC II are not well understood (Carmel, R., 2002) and it is not widely accepted for clinical use. Low holo-TC II was defined as concentration <35pmol/L (Lindgren et al., 1999).

A study of 204 men and women aged 27-55y from India showed that, serum B_{12} had a strong inverse correlation with tHcy (r = -0.59) and MMA (r = -0.54). 47% of the subjects had serum B_{12} <150pmol/L, 73% had low holo-TC II, 77% had hyperhomocysteinemia, and 73% had elevated serum MMA (Refsum, H. et al., 2001). There were 26% of the subjects with normal serum B_{12} but low holo-TC II and/or elevated serum MMA, which suggested holo-TC II and serum MMA were more sensitive markers for cobalamin deficiency. However, folate deficiency was rare in this study.

A study in Denmark evaluated whether measurement of holo-TC II concentrations or TC saturation (holo-TC II/total TC) reflects active B_{12} absorption in healthy individuals and patients after B_{12} intake (Bor et al., 2004). Blood samples of 31

healthy individuals and seven patients were obtained before and after oral administration of B_{12}. In healthy individuals after intake of B_{12}, the maximum median increase was in TC saturation (52% and 0.04 as a fraction), closely followed by holo-TC II concentrations (39% and 34pmol/L). Serum B_{12} showed a smaller increase (14% and 36pmol/L). Three patients with Crohn's disease had the lowest increases in holo-TC II concentration and in TC saturation in both groups. It drew a conclusion that, holo-TC II concentrations and TC saturation reflect recent B_{12} absorption better than serum B_{12}.

Wolfgang Herrmann investigated B_{12} status in vegetarians and non-vegetarians. Subjects were divided into 3 groups: lacto-vegetarians or lacto-ovo-vegetarians (LV-LOV group), vegan, and omnivores. Low holo-TC II (<35pmol/L) was found in 11% of the omnivores, 77% of the LV-LOV group, and 92% of the vegans. Elevated MMA (>271nmol/L) was found in 5% of the omnivores, 68% of the LV-LOV group, and 83% of the vegans. Hyperhomocysteinemia (>12µmol/L) was present in 16% of the omnivores, 38% of the LV-LOV group, and 67% of the vegans (Herrmann et al., 2003b).

A pilot study of 11 Indian preadolescent girls in New Zealand (six meat-eaters vs six non-meat-eaters) (Chhichhia, 2007) showed that holo-TC II was positively correlated to serum B_{12} ($r=0.86$, $p<0.001$), while negatively correlated to MMA ($r=-0.76$, $p=0.01$) by Spearman's rho. Mean holo-TC II of the meat-eating girls was significantly higher than non-meat-eating girls (mean±SD in the thesis, 84±33pmol/L vs 39±13pmol/L, $p=0.02$), as was serum B_{12} (543±201pmol/L vs 231±95pmol/L, $p=0.01$). Meat-eating girls tended to have lower MMA levels than non-meat-eating girls, but no significant difference was found (0.15±0.05µmol/L vs 0.30±0.18µmol/L, $p=0.10$). The results of holo-TC II were not published as these analyses were made after thesis submission. The presence of low serum B_{12} in these non-meat-eating preadolescent girls demonstrates that women may also be at risk of B_{12} depletion and if they became pregnant this will also affect their baby.

1.1.7 Imbalance between B_{12} and folate

The New Zealand Ministry of Health recommends that all women planning a pregnancy take a daily 800µg folic acid supplement (Public Health Commission, 1995), starting four weeks before conception and continuing through to the twelfth week of pregnancy (Ministry of Health, 1997), to prevent neural tube defects (NTDs). Except for the recommendation above, folic acid fortification of food is a potentially effective way of increasing folic acid intakes of most women throughout their childbearing years. However, others in the population are exposed to increased amounts of folic acid at the same time. Concerns had been expressed that an increased folic acid intake might delay the diagnosis of B_{12} deficiency or even also possible progression of neurological damage (Herbert, V 1994; Rush, D., 1994). High blood concentrations of folic acid may be related to decreased natural killer cell cytotoxicity, and high folate status may reduce the response to antifolate drugs used against malaria, rheumatoid arthritis, psoriasis, and cancer. Folate has a dual effect on cancer, protecting against cancer initiation but facilitating progression and growth of preneoplastic cells and subclinical cancers (Smith et al., 2008).

Since 1998, fortification of flour products in the US with folic acid has been mandated by the Food and Drug Administration, with the aim of preventing the NTDs (US Food and Drug Administration & Health and Human, 1996). Due to age-related declines in vitamin absorption and extraction of B_{12}, and increases in autoimmunity against intrinsic factor or the gastric parietal cells, the elderly are of particular concern. The consumption of folic-acid-fortified foods in the elderly increases the risk of B_{12} deficiency not being detected. Morris et al. (2005) reported an increased risk of cognitive decline in elderly persons (aged ≥65y) who took folic acid supplements in doses >400µg/day, while the cognitive decline was less marked in those who also took high-dose B_{12}-containing supplements. The 1999-2002 US National Health and Nutrition Examination Survey studied 1,459 healthy elderly person aged ≥60y (Morris, M. S. et al., 2007) and reported that, 20% of subjects had a high concentration of serum folate, defined as >59nmol/L. In subjects with a low B_{12} status, high serum folate was inversely related to anaemia and cognitive impairment. When B_{12} status was normal however, high serum folate was associated with protection against cognitive impairment.

In a study that investigated the effect of folate and B_{12} on homocysteine concentrations (Quinlivan et al., 2002), 30 men and 23 women in UK received sequential supplementation with increasing doses of folic acid. After supplementation, the usual dependency of homocysteine on folate diminished, and B_{12} became the main determinant of plasma homocysteine concentration. This finding suggests that a fortification policy based on folic acid and B_{12}, rather than folic acid alone, is likely to be much more effective at lowering homocysteine concentrations, with potential benefits for reduction of risk of vascular disease.

Canada is another country with folic-acid-fortified flour since mid-1997 and a recommendation of periconceptional folic acid supplements. It has been assumed that there is no problem of folic acid deficiency among their population since then. A population-based case-control study 1993-2004 (Ray et al., 2007) measured serum holo-TC II of women (89 with NTD vs 422 unaffected controls) at 15 to 20 weeks' gestation. The mean serum holo-TC II concentration was lower among cases (67.8pmol/L) than controls (81.2pmol/L). Comparing the lowest with the highest quartile of maternal holo-TC concentration, the adjusted odds ratio for NTD was 2.9 (95% CI = 1.2-6.9), which meant almost a tripling in the risk for NTD in the presence of low B_{12} status. And the corresponding population-attributable risk for NTD in relation to low B_{12} was 34%. This study also suggested that the benefits of adding B_{12} to the periconceptional folic acid supplements or folic-acid-fortified foods needed to be considered.

Provided by more evidence that maternal and neonatal B_{12} status was related, a cross sectional observation in Brazil (Guerra-Shinohara et al., 2002) of 69 pregnant women and their newborn babies at the time of delivery. Maternal blood was collected up to 8 hours before delivery and umbilical cord blood was collected after the expulsion of the placenta. The result showed that, there was a large positive association ($r=0.68$, $p<0.01$) between maternal and neonatal B_{12} level. Pregnant women with low B_{12} levels are unable to provide the necessary amount of B_{12} to their foetuses.

In India, a country with high prevalence of B_{12} deficiency due to its dietary patterns, the Pune Maternal Nutrition Study (Yajnik et al., 2008) was designed to study the relationship between maternal nutrition and foetal growth, size at birth, and postnatal

growth. Seven-hundred pregnant women were involved, whose maternal nutritional intake and circulating concentrations of folate, B_{12}, tHcy and MMA were measured at 18 and 28 weeks of gestation. Their offspring were studied for anthropometry, body composition and insulin resistance at the age of 6 years. Results showed that, two thirds of mothers had low serum B_{12} (<150pmol/L), 90% had high MMA (>0.26μmol/L), and 30% had increased tHcy concentrations (>10μmol/L). Only one had a low erythrocyte folate concentration. Higher maternal erythrocyte folate concentrations at 28 weeks predicted higher offspring adiposity and more insulin resistance (both $p<0.01$), while higher maternal B_{12} status predicted lower adiposity and less insulin resistance in the offspring. Children born to mothers with a combination of low B_{12} and high folate concentrations were the most adipose and the most insulin resistant.

In New Zealand, up to 60% of pregnancies may be unplanned (Ministry of Health, 2003b). Therefore, the recommendation of periconceptional supplements will not benefit this group of pregnant women, and some babies might not have an optimal start of life. To improve the nutrition status of all women of childbearing age would be an efficient and long-term strategy, as maternal dietary patterns are associated with profound and possibly detrimental effects on the future health of the offspring.

1.1.8 Dietary patterns

Rather than focusing on single nutrients it is more meaningful to look at dietary patterns because nutrients act in synergy (e.g. B_{12} and folate, vitamin C and non haem iron) or may be antagonistic (e.g. phytates inhibit zinc and iron absorption, a high protein diet increases urinary loss of calcium). Therefore to translate nutrient research to public health action and understanding it is important to consider the context of the nutrient intake in the terms of whole diet commonly eaten. In this section variations in the animal sources of meat, vegetarianism and Indian culture and dietary patterns are considered.

Red-meat-eating vs non-red-meat-eating

Red meat refers to meat from mammals, for example, beef and lamb are red meats. Pork is also red, although it turns to a whitish colour when cooked, while chicken and

fish are white meats. The main determinant of the colour of meat is the concentration of myoglobin, a protein that contains iron. Red meat has a higher concentration than white meat.

Red meat is a good source of complete protein, creatine, and minerals such as iron, zinc and phosphorus, and vitamins such as niacin, B_{12}, thiamine and riboflavin. Red meat is the main source of B_{12} in the food supply and non-red-meat-eating individual might be in risk of low intakes of nutrients mentioned above, especially protein, iron, and B_{12}. However, regular consumption of red meats has also been linked to increased cancer risk, cardiovascular diseases, bone loss, type 2 diabetes, hypertension and arthritis (Fraser, 1999) due to its high content of saturated fat. To obtain the optimal nutritional benefits from red meats the advice is to select lean cuts, use low-fat cooking methods, and consume moderate amounts.

Obviously, vegetarians belong to non-red-meat-eating group and most of non-vegetarians are of red-meat-eating group. However, a few non-vegetarians consume white meat but not red meat for personal reasons, such as religion, allergy, or just dietary habit, would also be classified as non-red-meat-eaters. In recent years, the vegetarian dietary pattern, absolutely belonging to non-red-meat-eating pattern, has been adopted by an increasing number of people in Western countries. There are five broad groups of vegetarians (Jayanthi, 2001):

- Lacto-vegetarians consume milk and milk products in addition to the usual plant foods (cereals, vegetables, and fruits).
- Lacto-ovo-vegetarians consume eggs, milk and milk products in addition to plant foods.
- Ovo-vegetarians consume eggs and all the plant foods, but not milk and milk products.
- Fruitarians eat only the parts of plants that can be obtained without destroying the plant itself, such as grains, nuts, and fruits.
- Vegans eat only plant foods. They abstain from all animal foods, even milk products.

Of these five groups, fruitarians and vegans might be at the greatest risk of B_{12} deficiency as they exclude all eggs, milk and milk products in their diets, which are the best sources of B_{12} for vegetarians.

Indian diet

In India, the family's meal pattern depends on geographical region, religion, community and family practices (Jayanthi, 2001), and also socio-economic status. Meat consuming habits are predominated by religious beliefs. Hindus, the largest part of the Indian population, consume chicken, lamb and pork, but not beef; Muslims do not consume pork; while the minority group Christians/Catholics consume all types of meat (from personal communication with Purvi Chhichhia, an Indian colleague). Meat-eaters from eastern, western coast and southern India consume more fish than meat, whereas those from northern and some parts of southern India consume more red meat and white meat compared to fish or sea foods. Consumption of meat is higher in higher socio-economic classes.

Traditional Indian vegetarians belong to the lacto-vegetarians, who include milk and milk products in their diet in addition to the usual plant foods (cereals, pulses, vegetables, and fruits). The Indian non-vegetarian pattern has a cereal preparation as the main course, with a fish, poultry, or meat dish as an accompaniment in addition to vegetable and dahl preparations and salad. Fish and meat is served 1-3 times weekly (Jayanthi, 2001). The 1998-1999 National Family Health Survey-2 (NFHS-2) ("India 1998-99: results from the National Family Health Survey," 2001) sampled 315,598 individuals 15 years or older in India, and reported that one third of women never consumed chicken, meat or fish, and one in ten women never consumed milk or curd, which are rich in protein and B_{12}. The Indian women's nutrition status was poor; about two fifths of women were malnourished, with a BMI below 18.5kg/m^2, and nearly one third of pregnant women had moderate to severe anaemia.

In addition to nearly half of the Indian population being vegetarian, the low consumption of meat and dairy products, even among non-vegetarians, puts a large number of people at risk of low protein quality diet, decreased B_{12} concentration and nutritional anaemia due to B_{12} deficiency. Women, especially in their childbearing years, need to be better nourished. Refsum et al. (2001) reported that the mean serum

B_{12} of 204 men and women aged 27-55y from Pune, India, was 154pmol/L, even though only 38% excluded meat, poultry, fish, and eggs from their diet. In contrast, serum folate concentration were relatively high (median: 12.2nmol/L) and only 5% of the subjects had folate deficiency. The non-vegetarian diet included little animal foods; only 38% ate animal products more than once per month. B_{12} nutrition was poor in both vegetarians and non-vegetarians. Therefore it is to be expected that Indian migrants would bring these dietary patterns to the countries that they emigrate to and would be exposed to similar risk. However as a minority population this may not receive as much attention as it deserves, particularly for women of childbearing age who if malnourished will also expose the developing foetus to risks of nutrient imbalance and deficiency.

1.2 Measurements used for assessing body composition and obesity

Low B_{12} status can adversely affect de novo protein synthesis and lipogenesis, which finally results in low fat free mass (FFM) and high fat mass (FM). Therefore, measurements of body composition are necessary to demonstrate these adverse effects.

1.2.1 Body mass index (BMI)

BMI is calculated as weight in kilograms divided by height in meters squared. Obesity and overweight are commonly defined by measurements of BMI, which has been used by most of the studies as a reasonable measure of adiposity given that body weight and stature are simple, inexpensive, safe, and practical measurements to acquire (Gallagher et al., 1996). BMI cut off points are used clinically to identify high risk individuals for screening and identify individuals for absolute risk assessment (World Health Organisation, 2004). Thus, BMI is a useful tool for the initial screening of overweight, obesity and associated risk for chronic disease.

Limitations of BMI

Although BMI is the most commonly used surrogate for measuring overweight and obesity, there are certain limitations to its application. BMI may be stature dependent over at least part of the age range, and also be affected by relative leg length or relative sitting height (Garn et al., 1986). The most important limitation is that BMI does not differentiate between obese and muscular individual (Rush, E. C. et al., 1997), i.e. it does not distinguish FM and FFM. This is particularly applicable to Asians, who had a high body fat percentage (BF%) compared to a Caucasian with the same BMI (Deurenberg et al., 2002). Deurenberg et al. (2002) also reported that the Asian population in their study (Chinese, Malay and Indian Singaporeans) for similar BMI exhibited 3-5% higher BF% and for the same BF% their BMI was 3-4kg/m^2 lower than Caucasians. There are differences in body composition amongst Asians as well. For the same age, gender and BMI, Indians have the highest BF% compared to Malay and Chinese (Deurenberg-Yap et al., 2000). Therefore, in the present thesis, BMI was used for an initial assessment of overweight and obesity.

1.2.2 Bioelectrical impedance analysis (BIA)

A more accurate method of assessing body composition is bioelectrical impedance (BIA). BIA is based on the theoretical relationship between the volume of a conductor and its electrical impedance. In biological systems, electrical conduction is related to the geometry of the conductor and its characteristics. For the human body this is water and ionic distribution. The FFM, including the non-lipid components of adipose tissue, contains virtually all of the water and electrolytes of the body and thus its electrical conductivity is far greater than FM (Lukaski et al., 1985). Following the electrical conduction principles the length of the body, height, is squared and divided by the impedance or resistance value as an index of conductivity. The impedance value combined with anthropometric data like weight and height in prediction equations validated against a more accurate method of body composition measurement e.g. isotope dilution is used to derive body compartment measures. Measurement of bioimpedance is relatively inexpensive and less time consuming compared to other techniques like underwater weighing, magnetic resonance imaging (MRI) and computed tomography (CT) scan.

It has been established that there is a constant relationship between bioimpedance measurements in the standing and lying position (Rush, E. C., Crowley et al., 2006). As space and time was limited, standing hand-to-foot bioelectrical impedance was chosen as the body fat assessment measurement in this study. Lying impedance was consistently higher than standing with the relationship (resistance lying/resistance standing) for aged 15 to 30 years measuring 1.024, while for aged 31 to 59 years, it was 1.018. Therefore, a correction factor of 1.021 (the average for 15 to 59 years) was used in this study.

In present thesis, BF% of 35% is used as a reference value to categorise increased body fat in women of childbearing age as suggested by Goh et al. (2004).

1.2.3 Waist circumference and waist-to-hip ratio (WHR)

As observed above, BIA provides an estimate of total body fat, but not body-fat distribution. Intra abdominal fat is clinically the most relevant type of fat in humans. Adverse effects of increased intra abdominal adipose tissue include insulin resistance, dyslipidaemia, glucose intolerance, hypertension, hypercoagulable state, and increased cardiovascular disease risk (Misra & Vikram, 2003). Intra abdominal adipose tissue can be measured accurately using techniques like MRI and CT scan, which are costly and inconvenient for a large sample size such as a population survey. It is believed that waist measurement reflects total and abdominal fat accumulation and as an index of adiposity is not greatly influenced by height.

In a cross-sectional study of 586 men and women living in Spain, it was shown that a high waist circumference was associated with high concentrations of circulating oxidized low density lipoprotein (LDL) independent of BMI (Weinbrenner et al., 2006). Oxidised LDL is a cardiovascular disease risk factor. The risk of high oxidised LDL concentrations in overweight (BMI=25.0-29.9kg/m^2) or obese (BMI≥30kg/m^2) subjects with a waist circumference <102cm in men or <88cm in women was not significantly different from that in normal-weight subjects with these waist circumferences. In contrast, overweight or obese subjects with higher waist circumferences (≥102cm for men and ≥88cm for women) were at significantly higher risk of increased oxidised LDL.

A case-control study on obesity and the risk of myocardial infarction in 27,098 participants (12,461 cases and 14,637 controls) from 52 countries assessed the relation between BMI, waist and hip circumferences, and WHR to myocardial infarction and reported that WHR showed a graded and highly significant association with myocardial infarction risk worldwide (Yusuf et al., 2005). A national survey of 11,247 Australians aged ≥25y showed that WHR had the strongest relationship with type 2 diabetes, dyslipidaemia (women only) and hypertension (Dalton et al., 2003).

The International Diabetes Federation (2005) recommended ideal waist circumference for Europids, South Asians and Chinese women was <80cm, while that for Japanese women was <90cm. These values were taken from various data sources. A cut off value of 0.85 for WHR is used as recommended by World Health Organization (2000).

1.3 Dietary and physical activity assessment

It is not possible to assess nutrient intakes of a person without asking them about what they eat and what they do. For individuals in energy balance, habitual energy intake must equal energy expenditure. Assuming that an individual is in energy balance, dietary assessment should provide a valid measure of habitual energy intake (EI) (Black et al., 1997) and therefore energy expenditure (EE). If basal metabolic rate (BMR) is known then physical activity level (PAL; EE/BMR) may be derived. A 24-hour recall provides a retrospective record of intake over the previous day or the 24-hour preceding the interview. Typically, an individual is asked to recall all foods eaten during the reference time period, describe the foods, and estimate the quantities consumed (Berdanier et al., 2007). It has become a favoured way of collecting dietary data (Buzzard et al., 1996) as recalls can be administered easily and quickly with low respondent burden. However, one major disadvantage of the 24-hour recall is that it relies on the respondent's memory and ability to estimate portion sizes. When conducted with a random sample population, a single 24-hour recall is appropriate for estimating group means, but is not a tool to predict individual level health outcome. Because of intra-individual variation in intake, multiple recalls, including weekends

and weekdays, are needed to accurately estimate usual nutrient intake (Berdanier et al., 2007).

Food records, also known as food diaries, provide a prospective account of foods and beverages consumed in a defined period of time, usually 1 to 7 days. Participants are asked to carry the record with them and to record foods as eaten. Records are useful for detecting imbalances in food intake and making dietary change recommendations. A 7-day diet diary (7DDD) has been shown to be a better representative tool to measure usual energy intake and dietary pattern than a single 24 hours diet recall (24HDR) (Livingstone & Robson, 2000). The ability to detect an invalid report is improved by using 7DDD as it provides a smaller coefficient of variation in the estimated EI/BMR: 95% confidence intervals range 1.05-2.28 compared to a 24HDR 0.87-2.75 (Black, 2000). A 7DDD gives additional information including weekends about the timing and frequency of eating occasions, foods and food combinations eaten and fluid intake, energy density, and sources of nutrients like B_{12} and folate. The respondent burden for a 7DDD is higher and requires better literacy than a 24HDR. However, the number of days of recording required to estimate intakes of individual nutrients varies from several days up to a month or even a year (Berdanier et al., 2007). As to B_{12}, a 34-day diet record for female adults or a 35-day diet record for male adults is required to determine B_{12} intake (Nelson et al., 1989). In the present study, in order to decrease error related to variation in food intake and also to balance subject tolerability and assessment objectives, a single 24-hour recall followed by a 7-day food record was used for data collection. Daily variability however, is needed for adjustment and detects patterns rather than absolutes.

Physical activity may be assessed by questionnaire, diary or more complex methods. Physical activity assessed using diaries is highly cost-effective compared to other methods like accelerometry and does not involve complicated procedures. It, however, requires literacy and attention to detail and is not so objective. Diaries can be used in literate individuals over a wide age range of age groups 10-50y (Bouchard et al., 1983). A physical activity diary developed and validated by Bratteby et al. (1997) requires the subject to record the level of physical activity for every quarter hour (15 minute period) of each 24 hour day. The activities are categorised into nine levels according to their average energy costs representing multiples of BMR. The

dominant activity of every quarter hour (15mins) is recorded. Thus, a physical activity diary presents a habitual energy expenditure pattern of levels of activity of an individual. Human energy expenditure varies every day, especially between weekdays and weekends, and therefore one weekend day should be included in any diary (Bouchard et al., 1983). Although a 7-day physical activity diary (7DPAD) does not present the same precision in measurements of energy expenditure as doubly labelled water, it is an inexpensive, convenient method of discriminating individual and group physical activity patterns (Rush, E. C. et al., 2008). Use of a 7DPAD in conjunction with a 7DDD, gives both an approximation of habitual energy intake and energy expenditure.

1.4 Aims

The present thesis proposed to examine the interrelationships of dietary patterns, body size and biomarkers of B_{12} status in women of childbearing age (18-50y). A preliminary investigation was to examine the 1997 National Nutrition Survey data and measurement of adequate intake of B_{12} in the New Zealand population determined from 24 hour dietary recall. The aims of the thesis were to

1. Determine the possible prevalence of possible B_{12} nutrient inadequacy from the NNS97- this is reported in chapter 2.
2. Examine the validity of 24HDR and 7DDD in relation to protein, B_{12}, and folate intakes.
3. Examine differences in biomarkers of B_{12} and folate status and haematological measurements between red-meat-eaters and non-red-meat-eaters, non-Indians and Indians, including B_{12}, folate, full blood cells count, blood glucose and lipids.
4. Examine the differences in body composition between non-Indians and Indians, red-meat-eaters and non-red-meat-eaters.
5. Evaluate the time spent on static and moving physical activity by these groups.

1.5 Hypotheses

1. The prevalence of B_{12} inadequacy in New Zealand is higher than that reported by NNS97 and is higher in some populations – people aged 65+ and women aged 15-18 years.
2. B_{12} intake assessed by 7DDD is correlated with that assessed by 24HDR.
3. Indians/non-red-meat-eaters have more body fat and larger waist circumference compared with non-Indians/red-meat-eaters.
4. Indians/non-red-meat-eaters have lower serum B_{12} and higher folate concentrations than non-Indians/red-meat-eaters.
5. Indians/non-red-meat-eaters have higher fasting glucose and less favourable lipids than non-Indians/red-meat-eaters.
6. Indians/non-red-meat-eaters consume a higher carbohydrate but lower protein diet compared to non-Indians/red-meat-eaters.
7. Non-red-meat-eaters consume a higher folate diet compared to red-meat-eaters.
8. Indians/non-red-meat-eaters are physically less active than non-Indians/red-meat-eaters.

1.6 Significance

Intergenerational associations of disease risk and the life course model predict that the health of the mother during pregnancy and breastfeeding will have profound life-long effect on the health of off spring. Maternal obesity, a product of excess calorie intake is recognised as a health risk but less evidence is available for other nutrient deficiencies or excesses. Prevention is the best treatment and therefore this study in women of childbearing age of varying dietary patterns, ethnicity and body size will have future implications for

1. Metabolic and cardiovascular disease risks associated with vegetarian style, Indian and non-red-meat-eating diets.
2. The association of Indians/non-red-meat-eaters diet and physical activity with body composition.
3. The importance of maternal nutrition status.

In order to meet the aims and the hypotheses of the present study, specific measurements were used to measure the body composition, biomarkers (risks for diabetes and cardiovascular diseases), nutrient intake (energy, macronutrients and micronutrients) and physical activity levels. The rationale for using these measurements has been discussed in the section 1.2.

CHAPTER 2: INVESTIGATION OF THE USE OF DIETARY RECALL TO DETERMINE PREVALENCE OF B_{12} NUTRIENT INADEQUACY

National Nutrition Surveys are voluntary sectional population surveys which provide information on food and nutrient intakes, dietary habits and nutrition-related clinical measures on a representative sample of the population. The NNS97 report provides a "snapshot" of the nutritional and health status of New Zealanders in 1997. One section was on nutrients derived from 24HDR. One day diet recall however, does not represent all other days and the distribution of nutrient intake is wider due to daily variability. All findings in NNS97 report were adjusted by intra- and between-individual variations and weighted to estimate national population characteristics.

Prevalence estimates of population inadequate intakes from 24HDR are influenced by a number of factors including:

1. Intra-individual day-to-day variation by repetition of 24HDR in a reasonable sample size and statistic adjustment by C-SIDE.
2. Accuracy of food composition tables
3. The cut-off point for determination of low dietary intake: EAR and RDI values
4. Characteristics and demography of the people surveyed

In the 1997 New Zealand National Nutrition Survey (NNS97) and the 2002 National Children's Nutrition Survey (CNS2002), breakfast cereals were erroneously included as a source of B_{12} and were reported as contributing 3% of the daily intake of B_{12} among New Zealand adults (15+ years) (Russell et al., 1999) and 6% of that among New Zealand children (5-14 years) (Parnell et al., 2003). B_{12} is only available in foods that are derived from animals or microbes. Plants may contain B_{12} analogues, but they have no B_{12} activity in the human body (Banerjee & Chatterjea, 1963). New Zealand cereals at present are not permitted to have B_{12} added. The nutritional information on New Zealand cereals product packets does not bear any information on fortification of B_{12}. Personal communication with Sanitarium, a major cereal products supplier, and

then the Food and Crop organization for the previous small pilot study of "Indian preadolescent girls: Lifestyle patterns and accumulated risk factors" (Chhichhia, 2007) confirmed that cereals in New Zealand were not fortified with B_{12} and that there was an error in the FOODfiles data entries (and also previous years) for B_{12} in some cereals.

In addition, at the time of the NNS97 report, the estimated average requirement (EAR_{1999}) of B_{12} was 1.25μg (from the UK Dietary Reference Values 1991) for all adult age and sex groups (Russell et al., 1999). However, the Nutrient Reference Values for Australia and New Zealand 2005, revised the B_{12} EAR_{2005} for male and female adults to 2.0μg/day (Ministry of Health, 2005), and this was used as the cut-off in this reanalysis. Moreover, due to the change of dietary pattern and demography, a higher proportion of population may become vulnerable. Therefore, the assumption that "*vitamin B_{12} intake appeared adequate for the New Zealand population*" as stated in the NNS97 report needs to be reconsidered. The impact of the change in both B_{12} in cereal and the revised EAR value on NNS97 data will be examined in this chapter. The purpose of this investigation was to better understand the reliability of a 24HDR and an up to 7 days diet record to determine risk of inadequacy.

The data set of NNS97 was supplied to Auckland University of Technology by the Ministry of Health in 1999. The contract between these two organizations was designed to maintain the confidentiality of the NNS97 unit record data, protect ownership of the unit record data set, and ensure the data set was widely used. All resources and materials used for this reanalysis are listed in Table 2.1.

Table 2.1: Resources and materials used for the NNS97 reanalysis

NNS97 Reports	NZ Food NZ People. Key results of the 1997 NNS
	Food Comes First. Methodologies for the NNS
NNS97 Tables	24 Hr Diet Recall (Initial & Repeat)
	Nutrient Analysis (Initial & Repeat Recalls)
	General Details
	Clinical Measures
	Qualitative Food Frequency Questionnaire (QFFQ)
Software	FoodWorks 2007 version 5.00
	FOODfiles 2004© Crop & Food Research NZ
	Excel 2003™
	SPSS 14.0™
	Sigma Plot 8.0™

2.1 Methodology

A detailed description of the survey design for the NNS97 is included in *Food Comes First: Methodologies for the National Nutrition Survey of New Zealand* (Quigley & Watts, 1997). Briefly an area based sampling frame was used with a three stage stratified design consisting of a selection of primary sampling units (PSUs), households within the selected PSU, and a single randomly selected respondent within a household.

Usual intake of a particular dietary component for an individual is defined as the long-run average for that individual. The distribution of a dietary component based on an individual's one day intake is wider than that of individual's usual intake, since an individual's day-to-day diet is likely to be highly variable. The software package C-SIDE was used in the NNS97 to estimate the distribution of usual intakes of dietary components (Russell et al., 1999). However, the software C-SIDE was not available to estimate the distribution of usual intakes of dietary components in this reanalysis. Without C-SIDE, the mean or median intake for the group may be adequately estimated, but the within-subject variability distorts estimates of the percentiles above and below the mean by increasing the total variance of the distribution. As a result, the distribution of observed intakes is wider and flatter than the truth. Therefore, the raw data was used for this reanalysis, and all the results from reanalysis represent only

the participants involved in this survey, not the whole New Zealand population. The validity of dietary intake was not assessed, as there was no B_{12} biomarker, e.g. serum B_{12}, measured for the sample represented in the NNS97.

Following are the steps in the process for reanalysis of B_{12} intake using the NNS97 raw data.

2.1.1 Systematic exclusion of B_{12} derived from cereals

All tables of NNS97 were exported into Excel™ files. All the food intakes for each participant were included in the table "Initial 24 Hr Diet Recall". The word "Cereal" appeared in two columns: "item" and "descriptor". Some cereals in column "item" had descriptors starting with "milk", "sugar", and "milo", this was because all additions to cereals are categorized into the item "cereal" and not all foods in item "cereal" were actually cereals. Using sort procedures, the column "descriptor", which described the food in details, was manipulated so that foods with the word "cereal" in column "descriptor" were selected, copied, and pasted into a new Excel file. Of totally 107,041 food items in the table, 2,328 of them contained the word "cereal".

2.1.2 Quantity of B_{12} from cereals for individuals

In the new spreadsheet, the column "product" was sorted in alphabetical order. Most records in column "product" were brand and product names, whose compositions could be easily found out by the software "FoodWorks", including the amount of B_{12} in per unit (gram or millilitre) in the product. Due to the low density of cereal, there were large (up to 10 times) differences in the amount of B_{12} between per gram and per millilitre. For example, one gram of corn flakes was reported to contain 0.02µg B_{12}, while one millilitre only contained 0.002µg. For those cereals without brand and product names, matched foods in New Zealand FOODfiles 2004 were found according to their descriptors. For instance, the matched food for the descriptor "Cereal, biscuits (weetbix-type..), regular/plain" (sic) was "Whole wheat biscuits, Weet-Bix" in FoodWorks. Then the documented amount of B_{12} of these products was calculated in the same way as above.

Some cereal products show in FoodWorks and the associated NZ food files with B_{12}. Then the B_{12} content of each cereal product the individual consumed was calculated by multiplying the B_{12} content in per measurement unit with the amount documented in the 24HDR. However, this was not the final B_{12} intake from cereals for an individual as some individuals had same or different cereals more than once in the 24HDR. These individuals were identified and their B_{12} intake from all cereals summed and then the final contribution of B_{12} from cereals was determined for each individual.

2.1.3 Adjusted B_{12} intake from foods reported by individuals

The modified file and the table "Nutrient Analysis Initial Recall" provided as part of the NNS data were both converted into SPSS files and merged by individual ID number (refnum) and a new file with both reported B_{12} intakes and B_{12} from cereals was created. The adjusted reported B_{12} was calculated by the following formula: Adjusted B_{12} Intake = Reported B_{12} intake – B_{12} from Cereals.

An anomaly was reported after the new calculation of B_{12}, as four participants resulted in a negative daily B_{12} intake. In the original file this was found to be associated with large amounts of cereals (>150g) in all cases and these amounts were large compared to the quantities of those same products reported by the other individuals. Once the unit "g" was changed to be "ml", their adjusted reported B_{12} intakes were positive. Therefore, their adjusted reported B_{12} intakes were recalculated by changing the measurement unit.

2.1.4 Ethnicity

In the original record, each participant was asked about his/her ethnicity three times during interview. According to New Zealand ethnicity priority, if the subject reports "Maori" in any of three answers, then he/she is Maori. If he/she reports "Pacific Islander" in any of three answers, but not "Maori", then he/she is Pacific Islander. If he/she reports "Asian" in any answer, but not "Maori" or "Pacific Islander", then he/she is Asian. If he/she reports "Other ethnicity" in any answer, but not "Maori" or "Pacific Islander" or "Asian", then he/she is that ethnicity. If he/she reports

"European" in any answer, but not "Maori" or "Pacific Islander" or "Asian" or "Other ethnicity", then he/she is European. Thus the hierarchical ethnicities of individuals were identified based on their three answers in the table "General Details".

2.1.5 Manipulation of the database

All individual clinical measures, such as height and weight, were recorded three times in the table "Clinical Measures". These measures were averaged and the eating patterns in table "QFFQ" were recoded into "red-meat-eaters vs non-red-meat-eaters". Finally, the adjusted reported B_{12} intakes file, the tables "General Detail", "QFFQ" and "Clinical Measures" were merged together. Nine hundred and seventy seven subjects who withdrew or did not complete the survey were removed from the merged file. Then, a revised spreadsheet was ready for reanalysis. The same process was carried out for the tables "Repeat 24 Hr Diet Recall" and "Repeat Nutrient Analysis".

2.2 Results and Discussion

The individuals were divided by sex, age, ethnicity, dietary pattern, and compared with their reported and adjusted B_{12} intakes. Individuals were identified with inadequate B_{12} intake by the old and revised EAR values.

2.2.1 Application of the revised estimated average requirement (EAR_{2005})

When the revised EAR_{2005} (2.0μg/day) was used as the cut-off, the total number of participants with B_{12} intakes lower than EAR increased dramatically, from 579 to 1,250, and the total percentage was raised from 12.5% (8.1% of males vs 15.6% of females) to 27.0% (18.2% of males vs 33.2% of females), by their adjusted B_{12} intakes (Table 2.2).

Table 2.2: Inadequate B_{12} intake by using different EAR values

	EAR_{1999}=1.25μg/day				EAR_{2005}=2.0μg/day			
	Reported		Adjusted		Reported		Adjusted	
Male	139	7.2%	157	8.1%	317	16.5%	351	18.2%
Female	380	14.0%	422	15.6%	827	30.5%	899	33.2%
Total	519	11.2%	579	12.5%	1,144	24.7%	1,250	27.0%

EAR, Estimated average requirement.

2.2.2 Effect of subtracting B_{12} from cereals

A total of 733 individuals (15.8% of population) were affected by subtracting B_{12} derived from cereals, which counted for 0.0036-9.72μg (median 0.60μg). Sixty more individuals were identified with inadequate B_{12} intake if the EAR_{1999} value was used, while 106 more individuals were identified if the EAR_{2005} value was used (Table 2.2). Reported B_{12} counted from cereals took up 50.0% - 97.4% of 49 individuals' daily B_{12} intakes (1.1% of population). Once the B_{12} intakes from cereals were subtracted, the median B_{12} intake of these 49 individuals reduced from 2.4μg/day (range 0.6–11.4) to 0.9μg/day (range 0.0–2.9). These individuals might not be identified with inadequate B_{12} intake when cereal was considered as one source of B_{12} in the NNS97 report. However, their adjusted B_{12} intakes demonstrated that, these individuals would possibly have inadequate B_{12} intake, no matter either the EAR_{1999} value or the revised one was used. In addition, B_{12} intakes from cereals of another 112 individuals (2.4% of population) took up 30.0% – 49.6% of their daily intakes. The median B_{12} intake of these 112 individuals was lowered from 3.3μg/day (range 0.9-7.8) to 2.0μg/day (range 0.6-5.2) by subtracting B_{12} from cereals, which meant half of these 112 individuals' adjusted B_{12} intakes were inadequate, according to the EAR_{2005} value.

2.2.3 Comparison of reported and adjusted B_{12} intakes

The average B_{12} intake of the population reduced from 4.8μg/day to 4.6μg/day. The number of individuals with B_{12} intake lower than EAR_{2005} increased from 1,144 to 1,250, counting for from 24.7% to 27.0% of the whole population (Table 2.3). Subjects were divided into five age groups of males and females using the same intervals as in the NNS97 report (Russell et al., 1999). In males, the percentages of

subjects with B_{12} intake lower than EAR_{2005} were significantly different among the five age groups (p=0.002 in reported B_{12} intake, and p=0.006 in adjusted B_{12} intake), while those of females were not significantly different (p=0.128 in reported results and p=0.103 in adjusted results), using Pearson Chi-Square. All medians in the table are smaller than means, which indicates a positive skewed distribution of the dataset, and more people were with lower B_{12} intakes than the mean values. The adjusted reported daily median intake of B_{12} for the New Zealand population was 3.1µg/day (males 3.9µg, females 2.7µg). Males 19-44 years had higher daily intakes (4.2µg/day) than males 65+ years (3.2µg/day), while females 19-24 years had higher daily intakes (3.0µg/day) than females 65+ years (2.3µg/day) (Figure 2.1). Overall, females had nearly twice the rate of inadequate intake in either individual age groups or total population than males (33.2% vs 18.2%, Table 2.3). Males 15-18 years had the lowest rate of inadequate B_{12} intake (14.7%), while males 65+ years had the highest (25.5%) of the five age groups. Females 65+ years also had the highest percentage of inadequate B_{12} intake (38.1%). Females aged 15-18 years had the second highest possibility (35.0%). And the lowest rate in five female groups was 25-44 years (31.1%), which was even higher than the males highest rate group (65+ years, 25.5%).

Figure 2.1: Daily reported & adjusted B_{12} intake in male and female age groups

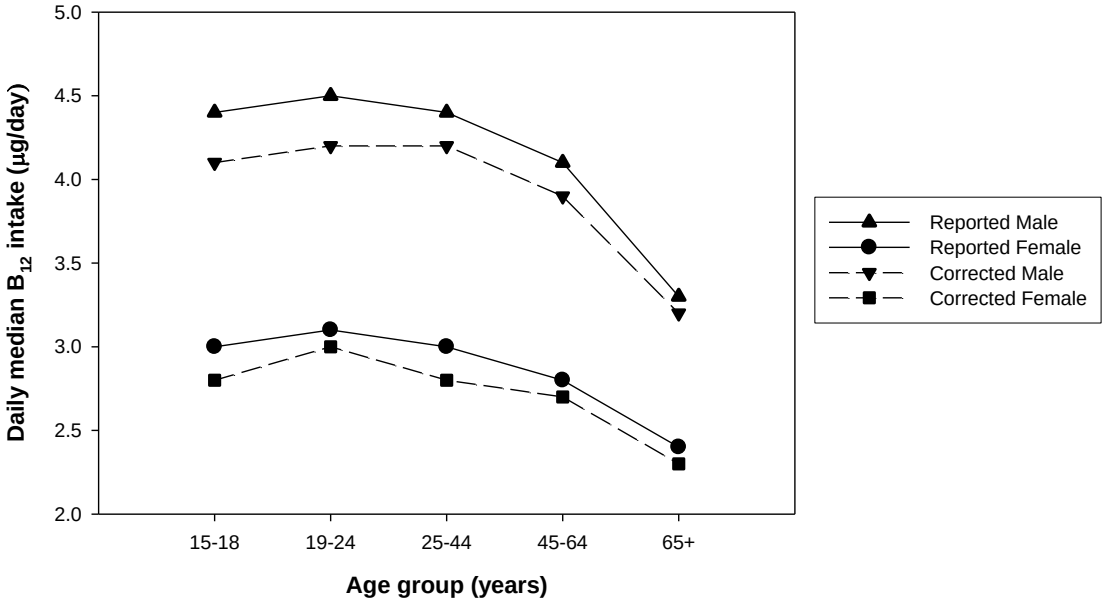


Table 2.3: Comparison of reported and adjusted B_{12} intakes for 4,635 people in the NNS97

		Reported B_{12} intakes (µg/day)							*Adjusted B_{12} intakes (µg/day)*					
		N	**Mean**	**SEM**	**10th [1]**	**50th [1]**	**90th [1]**	**Inadequate intake [n(%)] [2]**	**Mean**	**SEM**	**10th [1]**	**50th [1]**	**90th [1]**	**Inadequate intake [n(%)] [2]**
NZ Pop'n (Age 15+)		4,635	4.8	0.12	1.2	3.3	8.4	1,144(24.7%)	4.6	0.12	1.1	3.1	8.2	1,250(27.0%)
Male	**15-18**	109	5.0	0.29	2.1	4.4	9.4	10 (9.2%)	4.8	0.3	1.5	4.1	9.4	16 (14.7%)
	19-24	145	5.2	0.33	1.3	4.5	11.1	26 (17.9%)	5.0	0.32	1.2	4.2	10.7	27 (18.6%)
	25-44	759	6.5	0.34	1.7	4.4	11.6	116 (15.3%)	6.4	0.34	1.6	4.2	11.4	126 (16.6%)
	45-64	588	6.5	0.63	1.7	4.1	10.5	89 (15.1%)	6.4	0.63	1.5	3.9	9.8	99 (16.8%)
	65+	326	4.3	0.29	1.2	3.3	7.3	76 (23.3%)	4.2	0.29	1.2	3.2	7.0	83 (25.5%)
	Total	1,927	6.0	0.24	1.5	4.1	10.4	317 (16.5%)	5.8	0.24	1.4	3.9	10.2	351 (18.2%)
Female	**15-18**	137	3.4	0.20	0.9	3.0	6.4	46 (33.6%)	3.3	0.19	1.6	2.8	6.3	48 (35.0%)
	19-24	209	4.0	0.35	1.0	3.1	7.2	58 (27.8%)	3.9	0.35	0.9	3.0	7.0	70 (33.5%)
	25-44	1,204	4.0	0.13	1.1	3.0	7.1	352 (29.2%)	3.9	0.13	1.0	2.8	6.9	375 (31.1%)
	45-64	667	3.9	0.27	1.0	2.8	6.5	199 (29.8%)	3.7	0.27	0.9	2.7	6.4	219 (32.8%)
	65+	491	4.0	0.44	1.0	2.4	6.0	172 (35.0%)	3.9	0.44	0.9	2.3	6.0	187 (38.1%)
	Total	2,708	3.9	0.12	1.0	2.8	6.7	827 (30.5%)	3.8	0.12	1.0	2.7	6.5	899 (33.2%)

[1] Percentiles.

[2] With referent to reported B_{12} intake lower than EAR_{2005} in the raw data.

2.2.4 Dietary pattern

Thirteen percent of females 15-18 years were most likely to report eating a vegetarian diet or avoiding red meat (Russell et al., 1999). Four point two percent of the population (195 individuals) were non-red-meat-eaters (2.8% males vs 5.2% females) as determined from the QFFQ table. Nearly half of non-red-meat-eaters (49.7%) reported inadequate B_{12} intake, which was almost twice that of red-meat-eaters (25.8%). Within 195 non-red-meat-eaters, males 15-18 years had the lowest prevalence of reported inadequate B_{12} intake (0 of 4, 0.0%), while males 65+ years had the highest rate (5 of 7, 71.4%). There was no difference between originally analysed and adjusted rates of inadequate B_{12} intakes among non-red-meat-eaters, which might be due to the small sample size in each subgroup. Except for males 15-18 years, all the other non-red-meat-eaters subgroups had higher inadequate B_{12} intake rates than the comparative red-meat-eaters subgroups (Table 2.4). The distribution of red-meat-eaters was similar to the total population as they occupied 95.8% of the total population.

Table 2.4: Comparison of daily reported B_{12} intakes of red-meat-eaters and non-red-meat-eaters in sexual age groups of the NNS97

			Reported B_{12} intakes (µg/day)				*Adjusted B_{12} intakes (µg/day)*			
		N	**Mean**	**SEM**	**Median**	**Inadequate intake [n(%)]**	**Mean**	**SEM**	**Median**	**Inadequate intake [n(%)]**
Non-red-meat-eaters		195	2.7	0.20	2.0	97(49.7%)	2.6	0.20	2.0	97(49.7%)
Male	**15-18**	4	5.0	1.92	3.5	0(0.00%)	5.0	1.92	3.5	0(0.0%)
	19-24	7	2.6	0.63	2.3	3(42.9%)	2.4	0.49	2.3	3(42.9%)
	25-44	16	2.3	0.36	2.2	7(43.8%)	2.2	0.33	2.2	7(43.8%)
	45-64	19	2.9	0.50	2.3	8(42.1%)	2.6	0.42	2.3	8(42.1%)
	65+	7	4.9	3.89	0.7	5(71.4%)	4.9	3.89	0.7	5(71.4%)
	Total	53	3.1	0.56	2.3	23(43.4%)	2.9	0.55	2.3	23(43.4%)
Female	**15-18**	20	2.6	0.34	2.3	8(40.0%)	2.6	0.33	2.3	8(40.0%)
	19-24	11	2.6	0.73	1.8	6(54.5%)	2.6	0.73	1.8	6(54.5%)
	25-44	63	2.3	0.22	1.9	34(54.0%)	2.3	0.22	1.9	34(54.0%)
	45-64	31	3.1	0.63	2.0	16(51.6%)	3.0	0.64	2.0	16(51.6%)
	65+	17	2.0	0.26	1.7	10(58.8%)	2.0	0.25	1.7	10(58.8%)
	Total	142	2.5	0.19	1.9	74(52.1%)	2.5	0.19	1.9	74(52.1%)
Red-meat-eaters		4,334	4.9	0.13	3.4	1,016(23.4%)	4.7	0.13	3.2	1,118(25.8%)
Male	**15-18**	98	5.0	0.29	4.5	10(10.2%)	4.8	0.29	4.3	15(15.3%)
	19-24	135	5.3	0.34	4.6	23(17.0%)	5.1	0.33	4.3	24(17.8%)
	25-44	722	6.6	0.36	4.5	102(14.1%)	6.5	0.36	4.3	112(15.5%)
	45-64	565	6.6	0.65	4.1	80(14.2%)	6.4	0.65	4.0	90(15.9%)
	65+	315	4.4	0.29	3.3	70(22.2%)	4.2	0.29	3.2	77(24.4%)
	Total	1,835	6.0	0.25	4.2	285(15.5%)	5.9	0.25	4.0	318(17.3%)
Female	**15-18**	111	3.6	0.23	3.3	35(31.5%)	3.4	0.22	2.9	37(33.3%)
	19-24	187	4.1	0.39	3.1	50(26.7%)	4.0	0.39	3.1	60(32.1%)
	25-44	1,119	4.0	0.13	3.0	312(27.9%)	3.9	0.13	2.9	335(29.9%)
	45-64	617	3.9	0.29	2.8	176(28.5%)	3.8	0.29	2.7	196(31.8%)
	65+	465	4.1	0.46	2.5	158(34.0%)	4.0	0.46	2.3	172(37.0%)
	Total	2,499	4.0	0.13	2.9	731(29.3%)	3.9	0.13	2.7	800(32.0%)

2.2.5 Adjusted B_{12} intakes and dietary patterns of ethnicities

The NNS97 participants were divided into 15 ethnic groups in the report "NZ Food NZ People". However, the ethnic groups "European/Pakeha" and "Other European" were categorized into the group "European" during this reanalysis. As shown in Table 4.5, the three ethnic groups of highest rates of B_{12} inadequate intakes were Other Pacific Islander (3 of 5, 60.0%), Indian (14 of 26, 53.8%), and Fijian (9 of 18, 50.0%). Tongan seemed to have the lowest inadequate intake rate among all ethnic groups (6 of 40, 15.0%). Indian had the lowest B_{12} intake (2.1μg/day) among all ethnicities, which is close to the EAR_{2005} value and lower than the RDI value (2.4μg/day), followed by Other Pacific Islander (2.3μg/day) and Fijian (2.6μg/day). All the other ethnic groups were with mean values higher than 3.1μg/day, and the 33 Chinese had the highest average B_{12} intake (8.3μg/day). Regarding their dietary patterns, the Indians had extremely high prevalence of not eating red meat (10 of 26, 38.5%), compared to the rate of total population (4.2%), followed by Tokelauan (1 of 5, 20.0%). The percentages of non-red-meat-eaters of all the other ethnicities ranged between 0.0% and 8.7% (Table 2.5). However, the small numbers in these ethnicities means that generalisation is impossible but suggests further investigation is required.

Table 2.5: Adjusted B_{12} intake and dietary pattern in different ethnic groups of the NNS97

Ethnicity	*Total N (%)*	*Mean B_{12} intake*	*SEM*	*Inadequate intake [n (%)]*	*Age^ (y)*	*Weight^ (kg)*	*Non-red-meat-eaters [n (%)]*
European*	3,505 (75.6%)	4.5	0.15	946 (27.0%)	45.0	71.3	132 (3.8%)
New Zealand Maori	703 (15.2%)	5.3	0.29	171 (24.3%)	34.0	76.6	29 (4.1%)
Samoan	154 (3.3%)	4.9	0.50	48 (31.2%)	32.5	84.7	12 (7.8%)
Cook Island Maori	70 (1.5%)	5.7	1.05	17 (24.3%)	33.0	87.7	3 (4.3%)
Tongan	40 (0.9%)	4.7	0.48	6 (15.0%)	30.5	86.1	1 (2.5%)
Niuean	15 (0.3%)	5.4	1.85	5 (33.3%)	42.0	83.9	1 (6.7%)
Tokelauan	5 (0.1%)	3.6	0.72	1 (20.0%)	18.0	90.0	1 (20.0%)
Fijian	18 (0.4%)	2.6	0.44	9 (50.0%)	40.5	70.0	1 (5.6%)
Other Pacific Islander	5 (0.1%)	2.3	0.53	3 (60.0%)	32.0	70.3	0 (0.0%)
Southeast Asian	12 (0.3%)	3.7	1.24	5 (41.7%)	38.5	57.2	1 (8.3%)
Chinese	33 (0.7%)	8.3	3.17	6 (18.2%)	34.0	61.1	2 (6.1%)
Indian	26 (0.6%)	2.1	0.26	14 (53.8%)	35.0	59.2	10 (38.5%)
Other Asian	23 (0.5%)	4.0	0.85	9 (39.1%)	40.0	60.8	2 (8.7%)
Other Ethnic Groups	26 (0.6%)	3.1	0.38	10 (38.5%)	37.5	74.0	0 (0.0%)

*including European/Pakeha and Other European
^Median values

2.2.6 People with high B_{12} intake

In the NNS97 report, 19% of the New Zealand population consumed multi vitamin and/or mineral supplements and a further 10% reported consuming a vitamin B complex supplements. Both these groups of supplements are likely to contain B_{12}. The content of B_{12} in commercial supplements varies from 10μg to 100μg per tablet (gained from the labels of supplements available in the central Auckland shops). If the adjusted B_{12} intake >10μg/day was defined including consumption of B_{12} supplement, 319 people (7%) were identified. This percentage was reasonable because it was smaller than the total percentage (29%) of multi vitamin and/or mineral and vitamin B complex supplements consumption. The prevalence of inadequate B_{12} intake would be even worse if these 319 people were excluded. However, there might be overlap of B_{12} from dietary source and supplements using the cut-off of 10μg/day, as a person consuming a large amount of red meat (or other food contain a large quantity of B_{12}, such as pork liver) might exceed that cut-off a day. Table 2.6 shows that, more males reported a B_{12} intake >10μg/day than females in each age group and within sex group. The age group of 25-44 years reported the highest proportion of B_{12} intake >10μg/day in both males (13.8%) and females (5.1%). Table 2.7 suggests that, New Zealand Maori had greater percentage of B_{12} intakes >10μg/day than the other three major ethnicities. Also there was no individual of Tokelauan, Fijian, and Indian ethnicities with a reported B_{12} intake >10μg/day in this survey.

Table 2.6: People with adjusted reported B_{12} intakes >10µg/day in male and female age groups of the NNS97

		Adjusted B_{12} intakes (µg/day)							
		Number	**Mean**	**Range**	**SEM**	**10th [1]**	**50th [1]**	**90th [1]**	**Percentage[2]**
NZ Pop'n (Age 15+)		319	21.9	10.0- 290.9	1.44	10.6	14.3	33.8	6.9%
	15-18	9	11.6	10.0 – 16.6	0.71	10.0	10.7	16.6	8.3%
	19-24	17	13.2	10.2 – 24.5	0.86	10.3	12.0	19.2	11.7%
Male	**25-44**	105	20.7	10.1 – 121.2	1.89	10.3	13.2	35.9	13.8%
	45-64	56	28.8	10.5 – 290.9	5.76	11.1	16.5	46.9	9.5%
	65+	18	19.4	10.1 – 69.1	3.25	10.3	15.8	36.1	5.5%
	Total	205	21.8	10.0 – 290.9	1.89	10.3	14.2	334.4	10.6%
	15-18	2	12.5	12.1 – 12.8	0.35	12.1	12.5	12.8	1.5%
	19-24	6	25.3	10.8 – 53.8	7.67	10.8	15.4	53.8	2.9%
Female	**25-44**	61	18.2	10.1 – 64.0	1.26	10.7	14.9	30.4	5.1%
	45-64	26	22.1	10.0 – 162.8	5.81	10.2	14.5	34.1	3.9%
	65+	19	34.0	10.4 – 144.8	8.81	10.6	16.5	117.9	3.9%
	Total	114	22.0	10.0 – 162.8	2.16	10.6	14.9	34.2	4.2%

[1] Percentiles.
[2] Percentage of people with B_{12} intake >10µg/day.

Table 2.7: Adjusted B_{12} intakes >10µg/day in four major ethnic groups of the NNS97

Ethnicity	*Adjusted B_{12} intakes (µg/day)*				
	Number	**Mean**	**Range**	**SEM**	**Percentage***
European[1]	214	22.5	10.0 – 290.9	1.97	6.1%
New Zealand Maori	72	19.3	10.1 – 121.2	2.04	10.2%
Pacific Islander[2]	26	21.1	10.1 – 64.0	2.63	8.5%
Asian[3]	7	32.3	10.2 – 95.2	11.87	7.4%

* Percentage of people with B_{12} intake >10µg/day.
[1] including European/Pakeha and Other European
[2] including Samoan, Cook Island Maori, Tongan, Niuean, Tokelauan, Fijian, and other Pacific Islander
[3] including Southeast Asian, Chinese, Indian, and other Asian.

2.2.7 Changes of ethnic proportion in New Zealand 1996-2006

According to data of Statistics New Zealand (Table 2.8), the proportions of the total population decreased in European and New Zealand Maori from 1996 to 2006, while the other ethnic groups all increased during these ten years. The Indian ethnic group increased most; from 38,403 people in 1996 to reach 104,583 people in 2006 (almost trebled). The Fijian ethnic group also increased rapidly from the 1996 Census, up 48.2 percent to total 9,864 people.

Table 2.8: Ethnic groups 1996 & 2006 New Zealand Censuses

Ethnicity	*1996 Census*		*2006 Census*	
	Count	**Percentage**	**Count**	**Percentage**
European	2,594,694	71.71%	2,609,592	64.79%
New Zealand Maori	523,371	14.46%	565,329	14.04%
Samoan	83,718	2.31%	131,103	3.25%
Cook Island Maori	34,167	0.94%	58,008	1.44%
Tongan	26,061	0.72%	50,481	1.25%
Niuean	14,712	0.41%	22,476	0.56%
Tokelauan	4,461	0.12%	6,819	0.17%
Fijian	6,657	0.18%	9,864	0.24%
Chinese	70,227	1.94%	147,570	3.66%
Indian	38,403	1.06%	104,583	2.60%
Total	3,618,303		4,027,947	

Note: People can choose to identify with more than one ethnic group, therefore figures may not sum to totals.

2.2.8 Repeatability of 24-hour diet recall (24HDR)

Six hundred and ninety five (one person was not available in initial nutrients analysis table) participants in NNS97 repeated their 24HDR within three weeks in the first National Nutrition Survey interview. Their initial and repeat adjusted reported B_{12} intakes were both recoded into binary variables as 0 (<2.0μg/day) and 1 (≥2.0μg/day), and then compared by cross tabulations. Within these 694 participants, 11.1% of them reported B_{12} intakes lower than 2.0μg/day in both initial and repeat 24HDRs, which could be definitely defined as inadequate usual intake of B_{12}. An additional 32.7% reported low B_{12} intake in either initial or repeat 24HDR, which might be in risk of inadequate B_{12} intake. The remaining 56.2% reported adequate B_{12} intake on both

24HDRs. The B_{12} intakes of initial and repeat 24HDRs were averaged and 19.9% of the population were lower than 2.0µg/day, which suggested that, except those 11.1% people with definite inadequate usual B_{12} intake, another 8.8% might have greater probability for their usual B_{12} intake to be lower than the EAR_{2005} value.

2.3 Conclusions

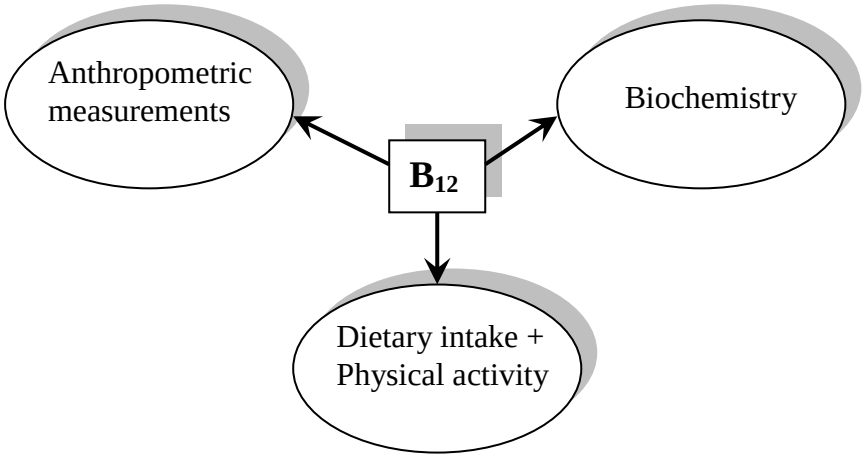
Cereals are not a source of B_{12} in New Zealand as at present there is no cereal fortified with B_{12} in the market. Investigation of the raw data provided for NNS97 and subtraction of the B_{12} derived from cereals, and application of the revised EAR_{2005} value (2.0µg/day) meant that, the apparent number and percentage of people with reported inadequate B_{12} intake might be more than doubled from that originally reported. Females had a higher prevalence of reported inadequacy than males of any age group. The median value of B_{12} intake in females (2.7µg/day) of all ages was 1.2µg lower than that in males (3.9µg/day). Non-red-meat-eaters (49.7%) had higher prevalence (almost twice) of inadequate B_{12} intake than red-meat-eaters (25.8%). The Indian population had an extremely high prevalence of not eating red meat (10 of 26, 38.5%), compared to the rate of total population (4.2%). Within all sexual age groups, females 15-18 years were most likely to report not eating red meat (13%). There was a greater percentage of males with B_{12} intake over 10.0µg/day than females (10.6% vs 4.2%), which might be due to a higher consumption of supplements containing B_{12} or of meat product portions. This was not investigated further for this report. Within all ethnic groups in New Zealand, Indian and Fijian populations were with highest rate of inadequate B_{12} intake. The number of these people in New Zealand more than doubled between 1996 and 2006, accounting for 1.24% to 2.84% of the whole population, small but significant in terms of health needs. There were 11.1% of individuals reporting B_{12} intake lower than the EAR_{2005} value in both initial and repeat 24HDRs. Another 8.8% reported average B_{12} intake lower than the EAR_{2005} value. Nowadays, the situation of inadequate B_{12} intake might be interpreted as more serious than ten years ago because of the EAR_{2005} value, the change in ethnic proportions and the increased tendency for young women to be vegetarian or avoid meat - females 15-18 years were most likely to report eating a vegetarian diet or avoiding red meat (13%) than the other sexual age groups in the NNS97 (Russell et

al., 1999). However, a blood biomarker is needed to validate B_{12} status, as there might be under-report or over-report in 24HDRs. Moreover, 24HDR is not enough to adequately determine B_{12} status. A 7DDD may be more appropriate for daily intake. According to the apparent high prevalence of risk for inadequate B_{12} intake in the survey individuals, a future survey to assess B_{12} status should use biomarkers to be more meaningful and accurate. In the present thesis, a validation study was designed to assess validity of 24HDR and 7DDD, and B_{12} status of women of childbearing age in New Zealand.

CHAPTER 3: VALIDATION STUDY DESIGN AND METHODS

In this chapter, the determination of subject number and participant recruitment of the validation study will be described first, followed by the design of the validation study, and then the methods of all measurements, and finally the statistical analysis. Based on Figure 1.1 and Figure 1.3, low level of B_{12} can adversely affect protein synthesis, lipogenesis, and insulin resistance, which result in decreased FFM and increased BF%, high fasting glucose and total cholesterol levels. Dietary intake should be positively related to B_{12} status. Body composition, such as FM and FFM, is associated with physical activity. All measurements of participants related to B_{12} are interpreted in Figure 3.1.

Figure 3.1: Measurements of participants related to B_{12}



3.1 Participants

3.1.1 Determination of subject number

This validation study was to determine the feasibility of the measurement techniques and the sample size necessary for a larger study to be carried out at a later date. A related pilot study (Chhichhia, 2007), supervised by Prof. Elaine Rush, looking at B_{12} status, showed that there was significant positive correlation ($r=0.66$, $p=0.02$) between

7DDD B_{12} dietary intake and blood level in 12 preadolescent Indian girls. The difference in means of reported B_{12} intake was 5μg/week (12.6 vs 17.7, non-meat-eaters vs meat-eaters) and the standard deviation was 5 so the effect size was 1.0. With the acceptable power being 85% and the alpha being 0.05, the power calculation predicts that 19 women would be needed to participate in the present validation study. Given that the proposed study will not be looking at an entirely Indian population, the number is doubled to 40 so that a range of dietary patterns are achieved and to account for the heterogeneity of the sample (ethnicity, age, diet).

Also, considering the period of study (1 year), the sample size of 40 could be realistically measured within the time frame. By recruiting 40, participants who could not complete the study were accounted for, and a bigger number meant there would be more variety in the dietary pattern as there would not be specific recruitment of vegetarian participants. However, only 38 participants were involved during the recruitment period (August – early December 2007). With the time frame in mind, 38 participants, with different ethnicities and dietary patterns, were recruited before the Health Clinic closed in the middle of December for Christmas and New Year holidays.

3.1.2 Inclusion and exclusion criteria for the study

It was essential that women were of childbearing age (18-50 yrs) and healthy. Participants were excluded if they were male; were not between 18 and 50 years; had chronic disease or major health conditions, malabsorption diseases; were physically disabled; were pregnant; or were consuming supplements including B_{12}. It was explained that if they could not or did not want to have any of the measurements conducted on them then they were not eligible for this study.

3.1.3 Participant recruitment

Participants were recruited by advertisement (Appendix 1) on Auckland University of Technology electronic staff and student notice board, personal contacts by word of mouth and establishing contacts through participants' network. More references and contacts were obtained through supervisors and colleagues who helped recruiting

subjects. Different ethnicities and a variety of dietary patterns including vegetarian were sought. As only three of the first 28 participants were vegetarians, personal contacts were mainly focused on recruiting vegetarians for the last ten subjects, in order to obtain enough power for analysing differences among varied dietary patterns. Ethical approval was gained from the Auckland University of Technology on July 13, 2007 (Appendix 2) and informed signed consent was gained before any participation.

Once a self-selected woman showed interest in taking part in the study, the participant information sheet (Appendix 3) was handed out and a follow up telephone or e-mail contact was made to explain the study and to answer any question. Exclusion criteria were emphasised to make sure she did not fit within them and then a timetable of available times for making an appointment for visit 1 was sent in the email to her. After making a decision to be involved in this study, she ticked an appointment time and sent it back to the researcher. Then the subject was then officially recruited on receipt of the appointment time.

3.2 Design

The study "Women of childbearing age: Dietary patterns and vitamin B_{12} status" was a cross-sectional validation study designed to provide pilot data on the feasibility of measuring B_{12} intake in dietary reports and validating this by measuring the serum level of B_{12} and holo-TC II in a group of women of diverse dietary habits. Anthropometric measurements, Bioelectrical Impedance Analysis (BIA) and fasting blood sample collections were made during visit 1. The previous 24-hour dietary and physical activity recalls were collected by researcher through interview during visit 1, followed by 7DDD and 7DPAD completed daily by participants. The dietary and physical activity data were collected by the participants over the one week between two field visits. Visit 2 was seven days following visit 1 (Table 3.1) and the participants came to Auckland University of Technology Akoranga campus for both visits.

Table 3.1: Research procedures in data collection

Step	*Body of work*
Visit 1	
Anthropometric measurements and health screening	• Weight and height • Waist and hip circumference • Grip strength • Mid upper arm circumference • Blood pressure and pulse • Standing bioimpedance for determination of body water and fat
Quick questionnaire	• Age • Ethnic group • List of foods not eaten (this will identify different types of diet including classes of vegetarianism) • Taking supplements or not • Menstruation conditions • Others: smoking or not, any medicine taken
24HDR	Structured interview
7DDD and 7DPAD	Explain how to complete diet and activity diaries
Fasting blood sample collection	Analyses: • Fasting serum folate and B_{12} • Fasting serum glucose • Fasting serum lipid profile • Full blood count • Holotranscobalamin II (Holo-TC II)
Visit 2	• Collect 7DDD and 7DPAD • Hand out blood assays results • Suggest someone with clinically abnormal results to visit a doctor.

Visit 1 procedure

This visit was arranged in the early morning as participants' fasting blood sample was collected during this visit. Participants were informed to be fasting at least eight hours but not more than 12 hours, and not to drink too much water before the visit, as their body water percentage would be measured. At the beginning of visit 1, the outline of the study and what would happen during this study were explained to the participants. Informed written consent was obtained before any measurement was conducted. During visit 1, anthropometric measurements of height, weight, waist circumference, hip circumference, and mid upper arm circumference (MUAC) were made and recorded. Bioelectrical impedance analysis was used for estimating FFM, FM and BF%. Functional measurements including right and left hand grip strength, blood

pressure and resting pulse rate were recorded. Personal information including date of birth, menstruation conditions, ethnicity, dietary pattern, food avoided, smoker or not, supplements and medication intake were recorded. Then the researcher collected the fasting blood sample. A previous 24 hours dietary and physical activity recall was completed through interview and the participants were instructed on how to record their daily activity and the food they ate. More explanation was given to the participants about the completing the 7DDD and 7DPAD. All the procedures of visit 1 occurred the Health Clinic of Auckland University of Technology Akoranga campus and a total time required for visit 1 was approximately 1 ½ hours.

Blood analysis

The fasting venous blood samples were drawn by the researcher, an experienced phlebotomist, at the Health Clinic in Auckland University of Technology Akoranga campus. Approximately 14ml of blood sample was drawn from each participant. Biochemical examination of the serum samples included fasting plasma glucose, Total Cholesterol, High Density Lipoprotein (HDL) cholesterol, Low Density Lipoprotein (LDL) cholesterol, total/HDL cholesterol ratio, triglycerides, folate, B_{12} and holo-TC II. Haematological examination included Full Blood Cell (FBC) count. Four millilitres blood samples were collected in EDTA coated sterile tubes for FBC, 2ml in Fluoride tube for fasting glucose, 4ml in SST tube for lipid profile, folate and B_{12}. These three tubes of blood samples were delivered to Diagnostic Medlab's Northcote collection room by 4:00pm on the same day of collection. A 4mL heparin tube was held back for storage of plasma and later holo-TC II analysis. They were then centrifuged using Z150A compact lab centrifuge (Labnet International, Inc., USA) at the high speed (5,800 rpm/3,120 × g) for five minutes, aliquoted and stored in 1.5ml microfuge tubes to be delivered to Body Composition and Metabolism Research Centre (Auckland University of Technology Akoranga Campus) for temporary storage at -4ºC before they were stored at -85ºC for further analysis. Negotiations were made with the help of Professor Elaine Rush with Dr Lindsay Plank at the body composition unit at the University of Auckland to grant the permission for storing the samples in their freezers. A batch of plasma specimens were send to Middlemore Hospital (Auckland) for further analysis of holo-TC II.

Methods used for blood analysis

Glucose and serum lipids were measured by Diagnostic Medlab Ltd; Auckland using Roche/Hitachi Modular P/D automated clinical chemistry analysers. FBC count was measured using the Sysmex-XE-2100 haematology analyser, while serum B_{12} and folate were measured by the Modular Analytics E170 immunoassay analyser. Holo-TC II was measured by Middlemore Hospital Laboratory using AxSYM microparticle enzyme immunoassay analyser.

The blood reports were checked by Dr Janet Rowan for a medical opinion with particular attention to any signs of anaemia and B_{12} deficiency. The final results and the medical advice, if necessary, were given to the participant in the form of a letter.

Visit 2 procedure

The second visit happened seven days after visit 1 (Table 3.1). Participants came back to Auckland University of Technology Akoranga campus with 7DDD and 7DPAD. The researcher checked the two diaries to make sure that the participant had filled them in correctly for the seven days. More details were discussed if there were any unclear records in the diaries. Blood assay results were given in written format to the participant and explained as necessary. Results out of normal range were pointed out and suggestion of visiting a doctor was given to anyone with clinically abnormal results. Personalised diet and activity advice was given to the participants according to their blood assays result (e.g. if B_{12} was low), and the analysis of their previous 24 hours dietary and physical activity recall. A book named "Eat Less, Move More" (Author: Professor Elaine Rush) was given to each participant as a token of thanks for participating in the project. If required participants were followed up by email or phone for more details of their diaries during the analysis phase.

3.3 Measurements

All the measurements were made in the Health Clinic of Akoranga Campus at the Auckland University of Technology. Most of the anthropometric measurements were repeated at least once to ensure precision was known for the measurements. The third reading was recorded if the two readings were beyond a specified tolerance level:

- Height: more than 0.5cm
- Weight: more than 0.5kg
- Waist: more than 1cm
- Bioimpedance: impedance, resistance or reactance more than 1 ohm, or if the phase is less than 4°. (Check the electrodes are properly placed and the person's hands and feet are warm.)
- Blood pressure: more than 10mmHg

3.3.1 Height

Height was measured using a stadiometer fixed on the wall of the treatment room, consisting of a movable measuring rod and a horizontal headboard that slides to contact the vertex of the head. The horizontal headboard had a spirit level to ensure the horizontal placement of the headboard. It was ensured that the horizontal board at the base of the stadiometer was placed on a hard flat surface. The participant was asked to remove her shoes and then stood on the floor against the wall with both heels firmly together and weight distributed evenly between the feet. Arms were hanging by the sides with palms facing the thighs. The back of the heels, buttocks, shoulders and back of the head were in a line with the back as straight as possible. The head was held straight with the participant looking directly forward. The rod was kept straight throughout the procedure and not bent or curved. As the participant inhaled deeply and stretched tall, the horizontal board of the stadiometer was lowered to the top of the head firmly without exerting extreme pressure and ensuring the horizontal plane of the board was levelled using the carpenter's level. The standing height was measured to the nearest ± 0.1cm.

3.3.2 Weight

The weight was measured using an electronic digital scale (Soehnle, Germany).The accuracy of the weighing scale was confirmed every fortnight by cross checking its readings at a number of measurement points with a standard weighing scale in the anthropometry laboratory at Auckland University of Technology. Before weighing the participant, she was asked to empty her bladder and to remove her shoes and any heavy outer clothing or objects in pockets which might make a difference to weights.

The scale was placed on a hard, flat surface. The researcher pushed the button on the front edge of the scale with foot. When the display screen reads 0.0, the participant was asked to step on to it with both feet. The participant stood still in the centre of the platform, with the body weight evenly distributed between both feet. The weight was read off the screen and recorded. The unit of measurement was kilograms (kg). If her clothes were thick enough to make a significant difference to the weight, a remark was made and then the weight of those clothes was subtracted from the recorded weight. At least two measurements were recorded and weight was measured to the nearest ± 0.1kg.

3.3.3 Mid Upper Arm Circumference (MUAC)

The mid upper arm circumference was measured at the midpoint of the distance between the lateral projection of the acromial process and the inferior margin of olecranon process on the lateral aspect of the arm using a measuring tape with the forearm kept straight in the supine position. The midpoint was marked on the lateral side of the arm. The MUAC was measured twice to the nearest ±0.1cm by using figure finder tape measure (Novel Products Inc. Rockton, IL). All measurements were made on the right side of the body.

3.3.4 Waist Circumference

The waist was measured using the midpoint between the inferior lateral margin of the ribs and top of the iliac crest using horizontal position. The participants were asked to stand erect, abdomen relaxed, arms at sides and feet together. Each measurement was taken at the end of normal expiration to the nearest ±0.1cm by using figure finder tape measure (Novel Products Inc. Rockton, IL).

3.3.5 Hip Circumference

The participants were asked to stand erect, buttocks relaxed, feet together. The measurement was taken by placing the tape around the hips at horizontal level of the greatest gluteal protuberance and anteriorly at the level of symphysis pubis.

Measurements were made to the nearest ±0.1cm by using figure finder tape measure (Novel Products Inc. Rockton, IL).

3.3.6 Grip Strength

The participants were asked to hold the hand dynamometer (Smedley's Dynamo Meter, 100kg, TTM, Tokyo) in their pronated hand and compress it to the best of their ability. Measurements to the nearest ±1kg were taken for right and left hand alternately and repeated at least twice. The highest measurement for each hand was used for analysis.

3.3.7 Bioelectrical Impedance Analysis (BIA)

A tetrapolar single frequency 50 kHz analyser (Imp DF50, Impedimed Ltd, Australia) was used to perform the measurement. The bioimpedance analyser was checked weekly against a standard resistance block and the checked resistance measurement was recorded 409±8Ω. The bioimpedance measures were taken on the right hand side of the body of the participants while they were standing with legs apart and thighs not touching each other. It was ensured that there was no contact between the thighs or the arm and the trunk as this would create a short circuit in the electrical path thereby dramatically affecting the impedance value. The room temperature was kept warm (20-25°C). An electric heater was used to keep the room warm in the winter.

The skin was cleaned at the electrode sites with isopropyl alcohol wipes (Kendall, Webcol®, Alcohol Preps, Recorder 6818), four electrodes (3M Red Dot™, 2330) with electro conducting gel were attached at the following places:

a) Just proximal to the dorsal surface of the third metacarpal-phalangeal joint on the right hand (red coloured connection).

b) On the dorsal surface of the right wrist adjacent to the head of ulna (yellow coloured connection).

c) On the dorsal surface of the right foot just proximal to the second metatarsal-phalangeal joint (blue coloured connection).

d) On the anterior surface of the right ankle between medial and lateral malleoli (black coloured connection).

It was assured that the distance between the centre of the proximal and distal electrodes was at least 5 cm. The participants were asked to remain still while the analyser was turned on and off. The readings were obtained for impedance (Z ohm), resistance (R ohm), reactance (Xc ohm) and phase angle (P°). At least two sets of measurements were recorded. As the standing position was used in this study, a correction factor of 1.021 (the average for 15 to 59 years) was required to multiply with standing bioimpedance to equate with lying in this study. The Lukaski equation was used to analyse FFM for all ethnicities in this study. The whole group can be categorized into three ethnicity subgroup: Indian, Other Asian, and European/Maori. The Lukaski equation is mainly suitable for European. Another equation was found for migrant Asian Indian (Rush, E. C., Chandu et al., 2006). However, no equation was published for Other Asian healthy adults. Moreover, different equations have different correlation coefficient and standard error of estimate so more errors would appear if multiple equations were used. As most of participants were European, the Lukaski equation was used for the whole group. The results for FM, FFM and BF% were obtained by substituting the above adjusted resistance in the Lukaski equation.

$$\text{FFM} = 0.756H^2/R + 0.110W + 0.107X - 5.463 \text{ (Lukaski et al., 1986)}$$

$$R^2 = 0.98, \text{ SEE} = 2.06 \text{ kg}$$

$$\text{FM} = W - \text{FFM}$$

$$\text{BF\%} = \text{FM}/W \times 100\%$$

H = height (cm); R = resistance; SEE = standard error of estimate; W = weight (kg); X = reactance

3.3.8 Blood pressure and pulse

The arterial blood pressure was measured using digital sphygmomanometer (Omron IA2, Omron Healthcare Co. Ltd, Japan). The cuff size was selected depending on the mid upper arm circumference. The Medium Cuff/Bladder Set was used for mid upper arm circumference 22-32cm, while the Large Cuff/Bladder Set was used for 32-42cm. All blood pressures were measured on the left arm while the participants were seated in a relaxed position with their left arm stretched out, arm and hand relaxed and fist closed comfortably. The left arm was kept at heart level during the measurements and a minimum of two readings were recorded; systolic and diastolic to + 10mmHg and pulse to within 10 bpm.

3.3.9 Blood samples collection

The participant sat comfortably, arm extended straight from shoulder to wrist and well supported. The tourniquet was firmly applied to arm 8-10cm above venepuncture site and the researcher palpated the arm for a suitable vein, usually in the antecubital (elbow) area. Sometimes superficial veins were not readily apparent so then the arm was massaged from wrist to elbow, applying a warm washcloth for several minutes, or lowering the extremity. If none of these worked, the same process was tried on the opposite arm and after deciding which arm to use, venepuncture site was cleansed with isopropyl alcohol wipe. The area was allowed to dry to prevent haemolysis of specimen and burning sensation when venepuncture was performed. The process of venepuncture was as following:

a) With thumb of one hand the participant's skin was drawn taut 3-5cm below venepuncture site.
b) Using the other hand, with the bevel of the needle (PrecisionGlide^{TM}, REF 360212, 0.8×25mm) facing upwards the needle was lined up parallel to the vein, the vein firmly and smoothly punctured until the bevel was well covered. The needle and holder (Vacutainer$^{®}$ Brand Pronto^{TM}, Japan) was held securely in place.
c) The evacuated tube was slid into the holder with the free hand, the flange of the needle holder grasped between the first 2 fingers and the tube pushed onto the needle butt with thumb until the stopper was pierced.
d) When blood commenced flowing, the tourniquet was loosened and the tube was allowed to fill. The tube was removed from the holder, which was held steady to make sure the needle did not become dislodged from the vein.
e) The tube contents were mixed thoroughly with the additive immediately after drawing by inverting gently 4-5 times.
f) The process was repeated with the remaining tubes.

When all required tubes were filled, the tourniquet was completely released from arm; the needle was withdrawn gently from participant's arm and the puncture site was pressed quickly and firmly with a dry gauze swab. The participant was instructed to hold gauze for 1-2 minutes, to hold their arm straight and press firmly until bleeding stops. The used needle was disposed immediately into an approved disposal unit to

prevent re-use or injury. Tubes were labelled with the participant's name, trial number and the date. The date and time of collection were recorded on the pre-printed request form and the specimens were placed in Biohazard plastic bags with the forms in the outside pocket. The samples were then delivered to Diagnostic Medlab Northcote collection room on the same day. A 4mL heparin tube blood sample was centrifuged, aliquoted into 1.5ml microfuge tubes to be stored in freezer for further analysis. A batch of plasma specimens were send to Middlemore Hospital (Auckland) for holo-TC II assay. The ideal range of holo-TC II concentration suggested by Middlemore Hospital Lab was 19-120pmol/L. However, the reference range of low holo-TC II concentration used in the present thesis is <35pmol/L, derived from Lindgren et al. (1999).

3.3.10 Assessment of diet

The previous 24HDR was used to record what the participant ate and drank the day before visit 1 (Appendix 4) following the method of Gibson (2005). Information was collected through interview about the timing of consumption, the meal and foods consumed, the method of cooking, and the quantity of foods. Standard and household measurements were shown to participants for quantity description. Household measures included a teaspoon, a tablespoon, a cup and a portion of a plate. For foods like bread, the type of bread (wholemeal, white and mixed grains), thickness; toast or sandwich was reported. For ethnic foods, like chapatti the size and recipe was noted. This interview helped participants understand what should be recorded and how to record details in their own food diaries.

The 7DDD format as used in a previous study by Rush, E. C. et al. (2004) was provided by Professor Elaine Rush (Appendix 5). The procedure for using the 7DDD was similar to those used in the previous 24HDR. A ruler and a series of circles to estimate food portion size, such as an apple, were printed on the back cover of the diary. The diary was kept by the participant for seven days starting from the day of visit 1. And the researcher collected the diaries and quickly viewed them in visit 2 so that questions could be asked during visit 2 if there was any clarification of the diary required by the researcher. Otherwise, a phone conversation was required if questions

came out during analysis. To control for observer effects and bias, all the interviews and dietary analyses were conducted by the researcher (SX).

All the information obtained from the diaries was entered into the FoodWorks® program (FoodWorks professional 2007 XyrisT Australia, Queensland). For some foods which were not available in the program, standardized recipes were developed using ingredients within the FoodWorks program. All B_{12} in breakfast cereals will be subtracted for individuals as explained in chapter 2 as there are no B_{12} fortified breakfast cereals in New Zealand, but there are in Australia.

3.3.11 Assessment of physical activity

The physical activity diary was adapted from Bratteby et al.(1997) and Professor Elaine Rush (2008) (Appendix 6). Some modifications were made with respect to the description of physical activities for some categories so that the participants could comprehend it more easily in terms of their daily life (Table 3.3). One hour was divided into four quarters representing 15 minutes each, with one hour spread across a row (Appendix 6). There were totally 96 periods of 15 minutes in a whole day. The activities were categorized into nine levels, with level 9 representing the maximum intensity of physical activity, while level 1 representing minimum intensity (Table 3.3). For each 15 minute period the participants were asked to enter the activity level into the appropriate square. In the case of more than one level of activity happened in 15 minutes, the dominant activity of that period was recorded. If an activity was not listed, the participants were asked to record the closest activity of comparable intensity or make a note of it in the space provided on the page. A previous 24 hours physical activity recall was completed in visit 1 and recorded on the first page of seven day diary. Then the participant kept and recorded the diary for seven more days, following the example in the first page. And they brought it with the dietary diary back to the researcher in visit 2.

The number of 15 minute periods for each activity was summed up for each day and entered into an Excel spreadsheet along with the measured resting energy expenditure for each person. The total number of coded numbers filled in the table for each day was ensured to be 96. By multiplying the number for each activity level by the

physical activity ratio (Table 3.2) and averaging over the seven days, the average total energy expenditure was determined. The sum of time in each level of activity was also calculated. An individual estimated basal metabolic rate (BMR) was used during calculation.

Table 3.2: Physical activity ratios and examples of physical activities*

Category	Activities	Physical Activity Ratio
1	Sleeping, resting in bed	0.95
2	Sitting, eating, writing, listening, sitting in a car or bus, watching television etc.	1.5
3	Standing, washing etc.	2
4	Walking indoors, light home activities	2.8
5	Walking outdoors, light work, carrying a small bag	3.3
6	Leisure activities, sports and relaxed movement, running i.e. light intensity	4.4
7	Leisure activities, sports and manual work of moderate intensity	6.5
8	Leisure activities, sports and manual work of high intensity- sweating and breathing hard	10.0
9	Sports activities and work of very high to maximal intensity.	15.0

*Table adapted from Bratteby et al. (1997)

3.4 Statistical analysis

Descriptive statistics of data are presented as mean, standard deviation and maximum and minimum values as ranges. Due to the small sample size, data were compared using unequal variance unpaired t test. Effect size (ES) is used to describe the size of the difference in mean values between two groups relative to the standard deviation:

$$\text{Effect size} = (\text{Mean}_2 - \text{Mean}_1)/\text{SD}$$

Using an effect size is a meaningful way of comparing variables as it can be used to describe the magnitude of the difference between two groups in either experimental or

observational study designs. An effect size of 0.2 is considered small, 0.5 is considered medium and 0.8 is considered large (Peat & Barton, 2005).

Bivariate correlations were used to investigate relationships between variables: anthropometry, body fat by BIA, fasting blood biochemical, dietary intake and physical activity parameters using the Pearson's correlation coefficient (r) and 95% confidence intervals (CI) were calculated using an Excel spreadsheet provided by Professor Will Hopkins.

Sensitivity and specificity are often used to describe the utility of diagnostic tests in clinical applications. Sensitivity indicates how likely patients are to have a positive test if they have the disease and specificity indicates how likely the patients are to have a negative test if they do not have the disease (Peat & Barton, 2005). They are used for the validity of dietary assessments – 24HDR and 7DDD – on nutrition status of individual.

Subjects would be categorized into groups compared for ethnicities (Indians vs non-Indians) and dietary patterns (red-meat-eating vs non-red-meat-eating). The odds ratio is the odds of a person having a disease if exposed to the risk factor divided by the odds of a person having a disease if not exposed to the risk factor, or the odds of a person having been exposed to a factor when having the disease compared to the odds of a person not having been exposed to a factor when not having the disease. It is used for the analysis of the odds of an Indian/non-red-meat-eater being low in B_{12} status compared to the odds of a non-Indian/red-meat-eater.

All data were analysed using the statistical program SPSS 14.0™ provided by SPSS Inc, Chicago and Excel program™. The significance level was set at $p<0.05$.

CHAPTER 4: VALIDATION STUDY RESULTS

In this results chapter, demographics of the participants will be described first, then characterising anthropometry and physiological measurements followed by the blood biochemistry, and the dietary analysis 24HDR and 7DDD, and finally the analysis of the physical activity diaries. Supplementary information has been provided in appendices 7 and 8.

All measurements were completed for 38 women of childbearing age (30 ± 8y), except one European red-meat-eater did not complete the7DDD and 7DPAD. For this presentation of the findings participants were categorized in two ways based on ethnicity and red-meat-eating pattern:
6 Indians (28 ± 4y) & 32 non-Indians (31 ± 8y),
12 Non-red-meat-eaters (32 ± 6y) & 26 red-meat-eaters (30 ± 8y)
These two groups were not mutually exclusive as one Indian did eat red meat. The non-red-meat-eating group included 10 vegetarians and another two non-vegetarian non-red-meat-eaters. Vegetarians and non-vegetarians however, were not characteristic for the following analyses as the comparisons of these two subgroups were similar to those of red-meat-eaters and non-red-meat-eaters.

These independent categories were chosen given the original hypothesis and the supporting evidence provided in the introduction and chapter 2 to explore differences in B_{12} status of red-meat-eating and Indian culturally-based eating patterns. The differences between these grouping are summarised in section 4.8.

4.1 Ethnicity

This validation study was to cover a range of ethnic groups, however any healthy woman of childbearing age was welcome to take part and Indian women in particular were encouraged. The self-reported ethnicities are shown in Table 4.1 and they were categorized into two main ethnic groups: Indians and non-Indians (including Europeans & Other Asians) for the following analysis.

Table 4.1: Self-reported main ethnicities of 38 women of childbearing age

Categorised Ethnic groups	*Number*	*Reported Ethnicity*	*Frequency*
Indians	6	Asian Indian	5
		Indo Fijian	1
Europeans	26	Australian	1
		Canadian	1
		European	10
		New Zealander	10
		South African	2
		Maori+European	2
Other Asians	6	Indonesia	1
		Japanese	1
		Chinese	3
		Filipino	1
Total	38		38

4.2 Anthropometry

Body size was assessed by weight, height, waist, hip, mid upper arm circumference (MUAC) and fat free mass (FFM) by hand-to-foot single frequency bioimpedance analysis.

4.2.1 Weight and Height

Weight, height, BMI were compared with the recently published New Zealand and Australian reference values obtained from the New Zealand Ministry of Health (2005). According to BMI cut-off criteria (World Health Organisation, 2000), two subjects were underweight (BMI<18.5kg/m^2), while seven were overweight (BMI 25-30kg/m^2) and another three were obese (BMI>30kg/m^2). The cut-off point for overweight in Asian is 23kg/m^2 (Abbott & Young, 2006), lower than that for European. However, there were no Asian subjects in this study with a BMI between 23-25kg/m^2. Therefore, the reference range of BMI (18.5-25kg/m^2) was used for the analysis. One Indian woman, also a non-red-meat-eater, was 85.0kg heavier than the reference value of 61.0kg. Her BMI was 49.0kg/m^2 and at 172.6cm she was the tallest

Indian subject. If she was included in this analysis, the average weight and BMI would be higher than the reference values and a tendency was shown for Indian to be shorter than non-Indian (p=0.18, ES=0.71), while their BMI were larger than non-Indian (p=0.26, ES=0.69) (Table 4.2). However, once she was excluded, the average weight and BMI were close to the reference values, and a significant difference in height (p=0.04, ES=1.22) was observed between Indian (157.8±6.2cm) and non-Indian (165.6±6.6cm) groupings only (Appendix 7).

Table 4.2: Physical characteristics of 38 women of childbearing age categorized in different comparative groups

Physical characteristics	*Reference value*	*Total N=38*	*Indians N=6*	*Non-Indians N=32*	**p value*	*Effect size*	*Non-red-meat-eaters N=12*	*Red-meat-eaters N=26*	**p value*	*Effect size*
Age (y)		30 ± 8 (19 - 48)	28 ± 4 (24 - 33)	31 ± 8 (19 - 48)	0.13		32 ± 6 (24 – 42)	30 ± 8 (19 – 48)	0.34	
Height (cm)		164.8 ± 7.0 (149.6 – 186.0)	160.3 ± 8.2 (149.6 – 172.6)	165.6 ± 6.6 (155.5 – 186.0)	0.18	-0.71	164.4 ± 6.5 (149.6 – 172.6)	165.0 ± 7.4 (153.8 – 186.0)	0.83	-0.09
Weight (kg)	61.0[†]	64.5 ± 17.4 (42.9 – 146.0)	74.6 ± 36.2 (48.6 – 146.0)	62.6 ± 11.2 (42.9 – 88.9)	0.46	0.45	68.8 ± 25.3 (50.9 – 146.0)	62.4 ± 12.3 (42.9 – 88.9)	0.42	0.32
BMI (kg/m^2)	22.0[†] (18.5 – 25.0)[‡]	23.6 ± 5.5 (16.5 – 49.0)	28.3 ± 10.6 (20.4 – 49.0)	22.8 ± 3.6 (16.5 – 31.1)	0.26	0.69	25.3 ± 8.1 (19.1 – 49.0)	22.9 ± 3.8 (16.5 – 31.1)	0.33	0.38

Mean ± SD; Range in parenthesis; *p value calculated using independent unequal variance t test; BMI, Body mass index; [†]Reference from Ministry of Health (2005); [‡]Reference range from WHO (2000).

4.2.2 Girths

Average waist circumference (78.8cm, range 63.2-132.5cm) was smaller than the cut-off point (80cm) however 14 of 38 participants (four of six Indians) were above the reference range and one had an extremely large waist (132.5cm). This outstanding subject also had an extremely large hip circumference (147.5cm) and MUAC (46.7cm). The cut-off criteria used for waist circumference (<80cm) in the present study is that recommended by the International Diabetes Federation (2005), for Europids, South Asians, and Chinese women. Waist-to-hip ratio (WHR) >0.85 in women indicated abdominal fat accumulation and an increased risk of cardiovascular complications and related deaths (Bjorntorp, 1987). This cut-off was accepted and is widely used now (World Health Organisation, 2000). Within the whole group, there were three participants (all Indian) with WHR >0.85. Indians tended to have larger waist circumference ($p=0.19$, ES=0.84) and MUAC ($p=0.22$, ES=0.74) than non-Indians. Non-red-meat-eaters also tended to have greater waist circumferences than red-meat-eaters ($p=0.13$, ES=0.62). Significantly higher WHRs were observed in Indians ($p=0.02$, ES=1.55) and the non-red-meat-eaters ($p=0.06$, ES=0.78), than the comparative groups (Table 4.3). Similar results were found if the subject with extremely heavy weight (146.0kg) was excluded, as the Indians still tended to have higher waist circumference than non-Indians ($p=0.29$, ES=0.63). There was no difference in MUAC between Indians and non-Indians ($p=0.37$, ES=0.47) (Appendix 7).

Table 4.3: Girth measurements of 38 women of childbearing age categorized in different comparative groups

Girth measurements	*Reference value*	*Total N=38*	*Indians N=6*	*Non-Indians N=32*	**p value*	*Effect size*	*Non-red-meat-eaters N=12*	*Red-meat-eaters N=26*	**p value*	*Effect size*
Waist circumference (cm)	< 80†	78.8 ± 12.1 (63.2 – 132.5)	90.7 ± 22.5 (68.4 -132.5)	76.6 ± 7.9 (63.2 – 92.0)	0.19	0.84	84.5 ± 17.3 (66.5 – 132.5)	76.2 ± 8.0 (63.2 – 92.0)	0.13	0.62
Hip circumference (cm)		102.0 ± 11.1 (85.2 – 147.5)	108.0 ± 20.4 (90.9 – 147.5)	100.9 ± 8.4 (85.2 – 118.4)	0.44	0.46	105.3 ± 14.6 (91.6 – 147.5)	100.5 ± 8.9 (85.2 – 118.4)	0.31	0.40
WHR	<0.85‡	0.77 ± 0.05 (0.68 – 0.90)	0.83 ± 0.05 (0.75 – 0.90)	0.76 ± 0.04 (0.68 – 0.84)	0.02	1.55	0.80 ± 0.06 (0.68 – 0.90)	0.76 ± 0.04 (0.69 – 0.84)	0.06	0.78
MUAC (cm)		29.4 ± 4.3 (20.2 – 46.7)	32.9 ± 7.3 (26.5 – 46.7)	28.7 ± 3.3 (20.2 – 35.2)	0.22	0.74	30.9 ± 5.6 (25.0 – 46.7)	28.7 ± 3.5 (20.2 – 35.2)	0.21	0.47

Mean ± SD; Range in parenthesis; *p value calculated using independent unequal variance t test; WHR, waist to hip ratio; MUAC, mid upper arm circumference; †Reference range from International Diabetes Federation (2005); ‡Reference from WHO (2000)

4.2.3 Body fat percentage determined using bioelectrical impedance analysis

The mean body fat percentage of eight of 38 subjects was higher (~1.2–18.1%) than the cut-off criteria recommended for women (35%) (Goh et al., 2004). Four of the six Indians had BF% higher than 35% (Table 4.4). The average BF% of Indians (39.7%) was significantly higher (p=0.01, ES=1.64) than non-Indians (26.0%) while Indians (p=0.18, ES=0.87) and non-red-meat-eaters (p=0.17, ES=0.58) tended to have more FM than their comparative groups. Non-red-meat-eaters (33.1%) also had higher BF% (p=0.04, ES=0.79) than red-meat-eaters (25.9%, Table 4.4). Slightly different results were found if the subject with extremely heavy weight (146.0kg) was excluded (Appendix 7); Indians had significantly less FFM (p=0.03, ES=1.32) and higher BF% (p=0.02, ES=1.51) than non-Indian, but non-red-meat-eaters and red-eat-eaters were not different in FFM (p=0.07, ES=0.60) or BF% (p=0.09, ES=0.67).

Table 4.4: Body composition by bioimpedance analysis of 38 women of childbearing age categorized in different comparative groups

Body composition	*Reference value*	*Total N=38*	*Indians N=6*	*Non-Indians N=32*	**p value*	*Effect size*	*Non-red-meat-eaters N=12*	*Red-meat-eaters N=26*	**p value*	*Effect size*
FM (kg)		19.2 ± 12.1 (6.3 – 77.5)	31.8 ± 23.3 (14.1 – 77.5)	16.8 ± 7.2 (6.3 – 34.9)	0.18	0.87	24.6 ± 17.9 (10.1 – 77.5)	16.7 ± 7.5 (6.3 – 34.9)	0.17	0.58
FFM (kg)		45.3 ± 7.8 (30.7 – 68.5)	42.8 ± 13.6 (31.0 – 68.5)	45.8 ± 6.4 (30.7 – 64.3)	0.62	-0.28	44.3 ± 8.5 (33.1 – 68.5)	45.7 ± 7.5 (30.7 – 64.3)	0.62	-0.17
BF%	<35%†	28.2 ± 9.2 (12.2 – 53.1)	39.7 ± 9.1 (27.5 – 53.1)	26.0 ± 7.5 (12.2 – 44.2)	0.01	1.64	33.1 ± 10.1 (19.8 – 53.1)	25.9 ± 7.9 (12.2 – 44.2)	0.04	0.79
BF% ≥ 35% (percentage of the subgroup)		N = 8 (21.1%)	N = 4 (66.7%)	N = 4 (12.5%)			N = 4 (33.3%)	N = 4 (15.4%)		

Mean ± SD; Range in parenthesis; *p value calculated using independent unequal variance t test; FM, Fat mass; FFM, Fat free mass; BF%, Body fat percentage; †Reference value obtained from Goh et al. (2004).

4.3 Functional measurements

Body function was measured through hand grip strength (right and left), blood pressure and pulse rate then group comparisons made Table 4.5. The average blood pressure reading was below the recommended cut-off for hypertension, systolic blood pressure <130mmHg and diastolic blood pressure <85mmHg (International Diabetes Federation, 2005). However, one participant had high systolic blood pressure (134mmHg) but normal diastolic blood pressure; while another participant had normal systolic blood pressure but high diastolic blood pressure (89mmHg). And one more participant was high in both systolic (137mmHg) and diastolic (87mmHg) blood pressure. No difference was found in blood pressure by group, except that Indians tended to have higher diastolic blood pressure than non-Indians ($p=0.32$, ES=0.55). Indians also tended to have a higher ($p=0.06$, ES=0.94) pulse rate than non-Indians. A significantly larger grip strength of both hands was measured in non-Indians (Left: $p=0.02$, ES=0.98; Right: $p<0.005$, ES=1.46) and red-meat-eaters (Left: $p=0.01$, ES=0.94; Right: $p=0.03$, ES=0.72), than the comparative groups.

Table 4.5: Functional measurements of 38 women of childbearing age categorized in different comparative groups

Functional Measurements	*Reference value*	*Total N=38*	*Indians N=6*	*Non-Indians N=32*	**p value*	*Effect size*	*Non-red-meat-eaters N=12*	*Red-meat-eaters N=26*	**p value*	*Effect size*
Systolic blood pressure (mmHg)	<130†	113 ± 10 (96 – 137)	111 ± 10 (103 – 127)	114 ± 10 (96 – 137)	0.62	-0.30	112 ± 10 (101 – 134)	114 ± 10 (96 – 137)	0.67	-0.20
Diastolic blood pressure (mmHg)	<85†	70 ± 8 (53 – 89)	74 ± 10 (62 – 89)	69 ± 8 (53 – 87)	0.32	0.55	69 ± 9 (60 – 89)	70 ± 8 (53 – 87)	0.76	-0.12
Pulse (bpm)		67 ± 10 (52 – 91)	74 ± 8 (60 – 82)	66 ± 9 (52 – 91)	0.06	0.94	69 ± 7 (58 – 82)	66 ± 10 (52 – 91)	0.22	0.35
Grip strength left hand (kg)		30.1 ± 5.9 (22.2 – 44.9)	25.9 ± 3.8 (22.5 – 33.2)	30.8 ± 6.0 (22.2 – 44.9)	0.02	-0.98	26.8 ± 3.7 (22.2 – 33.2)	31.6 ± 6.2 (23.0 – 44.9)	0.01	-0.94
Grip strength right hand (kg)		33.3 ± 5.7 (24.0 – 45.2)	27.7 ± 3.3 (24.0 – 33.7)	34.3 ± 5.5 (25.2 – 45.2)	<0.005	-1.46	30.7 ± 4.4 (25.2 – 37.0)	34.5 ± 6.0 (24.0 – 45.2)	0.03	-0.72

Mean ± SD; Range in parenthesis; *p value calculated using independent unequal variance t test; bpm, beats per minute; †Reference from IDF (2005).

4.4 Fasting blood measurements

The fasting blood biochemistry allowed assessment of the cardiovascular risk factors of serum lipids and glucose, the full blood cell count to screen for possible anaemia, and one carbon metabolism serum variables B_{12}, holo-TC II, and folate concentrations (Tables 4.6-4.10) for assessment of B_{12} status..

4.4.1 Lipids and glucose

Fasting HDL cholesterol appeared to be higher on average by 0.3mmol/L in non-Indians (p=0.04, ES=0.95) compared to Indians (Table 4.6) and two (one Indian) of 12 non-red-meat-eaters and eight (all were non-Indians) of 26 red-meat-eaters had LDL cholesterol higher than the reference value (3.0mmol/L). Although the mean values for total cholesterol were within the reference range for all subgroups, three (one Indian) of 12 non-red-meat-eaters and nine (all were non-Indian) of 26 red-meat-eaters had a total cholesterol ≥5.0mmol/L. Indians tended to be higher in total/HDL cholesterol ratio than non-Indians (p=0.27, ES=0.63). Except for one non-Indian red-meat-eater (5.2), total/HDL cholesterol ratios were within the reference range (<4.5). All individual triglyceride results were within the normal range (<2.0mmol/L), although non-red-meat-eaters (p=0.30, ES=0.57) were slightly higher in triglycerides than red-meat-eaters. Fasting glucose levels were all were within the reference range (3.5-5.4mmol/L) and not different by group.

Table 4.6: Fasting lipids and glucose in 38 women of childbearing age categorized in different comparative groups

	Reference value	*Total N=38*	*Indians N=6*	*Non-Indians N=32*	**p value*	*Effect size*	*Non-red-meat-eaters N=12*	*Red-meat-eaters N=26*	**p value*	*Effect size*
HDL cholesterol (mmol/L)	>1.0†	1.8 ± 0.4 (1.0 – 2.9)	1.5 ± 0.2 (1.1 – 1.8)	1.8 ± 0.4 (1.0 -2.9)	0.04	-0.95	1.7 ± 0.5 (1.1 – 2.9)	1.8 ± 0.4 (1.0 – 2.5)	0.58	-0.22
LDL cholesterol (mmol/L)	<3.0†	2.4 ± 0.8 (0.8 – 4.1)	2.6 ± 0.8 (2.0 – 4.1)	2.4 ± 0.8 (0.8 – 3.8)	0.63	0.25	2.4 ± 0.8 (1.3 – 4.1)	2.4 ± 0.8 (0.8 – 3.8)	0.95	0.00
Total cholesterol (mmol/L)	<5.0†	4.6 ± 0.8 (3.0 – 6.5)	4.6 ± 1.0 (4.1 – 6.5)	4.6 ± 0.7 (3.0 – 6.0)	0.99	0.00	4.5 ± 0.8 (3.7 – 6.5)	4.6 ± 0.8 (3.0 – 6.0)	0.94	-0.13
Total/HDL cholesterol ratio	<4.5†	2.7 ± 0.8 (1.5 – 5.2)	3.1 ± 0.8 (2.3 – 4.3)	2.6 ± 0.8 (1.5 – 5.2)	0.27	0.63	2.8 ± 0.8 (1.5 – 4.3)	2.7 ± 0.8 (1.5 – 5.2)	0.60	0.13
Triglyceride (mmol/L)	<2.0†	0.8 ± 0.4 (0.3 – 1.9)	1.0 ± 0.5 (0.7 – 1.9)	0.8 ± 0.3 (0.3 – 1.6)	0.31	0.49	1.0 ± 0.4 (0.3 – 1.9)	0.8 ± 0.3 (0.4 – 1.6)	0.30	0.57
Fasting glucose (mmol/L)	3.5 – 5.4†	4.4 ± 0.4 (3.8 – 5.2)	4.3 ± 0.4 (3.8 – 5.0)	4.5 ± 0.4 (3.8 – 5.2)	0.42	-0.50	4.4 ± 0.4 (3.8 – 5.2)	4.4 ± 0.4 (3.8 – 5.2)	0.97	0.00

Mean ± SD; Range in parenthesis; *p value calculated using independent unequal variance t test; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; †reference values provided by Diagnostic Medlab Ltd.

4.4.2 Haematological measurements

Haematological measurements (full blood cell count) to check for anaemia are reported in Tables 4.7 and 4.8. Although the average level of haemoglobin was within the ideal range (115-160g/L) for women, two participants had mild anaemia as their haemoglobin was low; 97g/L and 105g/L respectively. Both of them also had a low packed cell volume (PCV, ideal range 0.35-0.47L/L), 0.32L/L and 0.33L/L. However, their serum B_{12} concentrations were adequate; 461pmol/L and 613pmol/L. All RBC counts were within the normal range and no difference was found in haemoglobin or RBC between groups. One participant had a platelet count lower than 150b/L, while two had platelet count higher than 450b/L however there was no difference between groups. The subject with low haemoglobin (97g/L) had low mean cell volume and mean corpuscular haemoglobin (MCV, 73fl; MCH, 22pg). Diagnostic Medlab reported that her red cell appearance suggested iron deficiency. One other participant had a high MCV of 102fl. All other participants were within the ideal range of PCV, MCV and MCH. Indians (p=0.10, ES=0.53) and non-red-meat-eaters (p=0.17, ES=0.54) appeared to have smaller MCV than the comparative groups and also Indians had less MCH as well (p=0.10, ES=0.66) (Table 4.7).

One participant had a slightly elevated white blood cell (WBC) count due to an increase in segmented neutrophils, while another two participants had slightly low WBC and segmented neutrophil counts. All counts of lymphocyte, basophil and monocyte were within normal ranges. Indians had higher lymphocyte counts (p=0.15, ES=0.71) but lower basophil counts (p=0.03, ES=1.32) than non-Indians (Table 4.8). One participant had an extremely high eosinophil (1.1b/L) than the ideal range (0.0-0.5b/L), which was probably because she had a mild cold. No significant differences in differential WBC counts were found between non-red-meat-eaters and red-meat-eaters.

Table 4.7: Haematological measurements in 38 women of childbearing age categorized in different comparative groups

Haematological measurements	*Reference value*	*Total N=38*	*Indians N=6*	*Non-Indians N=32*	**p value*	*Effect size*	*Non-red-meat-eaters N=12*	*Red-meat-eaters N=26*	**p value*	*Effect size*
Haemoglobin (g/L)	115 – 160†	134 ± 12 (97 – 157)	131 ± 7 (122 – 140)	134 ± 12 (97 - 157)	0.42	-0.31	131 ± 15 (97 – 157)	135 ± 10 (105 – 155)	0.52	-0.31
RBC (10E12/L)	3.8 – 5.8†	4.63 ± 0.33 (3.89 – 5.18)	4.70 ± 0.19 (4.43 – 4.91)	4.62 ± 0.35 (3.89 – 5.18)	0.42	0.28	4.68 ± 0.31 (4.14 – 5.18)	4.61 ± 0.34 (3.89 – 5.09)	0.56	0.22
Platelet Count (b/L)	150 – 450†	281 ± 73 (132 – 458)	281 ± 86 (151 – 388)	281 ± 72 (132 – 458)	0.99	0.00	291 ± 79 (151 – 458)	276 ± 71 (132 – 452)	0.58	0.20
PCV (L/L)	0.35 – 0.47†	0.41 ± 0.03 (0.32 – 0.46)	0.41 ± 0.02 (0.39 – 0.43)	0.41 ± 0.03 (0.32 – 0.46)	0.95	0.00	0.40 ± 0.04 (0.32 – 0.45)	0.41 ± 0.03 (0.33 – 0.46)	0.57	-0.28
MCV (fl)	80 – 98†	88 ± 5 (73 – 102)	86 ± 2 (83 – 88)	88 ± 5 (73 – 102)	0.10	-0.53	86 ± 6 (73 – 97)	89 ± 5 (82 – 102)	0.17	-0.54
MCH (pg)	25 – 34†	29 ± 2 (22 – 34)	28 ± 1 (26 – 29)	29 ± 2 (22 – 34)	0.10	-0.66	28 ± 3 (22 – 32)	29 ± 2 (27 – 34)	0.18	-0.40

Mean ± SD; Range in parenthesis; *p value calculated using independent unequal variance t test; RBC, Red blood cell count; PCV, Packed cell volume; MCV, Mean cell volume; MCH, Mean corpuscular haemoglobin; †reference values provided by Diagnostic Medlab Ltd.

Table 4.8: Haematological measurements count cont'd

Haematological measurements	*Reference value*	*Total N=38*	*Indians N=6*	*Non-Indians N=32*	**p value*	*Effect size*	*Non-red-meat-eaters N=12*	*Red-meat-eaters N=26*	**p value*	*Effect size*
WBC (b/L)	4.0 – 11.0†	6.5 ± 1.7 (3.7 – 11.1)	6.8 ± 1.8 (5.2 – 9.1)	6.4 ± 1.7 (3.7 – 11.1)	0.65	0.23	6.2 ± 1.9 (3.7 – 9.7)	6.6 ± 1.7 (3.9 – 11.1)	0.48	-0.22
Neutrophil seg (b/L)	2.2 – 7.5†	3.85 ± 1.42 (1.87 – 8.39)	3.79 ± 1.09 (2.77 – 5.44)	3.86 ± 1.49 (1.87 – 8.39)	0.89	-0.05	3.59 ± 1.25 (1.87 – 5.88)	3.97 ± 1.50 (2.17 – 8.39)	0.42	-0.28
Lymphocyte (b/L)	1.0 – 3.9†	1.92 ± 0.60 (1.17 – 3.57)	2.27 ± 0.56 (1.74 – 3.14)	1.86 ± 0.59 (1.17 – 3.57)	0.15	0.71	1.93 ± 0.62 (1.27 – 3.14)	1.92 ± 0.60 (1.17 – 3.57)	0.97	0.02
Basophil (b/L)	0 – 0.2†	0.03 ± 0.02 (0.00 – 0.09)	0.02 ± 0.01 (0.01 – 0.04)	0.04 ± 0.02 (0.00 – 0.09)	0.03	-1.32	0.03 ± 0.02 (0.01 – 0.09)	0.03 ± 0.02 (0.00 – 0.09)	0.62	0.00
Eosinophil (b/L)	0 – 0.5†	0.21 ± 0.18 (0.06 – 1.10)	0.27 ± 0.18 (0.06 – 0.48)	0.20 ± 0.18 (0.07 – 1.10)	0.45	0.39	0.18 ± 0.13 (0.06 – 0.48)	0.23 ± 0.20 (0.07 – 1.10)	0.35	-0.30
Monocyte (b/L)	0 – 0.9†	0.45 ± 0.15 (0.22 – 0.74)	0.45 ± 0.17 (0.27 – 0.74)	0.45 ± 0.15 (0.22 – 0.69)	0.98	0.00	0.43 ± 0.18 (0.22 – 0.74)	0.46 ± 0.14 (0.27 – 0.69)	0.59	-0.19

Mean ± SD; Range in parenthesis; *p value calculated using independent unequal variance t test; WBC, White blood cell count; †reference values provided by Diagnostic Medlab Ltd.

4.4.3 Biomarkers for B_{12} and folate

In this study, one participant reported when questioned after testing to regularly consume supplements with B_{12} (1 Berocca vitamin B and C tablet per day containing 10.0μg B_{12}). She was a European non-red-meat-eater and her serum folate was even higher (>45pmol/L) than the detection range of Diagnostic Medlab. Therefore, for analysis her serum folate value was replaced by 45pmol/L. Serum B_{12} was significantly higher (~180pmol/L and ~134pmol/L higher in mean respectively) in non-Indians (p=0.04, ES=1.26), and red-meat-eaters (p=0.01, ES=0.98), than the comparative groups (Table 4.9). Five of 38 participants had serum B_{12} concentrations lower than 170pmol/L, and all were non-red-meat-eaters (four Indians and one European). The lowest serum B_{12} concentration in red-meat-eaters group was 177pmol/L, and she was also Indian. Using serum B_{12} concentration =250pmol/L as the insufficiency cut point, the odds ratio that a non-red-meat-eater had B_{12} insufficiency was 5.5 times (95% CI 1.16 to 26.0) that of the odds of a red-meat-eater, while the odds of an Indian was even higher, 27 times (95% CI 2.58 to 283) that of a non-Indian. Overall, the odds of an Indian non-red-meat-eater being low in serum B_{12} was 29.3 times (95% CI 2.40 to 358) that of a non-Indian red-meat-eater. Holo-TC II was significantly higher in non-Indians (p=0.02, ES=1.19), and red-meat-eaters (p<0.005, ES=1.11), than the comparative groups. Seven participants had holo-TC II lower than 35pmol/L, five non-red-meat-eaters (four Indians vs one European) and two red-meat-eaters (one Indian vs one European).

No participant appeared to have folate deficiency as all except the one described previously had serum folate levels that were within the normal range (9-45pmol/L), No difference in serum folate was found between Indians and non-Indians. But the serum folate level tended to be higher in non-red-meat-eaters (p=0.10, ES=0.62).

Figure 4.1 shows no correlation between serum B_{12} and serum folate (r=0.09, 95% CI -0.23 to 0.40, p=0.57) while figure 4.2 shows strong correlation between serum B_{12} and holo-TC II (r=0.77, 95% CI 0.59 to 0.87, p<0.001).

Table 4.9: Biomarkers for B_{12} and folate of 38 women of childbearing age categorized in different comparative groups

Biomarkers	*Reference value*	*Total N=38*	*Indians N=6*	*Non-Indians N=32*	**p value*	*Effect size*	*Non-red-meat-eaters N=12*	*Red-meat-eaters N=26*	**p value*	*Effect size*
Serum B_{12} (pmol/L)	170 – 800[†]	355 ± 146 (112 – 613)	203 ± 157 (112 – 521)	383 ± 127 (148 – 613)	0.04	-1.26	263 ± 147 (112 -521)	397 ± 127 (177 – 613)	0.01	-0.98
Holo-TC Ⅱ (pmol/L)	≥35[‡]	66 ± 36 (17 – 198)	35 ± 26 (17 – 86)	72 ± 35 (33 – 198)	0.02	-1.19	43 ± 22 (17 – 86)	77 ± 37 (33 – 198)	<0.005	-1.11
Serum folate (pmol/L)	9 - 45[†]	26 ± 9 (12 – 45)	25 ± 11 (15 – 40)	26 ± 9 (12 - 45)	0.79	-0.13	29 ± 10 (15 – 45)	24 ± 8 (12 – 45)	0.10	0.62

Mean ± SD; Range in parenthesis; *p value calculated using independent unequal variance t test; holo-TC Ⅱ, holotranscobalamin Ⅱ; [†]reference values provided by Diagnostic Medlab Ltd; [‡]Reference value obtained from Lindgren et al (1999).

Figure 4.1: Scatter plot of serum B_{12} and folate concentrations

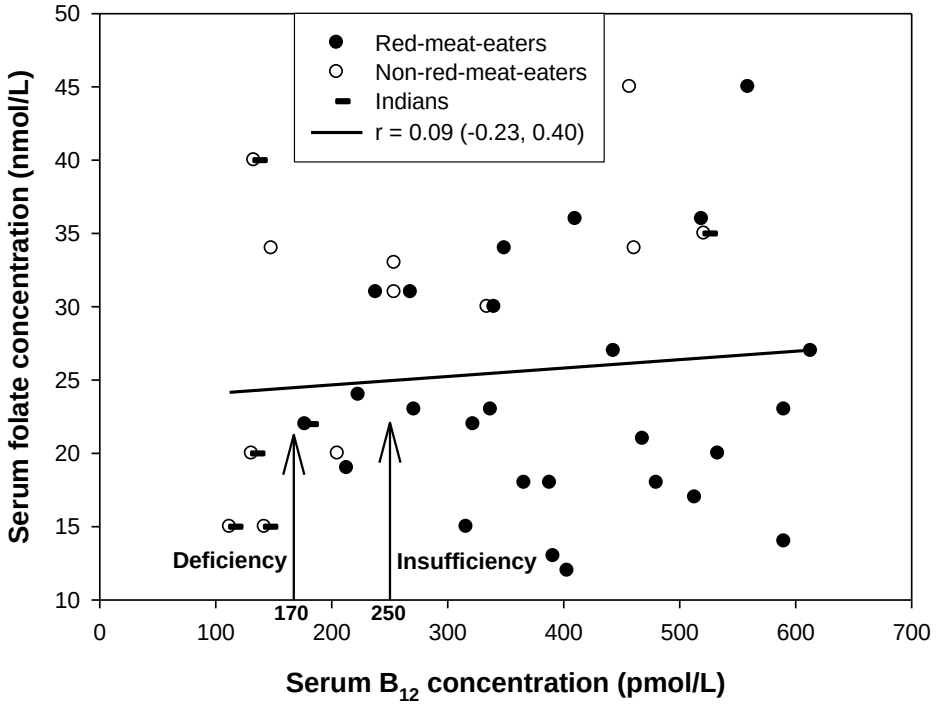
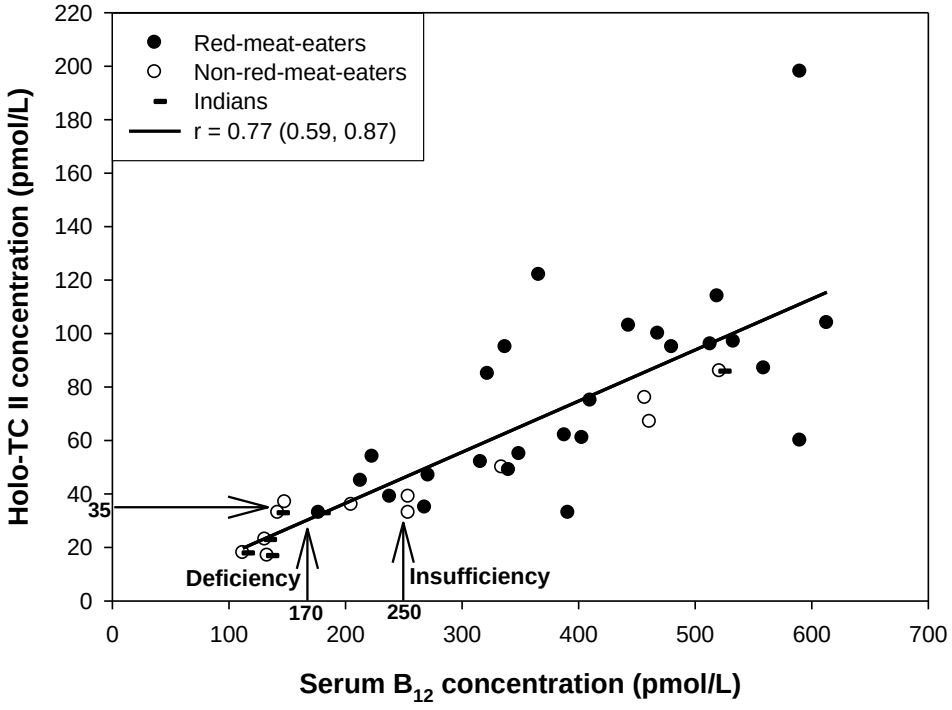


Figure 4.2: Scatter plot of serum B_{12} and holo-TC II concentrations



4.5 Dietary Analysis

As one participant did not return her 7DDD and 7DPAD, there were 38 participants in 24HDR and 37 participants in 7DDD. Dietary assessments (24HDR and 7DDD) will be compared in the section of micronutrients intake (B_{12} and folate) with biomarkers to validate which better represents the nutritional status of individuals. All the other dietary analyses, including the sections describing energy and macronutrient intake, were analysed using the average daily intake reported in the 7DDD unless 24HDR is stated. Between groups comparisons were made of Indians vs non-Indians and non-red-meat-eaters vs red-meat-eaters. Analyses of energy consumption and macronutrients from 7DDD are summarized in Table 4.10 and Table 4.11. Indians reported a more monotonous dietary pattern measured by the number of different foods and drinks reported (p=0.06, ES=1.04) and their diet contained fewer foods containing B_{12} (p=0.06, ES=1.10) than non-Indians in 7DDD. Although there was no difference in food variety between non-red-meat-eaters and red-meat-eaters (60 vs 63, p=0.52, ES=0.23), red-meat-eaters tended to consume a greater variety of foods containing B_{12} than non-red-meat-eaters (21 vs 16, p=0.02, ES=0.87).

Table 4.10: Reported dietary intakes from 7-day diet diary (7DDD) in 37 women of childbearing age

Measurements	*Reference value*	*Total N=37*	*Indians N=6*	*Non-Indians N=31*	**p value*	*Effect size*	*95% confidence limits*		*Non-red-meat-eaters N=12*	*Red-meat-eaters N=25*	**p value*	*Effect size*	*95% confidence limits*	
							Lower	**Upper**					**Lower**	**Upper**
Energy consumption (MJ/day)	9.1†	7.7 ± 2.2 (4.6 – 14.1)	6.8 ± 2.3 (4.6 – 10.7)	7.9 ± 2.2 (5.0 – 14.1)	0.36	-0.45			7.3 ± 2.0 (4.6 – 10.7)	7.9 ± 2.3 (5.0 – 14.1)	0.41	-0.28		
Energy density (kJ/g)		2.81 ± 0.96 (1.49 – 5.66)	2.23 ± 0.55 (1.49 – 2.95)	2.92 ± 0.98 (1.68 – 5.66)	0.03	-0.87	-1.65	-0.09	2.27 ± 0.56 (1.49 – 3.37)	3.07 ± 1.00 (1.75 – 5.66)	0.00	-0.99	-1.63	-0.34
%Energy carbohydrate	45-65%†	47.9 ± 7.5 (29.9 - 60.9)	55.8 ± 6.2 (47.3 – 60.9)	46.4 ± 6.8 (29.9 – 59.2)	0.01	1.45	0.43	2.47	52.2 ± 7.3 (37.5 – 60.9)	45.8 ± 6.9 (29.9 – 59.2)	0.02	0.90	0.16	1.64
%Energy protein	15-25%†	18.4 ± 3.3 (12.3 - 24.4)	16.7 ± 3.1 (13.7 – 21.8)	18.8 ± 3.3 (12.3 – 24.4)	0.19	-0.64	-1.67	0.40	16.0 ± 2.7 (12.3 – 21.8)	19.6 ± 2.9 (14.6 – 24.4)	0.00	-1.26	-1.98	-0.55
%Energy fat	20-35%†	31.3 ± 6.8 (17.6 – 48.3)	25.9 ± 5.6 (18.0 – 32.5)	32.3 ± 6.6 (17.6 – 48.3)	0.04	-1.06	-2.06	0.06	28.9 ± 6.5 (18.0 – 40.9)	32.4 ± 6.7 (17.6 – 48.3)	0.13	-0.54	-1.27	0.18

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §95% CI for effect size less than 0.5 not reported; †reference values obtained from Ministry of Health (2005).

Table 4.11: Reported dietary intakes cont'd

Measurements	*Reference value*	*Total N=37*	*Indians N=6*	*Non-Indians N=31*	**p value*	*Effect size*	*95% confidence limits*		*Non-red-meat-eaters N=12*	*Red-meat-eaters N=25*	**p value*	*Effect size*	*95% confidence limits*	
							Lower	**Upper**					**Lower**	**Upper**
Protein (g/day)	EAR 37g/day‡ RDI 46g/day‡	84 ± 28 (45 – 182)	66 ± 17 (45 – 88)	88 ± 28 (45 – 182)	0.03	-0.93	-1.73	-0.12	69 ± 17 (45 – 100)	92. ± 29 (45 – 182)	<0.005	-0.99	-1.64	-0.34
%Energy from Sucrose	<10%‡	8.1 ± 2.5 (3.7 – 15.5)	6.7 ± 2.9 (4.3 – 11.3)	8.3 ± 2.4 (3.7 – 15.5)	0.26	-0.59	-1.75	0.57	7.9 ± 3.0 (4.3 – 12.3)	8.1 ± 2.3 (3.7 – 15.5)	0.84	-0.07		
%Energy from Saturated Fat	<10%†	11.2 ± 2.9 (3.5 – 17.5)	8.5 ± 3.3 (3.5 – 12.2)	11.7 ± 2.6 (7.2 – 17.5)	0.07	-1.06	-2.24	0.12	9.4 ± 2.8 (3.5 – 13.8)	12.0 ± 2.6 (7.9 – 17.5)	0.01	-0.97	-1.72	-0.23
P:S ratio	0.7-1.0¶	0.53 ± 0.32 (0.26 – 2.13)	0.81 ± 0.67 (0.32 – 2.13)	0.48 ± 0.17 (0.26 – 1.17)	0.29	0.67	-0.78	2.12	0.72 ± 0.50 (0.32 – 2.13)	0.44 ± 0.10 (0.26 – 0.63)	0.08	0.79	-0.10	1.67
#Food variety		62 ± 15 (36 – 105)	50 ± 16 (36 – 70)	65 ± 13 (39 – 105)	0.06	-1.04	-2.18	0.10	60 ± 15 (36 – 77)	63 ± 15 (39 – 105)	0.52	-0.23		
Different foods with B_{12}		20 ± 6 (5 – 37)	14 ± 7 (5 – 24)	21 ± 6 (13 – 37)	0.06	-1.10	-2.25	0.05	16 ± 5 (5 - 24)	21 ± 6 (13 – 37)	0.02	-0.87	-1.58	-0.15

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §95% CI for effect size less than 0.5 not reported; P:S ratio, polyunsaturated fat-to-saturated fat ratio; EAR Estimated average requirement; # measured as number of different foods reported over seven days, RDI Recommended dietary allowance; † reference values obtained from Ministry of Health (2005); ‡ reference value obtained from World Health Organisation (2003); ¶ reference value obtained from Oliver (1997)

4.5.1 Energy

Indians tended to report a lower energy intake than non-Indians (6.8MJ/day vs 7.9MJ/day) but the difference was not significant (p=0.36, ES=0.45, Table 4.10). Red-meat-eaters had a slightly higher energy intake than non-red-meat-eaters (7.9MJ/day vs 7.3MJ/day) but also no significant difference was found (p=0.41, ES=0.28). The mean reported energy intake of the whole group (7.7MJ/day) was less than the recommended (Ministry of Health, 2005), i.e. 9.6MJ/day for women 63.3kg and 1.70m tall aged 19-30y and 9.1MJ/day for women with the same weight and height aged 31-50y. The percentage of energy obtained from reported carbohydrate:protein:fat intake of whole group was 48/18/31 (the remaining 3% of energy was derived from alcohol), which is within recommendations.

4.5.2 Macronutrients

The recommended proportions of energy from carbohydrate, protein and fat in the diet are in the ratio of 50/20/30 (Ministry of Health, 2005), ranging carbohydrate 45-65%, protein 15-25% and fat 20-35%.

Carbohydrate

The average proportion of energy from carbohydrate reported was 47.9%, which is close to the recommendation. No individual had a percentage of energy from carbohydrate over 65%, but 11 (two non-red-meat-eaters and nine red-meat-eaters; all were non-Indians) had percentages lower than 45%. Indians reported a significantly higher percentage of energy from carbohydrate than non-Indians (55.8% vs 46.4%, p=0.01, ES=1.45, Table 4.10). Non-red-meat-eaters also had higher percentage carbohydrate intake than red-meat-eaters (52.2% vs 45.8%, p=0.02, ES=0.90). The mean contribution of sucrose to the reported total energy intake (8.1%) was within the reference value (<10%) recommended by World Health Organisation (2003).

Protein

The mean protein intake of the whole group was 84g/day, accounting for an average of 18.4% of total energy intake. Indians had significant lower protein intake than non-Indians (66g/day vs 88g/day, p=0.03, ES=0.93, Table 4.11) while non-red-meat-eaters

also had lower protein intake than red-meat-eaters (69g/day vs 92g/day, p<0.005, ES=0.99). No individual had percentage of energy derived from protein over 25%, but five of them had a protein intake percentage lower than 15% (two Indians and three non-Indians; four non-red-meat-eaters and one red-meat-eater).

Fat

The mean fat intake of the whole group was within the reference range at 31.3% of energy but the range was wide. Eight reported percentage energy from fat of more than 35% (one non-red-meat-eater and seven red-meat-eaters; all of them were non-Indians), and two others had dietary fat intake lower than 20% of total energy intake; one Indian non-red-meat-eater and one Chinese red-meat-eater. The maximum recommended intake of saturated fat is 10% of total energy and Indians tended to have lower percentage of energy derived from saturated fat than non-Indians (8.5% vs 11.7%, p=0.07, ES=1.06, Table 4.11). Non-red-meat-eaters also had significant lower percentage of energy from saturated fat than red-meat-eaters (9.4% vs 12.0%, p=0.01, ES=0.97). Although no significant difference was found, non-Indians (0.48, Table 4.11) and/or red-meat-eaters (0.44) tended to have a lower polyunsaturated fat-to-saturated fat ratio (P:S ratio) than Indians (0.81) and/or non-red-meat-eaters (0.72).

4.5.3 Micronutrients (B_{12} and folate) and food sources of B_{12}

One participant consumed supplements with B_{12} regularly (10μg/day). Two participants reported not taking B_{12} supplements at interview and 24HDR but in the 7DDD recorded a total of 50μg and 10μg B_{12} from supplement respectively. All intakes of B_{12} from supplements were subtracted from their dietary intake for the following analysis of food sources. One participant ate pork liver (range 30-100g per time) and 50g pork liver contains 12.5μg B_{12}, which is already over five times of RDI value for individual. She mentioned pork liver was her favourite, and she ate liver three times in the 24HDR and twice in the 7DDD. This participant was included in the analysis as she her high B_{12} dietary intake was valid.

In 24HDR, Indians and/or non-red-meat-eaters reported lower B_{12} intake than non-Indians and/or red-meat-eaters (Table 4.12) but this was not significant. In 7DDD, Indians reported significantly lower B_{12} intake than non-Indians (p<0.001, ES=1.42)

and non-red-meat-eaters reported significantly lower B_{12} intake than red-meat-eaters ($p<0.001$, ES=1.15). The dietary B_{12} intake of 24HDR was positively correlated to that of 7DDD (r=0.33, p=0.06, Spearman's rho). However, adjusted reported 7DDD daily B_{12} intake by per unit (MJ) energy intake was not correlated to serum B_{12} (r=0.220, 95% CI -0.112 to 0.508, p=0.19) or holo-TC II concentration (r=0.26, 95% CI -0.073 to 0.536, p=0.12).

In 7DDD, the quartiles of reported daily B_{12} intake were 2.30μg/day (25 percentile), 3.40μg/day (50 percentile) and 5.14μg/day (75 percentile) respectively. There was no trend among daily B_{12} intake and B_{12} status in quartiles 2 to 4. However, the daily B_{12} intake in quartile 1 might be positively associated to B_{12} status, either serum B_{12} (r=0.62, p=0.08) or holo-TC II concentration (r=0.63, p=0.07, Table 4.13).

One participant consumed multivitamin and/or mineral supplement once during 7DDD, which contained 300μg folate but no B_{12}. She was still involved in the analysis of food sources of folate by subtracting folate from supplement. The dietary folate of 24HDR was significantly lower in Indians ($p<0.005$, ES=1.07, Table 4.12), while no significant difference was found between Indians and non-Indians in the daily folate intake by 7DDD (p=0.65, ES=0.18). Conversely, there was no difference in folate intake between non-red-meat-eaters and red-meat-eaters in 24HDR (p=0.32, ES=0.37). But non-red-meat-eaters reported a significantly higher folate intake in 7DDD (p=0.01, ES=0.94). The dietary folate intake of 24HDR was significantly positively correlated to that of 7DDD (r=0.49, $p<0.005$, Spearman's rho).

Reported daily protein intake in grams was strongly and positively correlated to reported daily B_{12} intake (r=0.79, 95% CI 0.63 to 0.89, $p<0.001$, Figure 4.3). Non-red-meat-eaters were largely represented in the lower B_{12} intake and lower protein intake section. The four individuals with lowest reported B_{12} daily intakes were Indians, all reporting values lower than the EAR value.

Table 4.12: Dietary B_{12} and folate intakes of different comparative groups excluding supplements

		Reference value	*Total N=37*	*Indians N=6*	*Non-Indians N=31*	**p value*	*Effect size*	*95% confidence limits*		*Non-red-meat-eaters N=12*	*Red-meat-eaters N=25*	**p value*	*Effect size*	*95% confidence limits*	
								Lower	**Upper**					**Lower**	**Upper**
Dietary B_{12} µg/day	**24HDR**	EAR 2.0µg/day†	4.0 ± 6.1 (0.5 – 38.0)	2.0 ± 1.4 (0.7 – 4.3)	4.4 ± 6.6 (0.5 – 38.0)	0.07	-0.51	-1.07	0.05	2.0 ± 1.0 (0.7 – 4.3)	5.0 ± 7.3 (0.5 – 38.0)	0.06	-0.57	-1.15	0.02
	7DDD	RDI 2.4µg/day†	4.0 ± 2.7 (0.6 – 13.2)	1.6 ± 0.8 (0.6 – 2.6)	4.5 ± 2.7 (1.7 – 13.2)	<0.001	-1.42	-2.02	-0.82	2.3 ± 1.1 (0.6 – 4.0)	4.8 ± 2.9 (1.9 – 13.2)	<0.001	-1.15	-1.76	-0.54
B_{12} milk µg/day	**7DDD**		0.52 ± 0.35 (0.02 – 1.61)	0.39 ± 0.24 (0.15 – 0.72)	0.55 ± 0.37 (0.02 – 1.61)	0.21	-0.51	-1.35	0.34	0.48 ± 0.37 (0.14 – 1.51)	0.54 ± 0.35 (0.02 – 0.16)	0.66	-0.16		
Dietary folate µg/day	**24HDR**	EAR 320µg/day†	290 ± 162 (119 – 761)	179 ± 55 (129 – 284)	312 ± 167 (119 – 761)	<0.005	-1.07	-1.69	-0.45	332 ± 182 (147 – 761)	270 ± 151 (119 – 740)	0.32	0.37		
	7DDD	RDI 400µg/day†	292 ± 122 (104 – 739)	276 ± 85 (164 – 391)	295 ± 129 (104 – 739)	0.65	-0.18			359 ± 81 (213 – 454)	260 ± 126 (104 – 739)	0.01	0.94	0.28	1.60

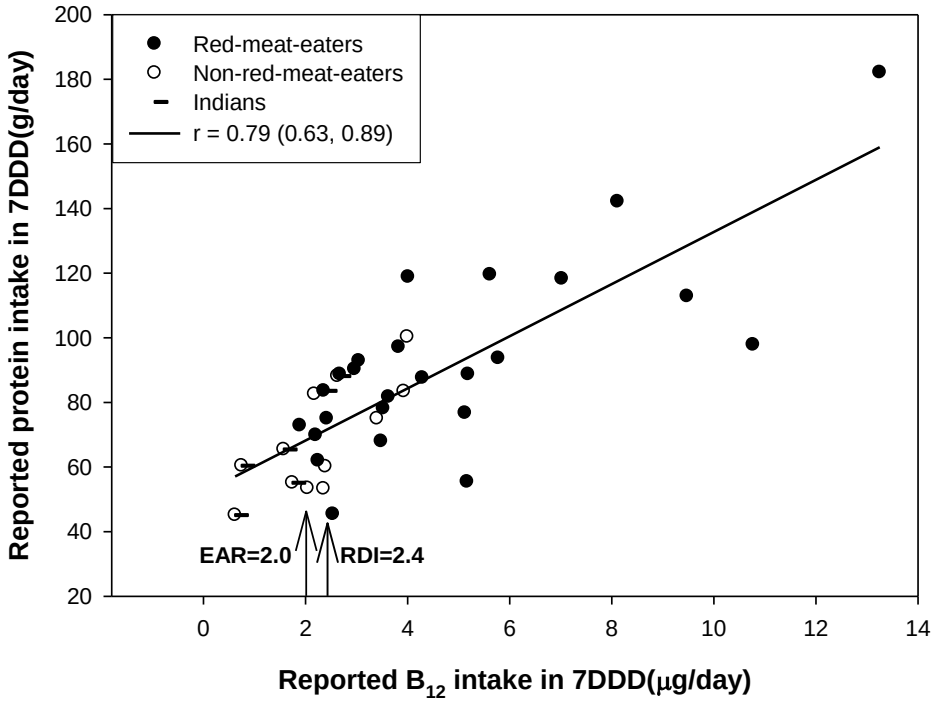
Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §95% CI for effect size less than 0.5 not reported; EAR Estimated average requirement; RDI, Recommended dietary intake; †reference value obtained from Ministry of Health (2005)

Table 4.13: Correlation of quartiles of daily B_{12} intake in 7DDD and B_{12} status

Daily B_{12} intake in 7DDD		*Serum B_{12} concentration (pmol/L)*		*Holo-TC II concentration (pmol/L)*	
		***r**	***#p value**	***r**	***#p value**
Quartile 1 (<2.3μg/day)	n=9	0.62	0.08	0.63	0.07
Quartile 2 (2.3-3.4μg/day)	n=10	0.05	0.90	0.43	0.22
Quartile 3 (3.4-5.14μg/day)	n=9	-0.15	0.69	-0.27	0.49
Quartile 4 (>5.14μg/day)	n=8	-0.58	0.10	-0.43	0.25

*r, pearson correlation coefficient; #p value calculated using Bivariate Correlations.

Figure 4.3: Scatter plot of reported daily protein and B_{12} intakes in 7-day diet diary (7DDD)



Daily B_{12} intake in 7DDD of Indians vs non-Indians

The reported daily dietary B_{12} intakes of 7DDD, by the Indians and non-Indians, are given in Table 4.12. The quantities and proportions of different B_{12} food sources in Indians and non-Indians are shown in table 4.14. Indians consumed less B_{12} from all food sources than non-Indians, except slightly more in yoghurt consumption. Indians, even the only one red-meat-eater, were found with no consumption of beef, fish and other seafood within the researched seven days.

The mean B_{12} intake of six Indians (1 red-meat-eater vs 5 non-red-meat-eaters) was 1.6µg/day, which was lower than the EAR value and two of them had B_{12} intake lower than 1.0µg/day. Another two Indians consumed between 1.5-2.0µg/day and one was between EAR and RDI and one above the RDI value. Milk (33.7%) and egg (18.8%) contributed more than half the B_{12} intake in Indians while yoghurt (7.4%) and marmite (5.1%) contributed a greater proportion than lamb (4.2%), cheese (2.9%), chicken (2.8%) and pork (0.8%) in this group.

The mean B_{12} intake in 7DDD of 31 non-Indians (24 red-meat-eater vs 7 non-red-meat-eaters) was 4.5µg/day, which was higher than the RDI value and significantly higher than that of Indians ($p<0.001$, ES=1.42). One had B_{12} intake slightly lower than 2.0µg/day while six were between EAR and RDI values. The remaining 24 were above the RDI value for B_{12} intake. Beef (16.2%) and milk (15.8%) contributed the largest proportion of B_{12} intake in non-Indians. Fish (12.0%) and seafood (10.4%) contributed a greater proportion than egg (9.9%), followed by cheese (6.3%). The contribution of chicken (4.8%) was slightly higher than lamb (4.2%) and pork (3.6%), which might be because of the high chicken thigh consumption of the only Japanese participant in this group as she had the highest B_{12} from chicken (8.62µg), while the second highest one was 4.76µg. The same amount of chicken thigh, for example 100g, contains more B_{12} (1.31µg) than chicken breast (0.23µg) and chicken wing (0.23µg), even compared to pork leg (0.53µg).

Daily B_{12} intake in 7DDD of non-red-meat-eaters vs red-meat eaters

Table 4.14 also compares the quantities and proportions of different B_{12} food sources in non-red-meat-eaters and non-Indians. Comparing all food sources of B_{12} excluding red meats between the two groups, red-meat-eaters had higher consumption of all food sources of B_{12}, except slightly lower consumption of cheese and marmite than non-red-meat-eaters.

The mean intake of B_{12} in 12 non-red-meat-eaters was 2.3µg/day, which was in agreement with the EAR of 2.0µg/day, but lower than the RDI 2.4µg/day (Ministry of Health, 2005). Within this group, two of them had extremely low daily B_{12} (<1.0µg/day) intake, while another two were between 1.5-2.0µg/day, and four more individuals had B_{12} intakes between EAR and RDI values. Only four reported B_{12}

intakes higher than RDI. Over a quarter (26.7%) of the B_{12} intake in 7DDD was obtained from milk, followed by egg (17.2%), fish (11.8%) and cheese (9.7%), while the contributions of seafood, marmite, yoghurt, and chicken were 4.9%, 4.8%, 3.8%, and 2.1% respectively (Table 4.14).

Red-meat-eaters had significantly high B_{12} intake than non-red-meat-eaters ($p<0.001$, ES=1.15). The mean B_{12} intake of the 25 red-meat-eaters was 4.8µg/day, which was dramatically above the RDI value provided by Ministry of Health (2005). Only one individual had B_{12} intake (1.9µg/day) slightly lower than EAR value, and another three were between EAR and RDI values. All the rest red-meat-eaters were above RDI value. Over a third (36.9%) of the B_{12} intake in 7DDD was obtained from meat, of which 5.6% was chicken (white meat). The contributions of red meat like beef, lamb and pork were 20.3%, 6.3% and 4.7% respectively. Milk, including ice cream and cream, contributed 14.8%, followed by seafood, including prawn and shellfish, 10.5%, fish 9.1% and egg 8.4%. The other major sources cheese, yoghurt, and marmite contributed 3.8%, 2.6% and 2.0% respectively.

Table 4.14: Comparison of B_{12} food sources and intakes in 7DDD

	Indians ***N=6***	***Non-Indians*** ***N=31***	***Non-red-meat-eaters*** ***N=12***	***Red-meat-eaters*** ***N=25***
Beef	0	0.73 (16.2%)	0	0.97 (20.3%)
Lamb	0.07 (4.2%)	0.19 (4.2%)	0	0.30 (6.3%)
Pork	0.01 (0.8%)	0.16 (3.6%)	0	0.22 (4.7%)
Milk	0.54 (33.7%)	0.71 (15.8%)	0.61 (26.7%)	0.71 (14.8%)
Fish	0	0.54 (12.0%)	0.27 (11.8%)	0.44 (9.1%)
Seafood	0	0.47 (10.4%)	0.11 (4.9%)	0.50 (10.5%)
Egg	0.30 (18.8%)	0.44 (9.9%)	0.40 (17.2%)	0.40 (8.4%)
Cheese	0.05 (2.9%)	0.28 (6.3%)	0.22 (9.7%)	0.18 (3.8%)
Chicken	0.05 (2.8%)	0.22 (4.8%)	0.05 (2.1%)	0.27 (5.6%)
Marmite	0.08 (5.1%)	0.11 (2.5%)	0.11 (4.8%)	0.10 (2.0%)
Yoghurt	0.12 (7.4%)	0.10 (2.2%)	0.09 (3.8%)	0.13 (2.6%)
Others	0.39 (24.3%)	0.55 (12.2%)	0.43 (18.9%)	0.57 (11.9%)
Total	**1.6 µg/day**	**4.5 µg/day**	**2.3 µg/day**	**4.8µg/day**

Dietary folate

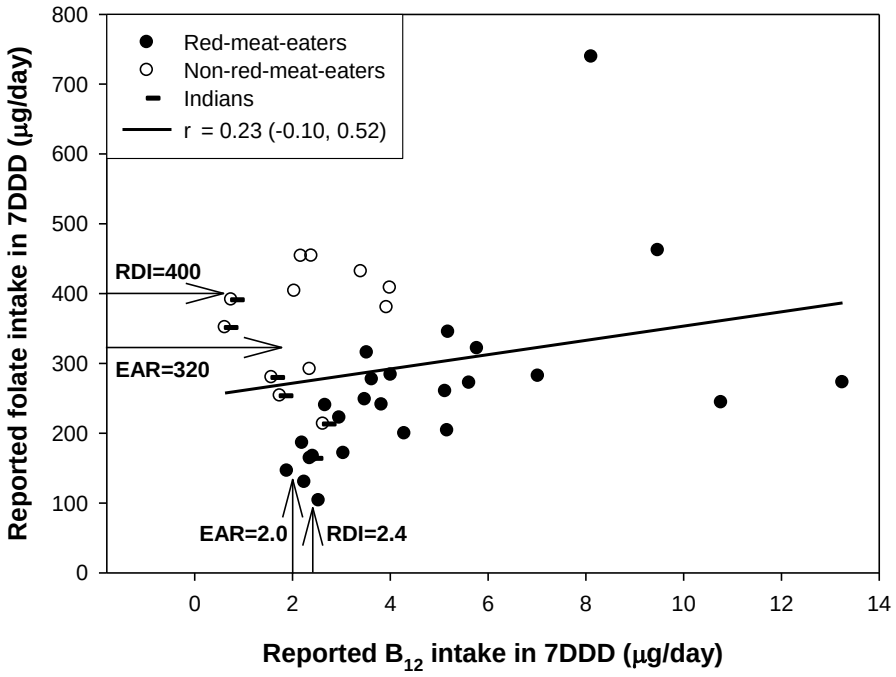
Non-red-meat-eaters reported a significantly higher folate intake in 7DDD than red meat eaters (359μg/day vs 260μg/day, p=0.01, ES=0.94, Table 4.12). Within the 25 red-meat-eaters, one had significantly high folate intake (739μg/day) in 7DDD, another one was above the RDI value (400μg/day), two more were between the EAR (320μg/day) and RDI values, and all remaining 21 were lower than the EAR value (eight of them were below 200μg/day). Five of the 12 non-red-meat-eaters had folate intakes above the RDI value, another three were between the EAR and RDI values, and the remaining four individuals were between 200μg/day and the EAR value. No extremely high or low folate intakes were reported in the non-red-meat-eating group.

In 7DDD, Indians reported just over one third of the daily B_{12} intake compared to non-Indians but there was no significant difference between these groups for reported folate intake. However, the imbalance between B_{12} and folate intakes was more serious with non-red-meat-eaters, which reported less than half of the daily B_{12} intake accompanied with 38% higher daily folate intake than red-meat-eaters.

Correlation between B_{12} intake and folate intake in 7DDD

Reported daily B_{12} intake was not associated with reported daily folate intake in 7DDD (r=0.23, 95% CI -0.10 to 0.52, p=0.17, figure 4.4). Most non-red-meat-eaters reported higher daily folate intake but lower daily B_{12} intake than red-meat-eaters. Indians' results accumulated at the lower end of B_{12} intake section.

Figure 4.4: Scatter plot of B_{12} and folate intakes in 7-day diet diary (7DDD)



Validity of dietary assessments for nutrition status of individual

There were a total of 38 participants who contributed to the results in the 24HDR in the left-hand side graphs of figure 4.5, while 37 participants in the 7DDD in the right-hand side graphs. B_{12} derived from breakfast cereals was subtracted from both 24HDR and 7DDD for all participants. The content of B_{12} from supplementation was subtracted for the two individuals with occasional consumption during 7DDD, as the blood sample was collected before they took the supplement, while B_{12} from supplementation was not subtracted for the individual with regular consumption as she took supplement both before and after blood collection and the dose should realistically be reflected in her serum B_{12} and holo-TC II concentrations. The individual with serum folate >45nmol/L was replaced by 45nmol/L for analysis. All folate from supplementation was subtracted as they were consumed after blood collection.

a) Serum B_{12} concentration

There was no significant correlation between serum B_{12} concentration and reported daily B12 intake in either 24HDR (r=0.03, 95% CI -0.29 to 0.35, p=0.86) or in 7DDD (r=0.22, 95% CI -0.11 to 0.51, p=0.19). 7DDD had the same sensitivity but higher

specificity than 24HDR for indicating low serum B_{12} concentration. The sensitivity of 7DDD indicated that 30% of participants with low serum B_{12} concentration (<250pmol/L) had B_{12} intakes <2.0μg/day and the specificity of 7DDD indicated that 93% of participants with normal serum B_{12} concentration had B_{12} intakes ≥2.0μg/day. The specificity of 24HDR to indicate low serum B_{12} concentration was only 64%.

b) Holo-TC II concentration

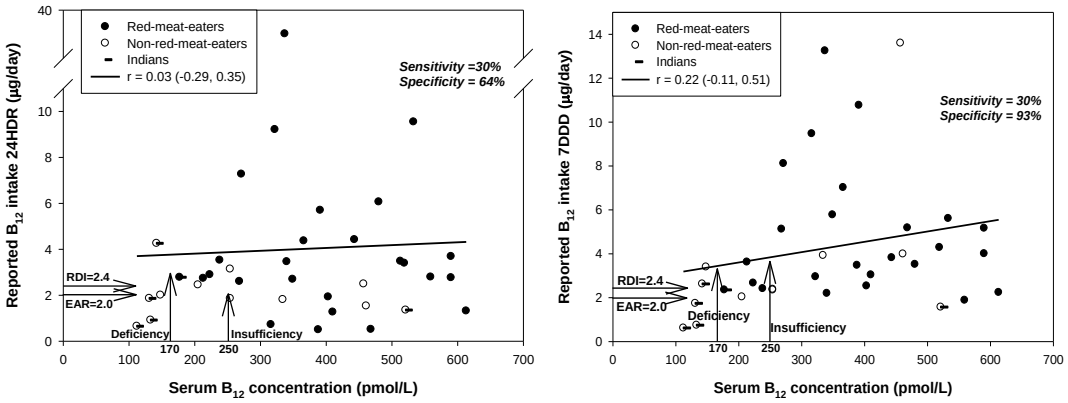
No correlation was found between holo-TC II concentration and reported daily B12 intake in either 24HDR (r=0.19, 95% CI -0.14 to 0.48, p=0.26) or 7DDD (r=0.23, 95% CI -0.10 to 0.52, p=0.16). They had the same sensitivity – 43% of participants with low holo-TC II concentration (<35pmol/L) had B_{12} intakes <2.0μg/day. However, 7DDD had a higher specificity than 24HDR in indicating low holo-TC II concentration. Ninety three percent of participant with normal holo-TC II concentration had B_{12} intakes ≥2.0μg/day in 7DDD, while only 68% of participant with normal holo-TC II concentration had B_{12} intakes ≥2.0μg/day in 24HDR.

c) Serum folate concentration

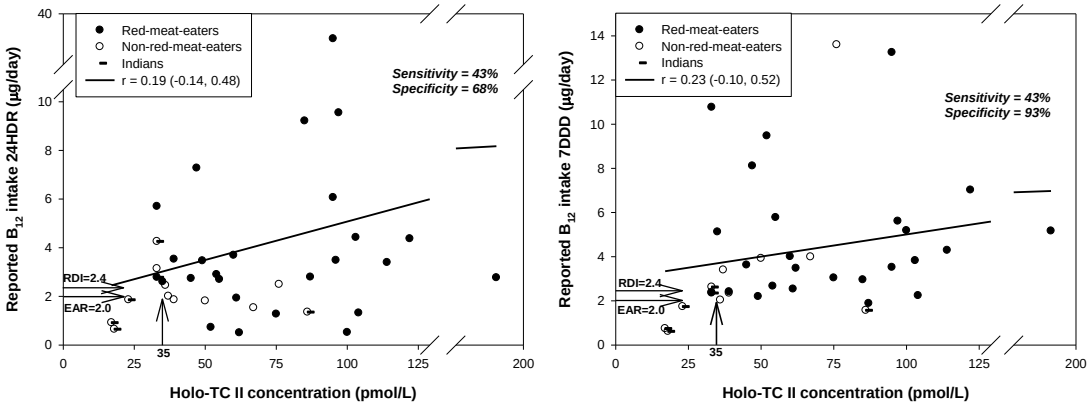
There was no association between serum folate concentration and reported daily folate intake in either 24HDR (r=0.01, 95% CI -0.31 to 0.33, p=0.93) or 7DDD (r=0.07, 95% CI -0.26 to 0.39, p=0.67). Neither 24HDR nor 7DDD had sensitivity in indicating low serum folate concentration (<9nmol/L) as nobody in the study group had folate deficiency. 7DDD had a slightly higher specificity of 32% than 24HDR of 29%.

Overall, 7DDD had the same sensitivity but higher specificity than 24HDR regarding these three biomarkers. Daily dietary intake in 7DDD had slightly better correlation with biomarkers than 24HDR, but was moderate to small and not significant. More discussion on validity of dietary assessment is in chapter 5.

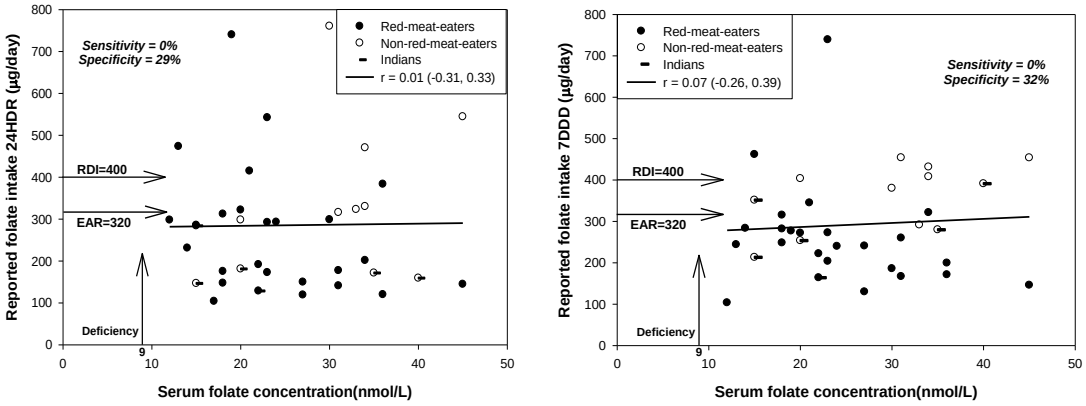
Figure 4.5: Validity of dietary assessments – 24-hour diet recall (24HDR) and 7-day diet diary (7DDD) – by B_{12} and folate biomarkers



a) Serum B_{12} concentration



b) Holo-TC II concentration



c) Serum folate concentration

Sensitivity indicates how likely participants are to have a positive test if they have insufficiency.

Specificity indicates how likely participants are to have a negative test if they do not have insufficiency.

r value is Pearson's correlation, followed by 95% confidence intervals in bracket.

4.6 Reported physical activity levels

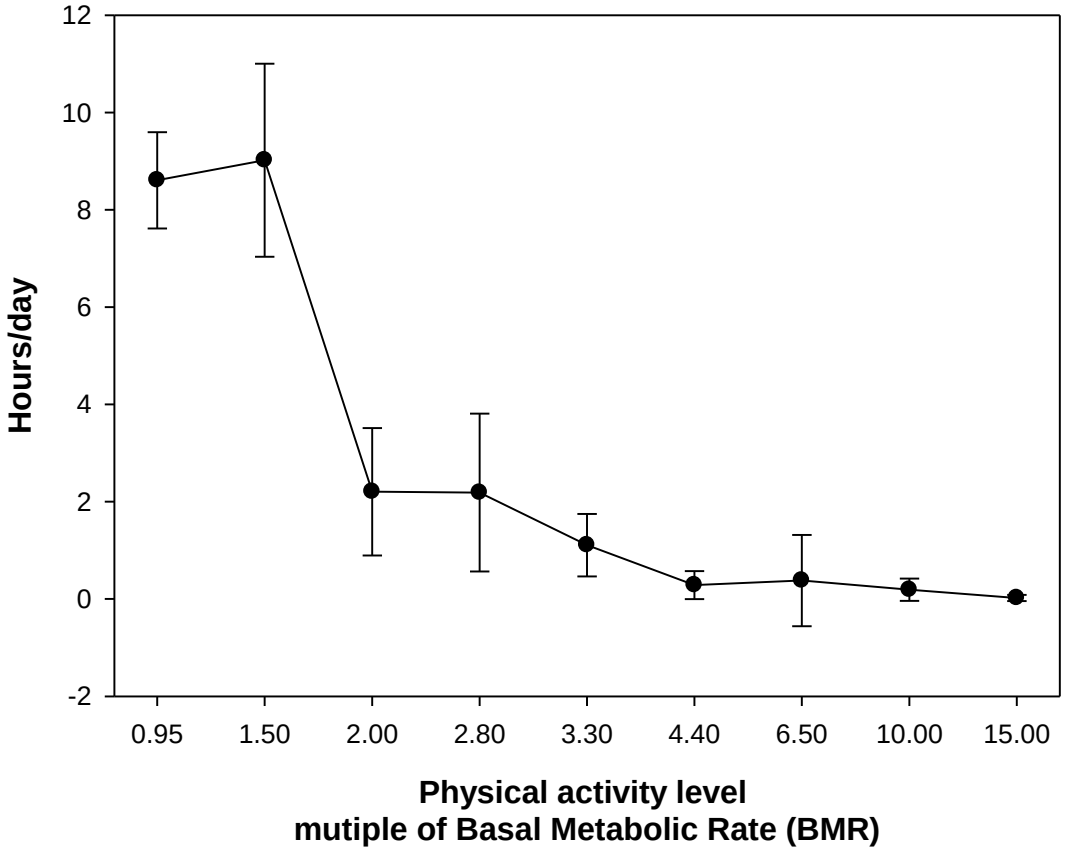
There was no difference between the two comparative groups in relation to energy expenditure measured in kilojoules per day derived from the 7DPAD (Table 4.15). The whole group spent a large proportion time in sedentary activities ~20 hours/day (including sleeping, sitting and standing) and 4 hours/day in moving activities (Figure 4.6) with no significant difference between Indians and non-Indians ($p=0.31$, $ES=0.52$), red-meat-eaters and non-red-meat-eaters ($p=0.71$, $ES=0.15$). As reported by the participants, moving activities involved more low intensity activities (215 minutes, categories 4, 5 and 6; Table 3.2) than moderate, high and maximal intensity activities (35 minutes, categories 7, 8 and 9; Table 3.2). On an average day they reported spending approximately 23 minutes in moderate intensity activity (category 7; Table 3.2), eleven minutes in high intensity activity (category 8; Table 3.2) and only one minute in maximal intensity activity (category 9; Table 3.2). However, the median time spent on moderate, high and maximal intensity activities was only 19 minutes; most of the participants were inactive.

Table 4.15: Reported physical activity from 7-day physical activity diary (7DPAD) in 37 women of childbearing age

	Total N=37	*Indians N=6*	*Non-Indians N=31*	**p value*	*Effect size*	*95% confidence limits*		*Non-red-meat-eaters N=12*	*Red-meat-eaters N=25*	**p value*	*Effect size*	*95% confidence limits*	
						Lower	**Upper**					**Lower**	**Upper**
Energy expenditure (MJ/day)	10.3 ± 2.5 (6.9 – 19.5)	11.6 ± 4.0 (8.8 – 19.5)	10.0 ± 2.1 (6.9 – 18.9)	0.39	0.50	-0.86	1.85	10.8 ± 2.9 (8.6 – 19.5)	10.0 ± 2.3 (6.9 – 18.9)	0.41	0.31		
Sedentary activity (hours/day)	19.8 ± 2.1 (14.0 – 23.0)	18.9 ± 2.4 (15.1 – 21.1)	20.0 ± 2.0 (14.0 – 23.0)	0.31	-0.52	-1.67	0.63	20.0 ±1.7 (15.1 – 21.8)	19.7 ± 2.3 (14.0 – 23.0)	0.71	0.15		
Moving activity (hours/day)	4.2 ± 2.1 (1.0 – 10.0)	5.1 ± 2.4 (2.9 – 8.9)	4.0 ± 2.0 (1.0 – 10.0)	0.31	0.52	-0.63	1.67	4.0 ± 1.7 (2.2 – 8.9)	4.3 ± 2.3 (1.0 – 10.0)	0.71	-0.15		

Mean ± SD; Range in parenthesis; *****p value calculated using unequal variance t test; §95% CI for effect size less than 0.5 not reported;

Figure 4.6: Mean total time ± SD spent in static and physical activities per day



4.7 Associations among measurements of participants

Bivariate associations were explored between measures of body composition, physiological measurements, biomarkers, diet and physical activity. Selected significant associations are grouped and summarised in Tables 8.1-8.7 in Appendix 8. As expected, there was good correlation within body composition measurements, e.g. BMI was correlated with waist, hip, WHR, MUAC, FFM, FM and BF% (all $r>0.50$, $p<0.001$, Table 8.1). Grip strength was negatively associated with WHR and BF% (Table 8.2). WHR was positively associated with MUAC, FM, BF%, pulse and Total/HDL ratio, but in turn negatively associated with HDL cholesterol (all $p<0.005$, Table 8.3). Furthermore, BF% had positive association with MUAC ($r=0.70$, $p<0.001$) and pulse ($r=0.49$, $p<0.005$). There were also positive associations between waist and diastolic blood pressure ($r=0.43$, $p=0.01$, Table 8.4). In terms of biomarkers, HDL cholesterol was negatively associated with waist circumference and FM (both

p<0.005). BF% was negatively associated with HDL cholesterol but positively associated with LDL cholesterol and Total/HDL ratio. There were strong positive associations of Total/HDL ratio with waist, MUAC, FM, BF% and serum triglyceride (Table 8.5). Although serum B_{12} was negatively associated with BMI, waist, WHR, MUAC, FM and BF%, the correlation was significant at the 0.05 level, but not at the 0.01 level (Table 8.6). Dietary B_{12} intake was positively associated with percentage energy from fat, but negatively associated with percentage energy from carbohydrate, while daily folate intake was positively associated with serum triglyceride, all with correlation significant at the 0.05 level (Table 8.7).

4.8 Summary

Indian vs Non Indian

In this small, convenience sample of 38 women, non Indians had a lower WHR and BF% and tended to be stronger with both hands than Indians. Being Indian or not made no apparent difference to fasting glucose, total cholesterol, triglyceride, LDL cholesterol, Total/HDL ratio or the full blood count measurements. However, HDL cholesterol was lower in Indians. Non-Indians had higher serum B_{12} and holo-TC II concentrations than Indians, but there was no difference observed in serum folate concentration. Non-Indians tended to report a higher energy density diet. Indians had a higher percentage of total energy from carbohydrate and a lower percentage of total energy from fat. They also reported significant lower daily B_{12} and protein intakes in 7DDD, as well as a lower folate intake in 24HDR (Table 4.16). Physical activity levels were not different between these groups.

Table 4.16: Summary of differences between Indians and non-Indians

Measurements	*Indians N=6*	*Non-Indians N=32*	**p value*	*Effect size*
WHR	0.83 ± 0.05 (0.75 – 0.90)	0.76 ± 0.04 (0.68 – 0.84)	0.02	1.55
BF%	39.7 ± 9.1 (27.5 – 53.1)	26.0 ± 7.5 (12.2 – 44.2)	0.01	1.64
Grip strength left hand (kg)	25.9 ± 3.8 (22.5 – 33.2)	30.8 ± 6.0 (22.2 – 44.9)	0.02	-0.98
Grip strength right hand (kg)	27.7 ± 3.3 (24.0 – 33.7)	34.3 ± 5.5 (25.2 – 45.2)	<0.005	-1.46
HDL cholesterol (mmol/L)	1.5 ± 0.2 (1.1 – 1.8)	1.8 ± 0.4 (1.0 – 2.9)	0.04	-0.95
Serum B_{12} (pmol/L)	203 ± 157 (112 – 521)	383 ± 127 (148 – 613)	0.04	-1.26
Holo-TC II (pmol/L)	35 ± 26 (17 – 86)	72 ± 35 (33 – 198)	0.02	-1.19
#Energy density (kJ/g)	2.23 ± 0.55 (1.49 – 2.95)	2.92 ± 0.98 (1.68 – 5.66)	0.03	-0.87
#%Energy carbohydrate	55.8 ± 6.2 (47.3 – 60.9)	46.4 ± 6.8 (29.2 – 59.2)	0.01	1.45
#%Energy fat	25.9 ± 5.6 (18.0 – 32.5)	32.3 ± 6.6 (17.6 – 48.3)	0.04	-1.06
#Protein (g/day)	66 ± 17 (45 – 88)	88 ± 28 (45 – 182)	0.03	-0.93
#Dietary B_{12} intake in 7DDD (µg/day)	1.6 ± 0.8 (0.6 – 2.6)	4.5 ± 2.7 (1.7 – 13.2)	<0.001	-1.42
#Dietary folate intake in 24HDR (µg/day)	179 ± 55 (129 – 284)	312 ± 167 (119 – 761)	<0.005	-1.07

Mean ± SD; Range in parenthesis; *****p value calculated using unequal variance t test; WHR, waist to hip ratio; BF%, body fat percentage; # 6 Indians vs 31 non-Indians.

Non-red-meat eating vs red-meat-eating

Non-red-meat-eaters tended to have a higher BF% but weaker grip strength of both hands compared to red-meat-eaters. There was no difference in fasting glucose, all forms of cholesterol, or full blood cell count but non-red-meat-eaters had lower serum B_{12} and holo-TC II concentrations in the presence of no difference in serum folate concentration. Non-red-meat-eaters reported a lower energy density diet, higher percentage energy from carbohydrate, and lower percentage energy from protein.

Furthermore, non-red-meat-eaters reported lower daily B_{12} and protein intakes and had a higher daily folate intake compared to red-meat-eaters. A higher percentage of energy obtained from saturated fat was observed in red-meat-eaters and they also had a better variety of foods containing B_{12} than non-red-meat-eaters (Table 4.17). There was no difference in physical activity levels between the groups.

Table 4.17: Summary of differences between red-meat-eaters and non-red-meat-eaters

Measurements	***Non-red-meat-eaters N=12***	***Red-meat-eaters N=26***	****p value***	***Effect size***
BF%	33.1 ± 10.1 (19.8 – 53.1)	25.9 ± 7.9 (12.2 – 44.2)	0.04	0.79
Grip strength left hand (kg)	26.8 ± 3.7 (22.2 – 33.2)	31.6 ± 6.2 (23.0 – 44.9)	0.01	-0.94
Grip strength right hand (kg)	30.7 ± 3.7 (25.2 – 37.0)	34.5 ± 6.0 (24.0 – 45.2)	0.03	-0.72
Serum B_{12} (pmol/L)	263 ± 147 (112 – 521)	397 ± 127 (177 – 613)	0.01	-0.98
Holo-TC II (pmol/L)	43 ± 22 (17 – 86)	77 ± 37 (33 – 198)	<0.005	-1.11
#Energy density (kJ/g)	2.27 ± 0.56 (1.49 – 3.37)	3.07 ± 1.00 (1.75 – 5.66)	<0.005	-0.99
#%Energy carbohydrate	52.2 ± 7.3 (37.5 – 60.9)	45.8 ± 6.9 (29.9 – 59.2)	0.02	0.90
#%Energy protein	16.0 ± 2.7 (12.3 – 21.8)	19.6 ± 2.9 (14.6 – 24.4)	<0.005	-1.26
#Protein (g/day)	69 ± 17 (45 – 100)	92 ± 29 (45 – 182)	<0.005	-0.99
#%Energy from saturated fat	9.4 ± 2.8 (3.5 – 13.8)	12.0 ± 2.6 (7.9 – 17.5)	0.01	-0.97
#Different food with B_{12}	16 ± 5 (5 - 24)	21 ± 6 (13 – 37)	0.02	-0.87
#Dietary B_{12} intake in 7DDD (µg/day)	2.3 ± 1.1 (0.6 – 4.0)	4.8 ± 2.9 (1.9 – 13.2)	<0.001	-1.15

Mean ± SD; Range in parenthesis; *****p value calculated using unequal variance t test; BF%, body fat percentage; # 12 non-red-meat-eaters vs 25 red-meat-eaters.

Overall, Indians and/or non-red-meat-eaters tended to have more BF% and weaker hand strength than their comparative groups. Their serum B_{12} and holo-TC II concentrations were significantly lower than non-Indians and/or red-meat-eaters, which was validated by their low B_{12} daily intakes. Moreover, Indians and/or non-red-meat-eaters had significantly lower daily protein intakes and a higher percentage of energy derived from carbohydrate.

CHAPTER 5: DISCUSSION

This thesis provides evidence that B_{12} intake is very unlikely optimal in many New Zealand people; and in particular those who do not eat red meat. B_{12} intake was re-examined in the 1997 National Nutrition Survey (NNS97) (n=4,635, mainly European and Maori, 75.6% and 15.2%) and a validation study of B_{12} reported in 24 hour diet recall (24HDR) and 7 day diet diaries (7DDD) with serum markers of B_{12} carried out with a sub analysis of dietary patterns and B_{12} status. The intakes of B_{12} reported in the 24HDR of the NNS97 were examined by subtracting the B_{12} derived from breakfast cereals and using the revised EAR value. Moreover, the validation study was designed to look at B_{12} status in New Zealand women of childbearing age in relation to the Indians vs non-Indians ethnicities and non-red-meat-eating vs red-meat-eating patterns. This thesis was successful in showing that:

1. Possible prevalence of B_{12} insufficiency assessed from NNS97 dietary data was 2.4 times that of the originally reported level, translating into a prevalence of up to 27% of the whole population (Chapter 2).
2. Factors associated with low B_{12} in the diet and markers of B_{12} status in the blood included being Indian, not eating red meat, low energy density, high carbohydrate, low protein, low fat and low B_{12} in the diet, low serum B_{12} and low holo-TC II concentrations in the blood. But nutrient analysis of the diet by 24HDR or 7DDD was not a reliable indicator of B_{12} insufficiency. Dietary pattern particularly red meat eating was the better way than the nutrient analysis.
3. Furthermore, body composition varied by dietary pattern. Indians/non-red-meat-eaters had higher body fat percentage and weaker grip strength than non-Indians/red-meat-eaters. In addition, Indians had a significantly higher waist-to-hip ratio than non-Indians.
4. Overall, the whole group reported that they were inactive. They spent approximately 20 hours of the day in sedentary activities (including sleeping) and four hours a day in any form of movement. The median time spent in moderate, high and maximal intensity activities was only 19 minutes a day, which did not meet the NZ guideline for adults of 30 minutes a day.

The implications and interrelationships of these findings: dietary analysis and patterns, biomarkers, body composition and physical activity are interpreted in the context of the life course model of disease (Uauy & Solomons, 2006) and the metabolic pathways that involve B_{12} and 3 carbon metabolism (Figure 1.3). Limitations of the study and future recommendations will then be described and conclusions stated.

It is recognised that in developing countries such as India, Mexico, Central and South America, and selected areas in Africa (Stabler & Allen, 2004) that there is a severe problem of dietary B_{12} deficiency with up to 75% of the population affected (Refsum, H. et al., 2001). The situation in developed countries has not been widely investigated particularly in minority ethnic groups. In migrant young Indians in New Zealand of both this study and Chhichhia (2007) using robust biomarkers showed that B_{12} was very low in New Zealand Indian. Similarly another small study in the United States reported significantly lower cobalamin levels in both Indian men and women than non-Indians (Carmel, R. et al., 2002). Indians are particularly at risk because their cultural/religious dietary patterns are intergenerational. Prenatal exposure to a suboptimal intrauterine environment including nutrition exposes the foetus to intrauterine programming signals that have life long consequences (Fowden et al., 2006).

In general in New Zealand, vegetarian practice in the three main ethnicities (European, Maori and Pacific) has not usually been lifelong or strict and has been within a supportive dietary education framework such as that provided by the Seventh-day Adventist church (Harman & Parnell, 1998). B_{12} deficiency in young New Zealand people has not been seen as a major problem (Parnell et al., 2003; Russell et al., 1999). In this study those who were not Indian were at less risk, while in particular those who do not eat red meat (largely Indian) were at more risk rather than vegetarian per se. Similarly, a study in France concluded that because of education there was no nutritional problem within vegetarian (Leblanc et al., 2000). The French study included Indian in their sample but no biomarkers were used to confirm B_{12} status. In this study of women of childbearing age it has been shown using biomarkers that those who are not Indian and eat red meat are at a lower risk of B_{12} insufficiency. While reanalysis of NNS97 data showed a possible prevalence of

up to 27%, the validity of dietary assessment to determine prevalence of nutrient deficiency must be considered.

The present study has demonstrated that B_{12} intake reported in both the 24HDR and 7DDD was not a valid indicator of B_{12} status measured either by serum B_{12} or holo-TC II concentration. Other ways to assess dietary validity include measures of energy intake and energy expenditure requirements necessary to maintain life. A basic test of dietary validity therefore is that sufficient energy intake is reported to ensure all foods and accurate quantities are recorded. It is recognized that energy intake is often under-reported (Westerterp & Goris, 2002) and physical activity is over-reported (Moy, 2005), which is supported by the low reported energy intake (7.7MJ/day, 7DDD) and the high reported energy expenditure (10.3MJ/day, 7DPAD) in this study. By international standards, New Zealanders' physical activity levels reported are considered favourable. The New Zealand Ministry of Health (2003a) recommends that, "*thirty minutes of physical activity of moderate intensity on most, if not all, days of the week can benefit health*". In this validation study, the whole group spent average 35mins/day but median of 19mins/day in moderate, high and maximal intensity activities. Compared to the 68% of population reported being active in the 2000/01 Hillary Commission Physical Activity Survey, the level of activity of the whole study group was low, as 46% (17 of 37, one Indian) of participants were physically active for 2.5hrs/week. Only 16% (6 of 37, all were non-Indians) of the whole group had met the recommendation of being physically active for 30 minutes per day on 5 or more days per week, compared to that (39%) in the 2000/01 survey of both sexes.

Physical activity has the effect of improving muscle function and driving an increase of muscle mass assisted by an increased influx of protein from diet (Wolfe, 2006). Decreased physical activity increases malonyl-CoA concentrations in the skeletal muscle, which further suppresses fatty acid oxidation and lipogenesis (Pan et al., 1997). Therefore, the decreased physical activity in these women of childbearing age would support poor muscle function and may add to the progression of increased body fatness and insulin resistance. Indians and/or non-red-meat-eaters are at a greater risk due to their poor protein intake and the accumulation of malonyl-CoA as a result

of B_{12} deficiency (Allen et al., 1998). Both of these groups had significantly more BF% than comparative groups, but no difference in fasting glucose concentration.

The dietary analysis was examined against energy intake criteria. The diaries were checked for reporting accuracy using the ratios of energy intake (EI, 7DDD) and estimated (Schofield, 1985) basal metabolic rate (BMR_{est}) to derive the physical activity level (PAL); the ratio of energy expenditure (EE, 7DPAD) and BMR_{est} value. The mean EI:BMR_{est} ratio of the whole group was 1.32 ± 0.40, while the mean PAL was 1.74 ± 0.25. As suggested by Goldberg cut-off using a ratio of 1.55 for recognizing under-reporters (Black, 2000), there might be some under-reported energy intake and/or some over-reported energy expenditure within this group.

Diet is very hard to measure but 7DDD is recognized as a reliable method of determining recent dietary intake (Livingstone & Robson, 2000) as the intake over a whole week including the weekend is averaged. It is preferred over semi-quantitative food frequency questionnaires (FFQ) when validated against urinary biomarkers like nitrogen, sodium and potassium (Day et al., 2001). The error variances for each of the three nutrients was more than twice as great with the FFQ than the 7DDD, and the correlation between errors in different nutrients was higher for the FFQ (0.77 – 0.80) than for the 7DDD (0.52 – 0.70). The measurement error and validity of dietary data has serious implications for nutritional epidemiology (Kipnis et al., 2002). The example that Kipnis et al. use in this later paper is validation of protein intake by the biomarker urinary nitrogen with more than 200% overestimation of the correlation. It follows that if protein is not well measured then it is very likely that B_{12} would not be either.

Therefore, the strength of the association between diet intake and biomarkers was compared between 24HDR and 7DDD. In this study both 24HDR and 7DDD reported B_{12} intake had trivial correlation to biomarkers and low sensitivity in indicating B_{12} insufficiency – 30% in serum B_{12} concentration and slightly higher at 43% in holo-TC II concentration. Validation of dietary intake using the biomarkers serum B_{12} and holo-TC II concentrations required a range of B_{12} dietary intake and therefore women with a range of dietary patterns were recruited in this validation study. There was

some “relative validity” between the 24HDR and 7DDD for B_{12}. Folate showed a similar pattern.

It is widely accepted that vegetarians might be at risk of dietary B_{12} deficiency because of low consumption of animal products (Stabler & Allen, 2004) but this often does not appear to be the case. In this investigation 10 vegetarian women volunteered and a further two non vegetarian (one Indian) did not eat red meat. Because red meat contains significantly more B_{12} than other animal products (Crop and Food Research, 2004) in this analysis red meat eating was selected as one defining characteristic and Indian ethnicity as the other. The purposive sampling enabled the hypothesis to be tested that red-meat-eaters (i.e. in general non-vegetarians) would have better vitamin B_{12} status than those who do not eat red meat. Red meat eating and not being Indian have been shown at the $p<0.001$ level to be very strong indicators of adequate B_{12} intake and adequate serum B_{12} concentrations.

Low consumption of meat has previously been associated with low B_{12} levels in Israel (Volkov et al., 2007) and it is likely that the same pattern of reduced meat eating, influenced by the current adverse media about beef and cholesterol, will result in more of the general population being at risk of B_{12} insufficiency. This is a particular concern in women of childbearing age as there is a proven impact on future child cardiovascular risk factors associated with a low maternal B_{12} intake in Indian (Yajnik et al., 2008). This study further shows that it is not just Indian that are at risk, more than one out of five (7/32) non-Indians had a serum B_{12} concentration of 250pmol/L or less and three of those ate red meat. Low B_{12} in the diet was supported by concomitant low serum B_{12} and holo-TC II concentrations. Indians also reported low intakes of eggs, milk and milk products, fish and sea foods, and all sorts of meats. Examination of the Indian diet for macro- and micronutrient analysis revealed that the Indian diet did not follow the New Zealand dietary guidelines and it was concluded that the diet quality of all Indians was “poor”, which will be explained in the next part of the discussion.

Among five Indian non-red-meat-eaters, three of them were born vegetarian, one became vegetarian in the last two years, and another one did not eat red meats because of her personal food choice. Many Indian have a family history of vegetarian practice.

In the 1998-1999 National Family Health Survey-2 (NFHS-2) ("India 1998-99: results from the National Family Health Survey," 2001), one third of women were born vegetarian and never consumed chicken, meat or fish. In this study no non-Indian non-red-meat-eater however, was born vegetarian. Non-Indian participants adopted a vegetarian dietary pattern between six months and twenty years ago, for a variety of reasons including health and animal rights. While vegetarian dietary practice may be associated with health benefits there is also evidence that vegetarianism is associated in more menstrual problems and poorer mental health (Baines et al., 2007).

Subsequent analysis of the data refined the analysis to answer the question to: "Are red-meat-eaters and non-red-meat-eaters different in their B_{12} status?" and "Are Indians and non-Indians different in their B_{12} status?". Food choices and patterns were also examined.

One measure of dietary pattern is macronutrient balance. The mean percentages of energy derived from carbohydrate, protein, and fat of the whole group were within the reference ranges. The ratio of carbohydrate:protein:fat was 48/18/31, similar to that in NNS97 report 45/16/34 for NZ European & Others (Russell et al., 1999).

The relatively high intake of carbohydrate was mainly due to the Indian diet, 56% of whose energy from carbohydrate, and/or non-red-meat-eating diet, 52% from carbohydrate. Popkin et al. (2001) have reported a large shift from consumption of coarse grain to rice in all income groups in India. This is also shown in the diet of Indian participants in present study, with high intakes of breakfast cereals, bread and rice, which contain great amount of carbohydrate. Non-red-meat-eaters ate more breakfast cereals than red-meat-eaters. The consumption of sucrose was within the recommended range of <10% of energy (World Health Organisation, 2003). But the main sources of sucrose in the diet were ready-to-eat foods like noodles, chips, biscuits, fruits juices and soft drinks.

The mean percent energy from protein was 18.4% for the whole group, which is within the recommended reference value 15-25% (Ministry of Health, 2005). Mean percentage protein intake for Indians and/or non-red-meat-eaters was on the borderline of the lower level of reference value; 16.7% and 16.0% respectively. As

expected protein intake in grams per day was positively correlated to B_{12} intake ((r=0.79, p<0.001), as food sources rich in protein, such as meat products, milk, and cheese, are also good sources of B_{12}. Indians and/or non-red-meat-eaters chose foods less rich in protein such as plants rather than animal products. This was associated with a higher daily intake of folate. Plant proteins may be less digestible (Stabler & Allen, 2004) plus are more likely to lack some essential amino acids.

The mean percent energy from fat of the whole group (31.3%) was within the reference range (20-35%) (Ministry of Health, 2005), and slightly less than that (34%) reported for NZ European & Others females in NNS97 (Russell et al., 1999). The mean percent energy from saturated fat was 11.2% for the whole group, which is higher than the recommendation (<10%) from Ministry of Health (2005). In subgroups, non-Indians and/or red-meat-eaters had mean percent energy from saturated fat >10%, while Indians and/or non-red-meat-eaters had that <10%, which was due to the substantial quantities of cholesterol and saturated fats from meat products (Fraser, 1999). However, serum LDL-cholesterol concentrations and total cholesterol concentrations were not significantly different between subgroups in the validation study.

The mean P:S ratio of the whole group was 0.53, similar to that, 0.52, found for 3,464 women in Cambridge, UK (Harding et al., 2001). Indians and/or non-red-meat-eaters tended to have higher P:S ratio than non-Indian and/or red-meat-eaters. The P:S ratio of non-Indians and/or red-meat-eaters was lower than the reference value, which implicated a risk for heart diseases and hyperglycaemia (Oliver, 1997). Oliver (1997) also concluded that it may be more important, and also more feasible to change the P:S ratio and increase polyunsaturated fat intake than decrease total fat and saturated fat intake.

As this study was limited by small numbers outliers were examined carefully. There was one participant with extremely high holo-TC II concentration. She reported Risperdal/Risperidone medicine consumption and daily multivitamin intake during visit 1 interview, but not multivitamin detail recorded in her diet diary. Another participant reported daily vitamin B complex supplement consumption as well, but with normal holo-TC II concentration. Therefore, the elevated holo-TC II

concentration of the previous participant might be due to a higher B_{12} quantity in the supplement she consumed, or the intake of Risperdal/Risperidone, an antipsychotic medication used to treat schizophrenia and symptoms of bipolar disorder. Although there is no report on the association between Risperdal and holo-TC II concentration, there might be some connection as Risperdal is dependent on renal clearance (Maxwell et al., 2002) and the concentration of holo-TC II can be affected by renal dysfunction (Herrmann et al., 2005). If she was excluded for the analysis, holo-TC II concentration had stronger positive correlation to serum B_{12} concentration ($r=0.79$, $p<0.001$).

A study on the effect of dietary intake on serum B_{12} concentrations in a cohort of human immunodeficiency virus-positive patients showed that, each 1μg/day increase in B_{12} intake was associated with a 1.06pg/ml (0.78pmol/L) increase in serum B_{12} concentrations (Woods et al., 2003). Although there was no significant correlation between dietary intake and serum B_{12} concentration in this validation study, the pattern of most Indians and/or non-red-meat-eaters with significant lower dietary intake gathering at the lower end of serum B_{12} concentration (Figure 4.5) suggested that, red meat consumption or consumption of foods rich in B_{12} is a feasible way to increase B_{12} in the blood and the next step would be a supplementation study to prove this.

In the present validation study, non-Indian and/or red-meat-eaters had a relatively higher serum concentration of haemoglobin than Indians and/or non-red-meat-eaters. There were two participants (one non-red-meat-eater and one red-meat-eater, both non-Indians) had haemoglobin concentrations lower than reference value. However, both of them had normal serum B_{12} and holo-TC II concentrations and no elevated MCV, so Diagnostic Medlab Ltd suggested that the iron deficiency indicated by their red cell appearances be further investigated (This advice was passed on to the participants). There was no evidence of macrocytosis which indicates B_{12} deficiency for all except one participant with an elevated mean cell volume (MCV, 102fl) but normal serum B_{12} and holo-TC II concentrations.

Five Indians, including one red-meat-eater, were low in serum B_{12} (<170pmol/L) and/or holo-TC II (<35pmol/L) concentrations, and the sixth had adequate serum B_{12}

(521pmol/L) and holo-TC II (86pmol/L) concentrations. Her mother was a non-vegetarian and the vegetarian dietary pattern had only been followed over the last two years. Her daily B_{12} intake in 7DDD was 1.57μg, around the mean intake of the six Indians, and lower than the EAR value. The reason for her high biomarker of B_{12} status and low reported B_{12} intake might be from accumulated body stores of B_{12} before the change in dietary pattern and the long biological half life of B_{12} in human body – more than 480 days (Butler et al., 2006).

The low levels of B_{12} in women in the present thesis are very likely to carry forward to their future offspring. A prospective study of hospitalized New Zealand children aged 8-23 months found that those children with a mother who restricted her meat intake during pregnancy were at increased risk of iron deficiency anaemia (Grant et al., 2003). Although B_{12} level was not detected in this prospective study, those children might be also at increased risk of B_{12} deficiency/insufficiency as meat is not only good source of iron but also rich in B_{12}. A limited supply of B_{12} during pregnancy has important consequences for foetal growth. The longer the mother being vegetarian, the greater likelihood she will maintain a low serum and breast milk concentration of B_{12}, which correlate with B_{12} insufficiency in the infant (Bjorke Monsen et al., 2001). Furthermore, the non-meat-eating habit of mothers might pass over to their children, which would further deteriorate their iron and B_{12} levels. The significant low B_{12} status among Indian non-red-meat-eaters, compared to non-Indian non-red-meat-eaters, might be due to both their poor diet and intergenerational transference as most of them were born vegetarian. Therefore, the adequate nutrient status of women of childbearing age is vital to both themselves and their offspring.

Folate is complementary to B_{12} metabolism. In the NNS97 report, the usual daily median intake of folate from food for the New Zealand population was 242μg (males 278μg, females 212μg). While the overall estimated prevalence of inadequate intake for the New Zealand population was 7.1%, the prevalence was consistently higher among females than males. Females 15-24 years had a higher prevalence of inadequate intake (21.2-22.2%) compared with females 45+ years (9.2-9.8%). The EAR value of folate used in the report was 150μg/day (Russell et al., 1999), while the one used in the validation study is 320μg/day, revised for Australian and New Zealander since 2005 (Ministry of Health, 2005). If the revised EAR value was used

to analyse the NNS97 database, the prevalence of inadequate intake of the population would be much more serious than reported, over half of the population would be at risk.

The mean daily folate intake in 7DDD of the validation study was 292μg/day, and 25 of 38 participants reported daily folate intake lower than the revised EAR value (320μg/day), which meant over half of the participants might be with inadequate intake of folate, and the prevalence was higher among red-meat-eaters (21 of 25) than non-red-meat-eaters (4 of 12). However, it was interesting to find that no one in the whole group had low serum folate concentration, no matter red-meat-eaters or non-red-meat-eaters, Indians or non-Indians. Studies in Pune have shown that B_{12} deficiency is common while folate deficiency is rare in India (Refsum, H. et al., 2001; Yajnik et al., 2006). As to the prevalence in New Zealand, though there is no national report on serum folate concentration of the whole population, Venn et al. (2002) studied 97 women of childbearing age (18-49 y old) in Dunedin and reported that the mean plasma folate concentration at baseline was 22.7nmol/L, which is slightly lower than that of the present validation study (26nmol/L). Except reported occasional consumption of folic acid supplement by three participants, some manufacturers in New Zealand voluntarily fortify their products with folic acid and this might contribute to the main reason of higher mean serum folate concentration in this validation study. Products permitted to add folate include breakfast cereals, juices and food drinks (such as liquid meal supplements) (New Zealand Food Safety Authority). Moreover, the phenomenon of low reported daily folate intake and normal serum concentration in the validation study suggests that their daily folate intake might be under-reported by themselves or because of the limitation of NZ food composition tables (not including completed information of folic acid fortified products) used for analysis, or the EAR value is higher than the one required for maintaining adequate serum concentration. However, due to the limited number of participants, the present validation study only represents the truth of this small group, not the whole population.

Long term imbalance of folate and B_{12} is associated with accumulation of risk for chronic disease. Although a few participants in the validation study had high systolic and/or diastolic blood pressure and some were high in LDL cholesterol and/or total

cholesterol concentrations, the mean values of fasting glucose and lipids in the whole group and even each subgroup were within the recommended ranges. No significant differences were found between subgroups for fasting glucose and lipids, expect that non-Indians had significant higher HDL cholesterol concentration than Indians. Since the Framingham Heart Study in USA first published significant inverse relation between HDL cholesterol and the incidence of coronary artery disease in both men and women 49-82 years of age in 1977, a number of other observational and epidemiologic trials have provided data that establish HDL cholesterol as an important risk factor for coronary artery disease, regardless of the level of total cholesterol or LDL cholesterol, and an increase of 1% in HDL cholesterol serum concentrations is associated with a 3% decrease in risk of heart disease (Boden, 2000). As the mean HDL cholesterol concentration of Indians was still within the reference value, the significant lower result of Indians could not draw a direct conclusion that Indians were in risk of heart disease, but they had more chance to be affected by heart disease in the future than non-Indians, according to the life course model (Figure 1.1), all the risk factors accumulating throughout life span. The lower HDL cholesterol concentration of Indians might be due to their high BF% and low consumption of fish and sea foods, the good sources of polyunsaturated fat and low physical activity.

In the present thesis, the mean weight was 64.5kg, lighter than that (67.1kg) of NZ European & Others females in NNS97 (Russell et al., 1999). Except for one Indian non-red-meat-eater (146.0kg), all the participants weighed between 42.9-88.9kg. Excluding this outstanding person, Indians had normal BMI but significant high BF%, which is similar to the high BF% at low BMI found in Asian Indians by Deurenberg et al. (2002), Rush et al. (2006) and Chhichhia (2007).

An increased BMI within a population is an important risk factor for type 2 diabetes. A diet, lifestyle, and the risk of type 2 diabetes mellitus study in USA women (Hu et al., 2001) reported that the relative risk for women with a BMI of 25-30 was 7.6, a BMI at the high end of normal range (23-25kg/m^2) increased the relative risk to 2.7, compared with women whose BMI was below 23kg/m^2. The insignificant higher BMI of Indians (28.3kg/m^2) and/or non-red-meat-eaters (25.3kg/m^2) suggested that they

were increasingly likely to have type 2 diabetes than non-Indians (22.8kg/m^2) and/or red-meat-eaters (22.9kg/m^2).

Another striking feature of increasing adiposity in Indians is increased abdominal adiposity, marked by increased waist circumference. In the present thesis, waist circumference was positively correlated to diastolic blood pressure, serum triglyceride, total/HDL ratio, and negatively correlated to HDL cholesterol and serum B_{12} concentrations. Similar associations of a relatively large waist circumference in men and women with cardiovascular disease risk factors, low HDL cholesterol concentration and high fasting triacylglycerol, insulin, and glucose concentrations, were found by Seidell et al. (2001). The high prevalence of large waist circumference (>80cm) in Indians (4 of 6) and/or non-red-meat-eaters (7 of 12) suggested their increasing likelihood to be affected by cardiovascular diseases in the future. Similar to waist, mid upper arm circumference (MUAC) is a standard technique used for assessment of body fatness to provide an estimate of nutritional status of an individual or population (Lukaski, 1987) and this was significantly positively related to BMI, WHR, FM, FFM, BF%, serum triglyceride, total/HDL ratio, diastolic blood pressure, and negatively related to HDL cholesterol and serum B_{12} concentration.

Vegetarians are often a little leaner compared with non-vegetarians and suffer less from obesity and its associated complication than non-vegetarians. Vegetarians were observed with significantly lower BMI (Kennedy et al., 2001; Key et al., 1999; Spencer et al., 2003) than non-vegetarians. However, the present validation study did not report a similar result to these published studies. Non-red-meat-eaters tended to have higher BMI, waist circumference, WHR, and BF% than red-meat-eaters. This unusual phenomenon might be due to the small sample size and the outstanding obese Indian woman driving the body composition results of the non-red-meat-eater group to the higher end. The high proportion of Indians in the non-red-meat-eater group (5 of 12) also contributed to the differences between non-red-meat-eaters and red-meat-eaters. Another reason is that some non-red-meat-eaters in the validation study had stopped eating red meats for a relatively short time for personal reasons like being overweight already or peer pressure. All the factors above may have contributed to the different body composition measurements between this validation study and other population studies.

Patterns of associations and interrelationships have been demonstrated that support the life course model and metabolic pathways that lead to changes in structure and function. The low B_{12} levels and protein intake of Indians and/or non-red-meat-eaters might result in long-term risk chronic disease. As shown in figure 1.3, results include high glucose concentration, insulin resistance, lipogenesis, high body fatness and poor muscle mass. The present validation study did not show any hyperglycaemia, but some participants were observed with hyperlipidaemia (high total cholesterol and/or LDL cholesterol concentration). Their low serum B_{12} concentrations were significantly associated with BMI, waist circumference, WHR, MUAC, FM, and BF% (Table 9.6, Appendix 9). The long-term low B_{12} level would decrease fat oxidation (Figure 1.3), which is associated with increased fat deposition (lipogenesis). This might explain why Indians and/or non-red-meat-eaters had higher BF% than comparative groups.

Body composition is mediated by the relationship between energy intake, macronutrient balance, and energy expenditure. Low energy expenditure as reported by the study group could be associated with decreased fatty acid oxidation (and an increase in Malonyl-CoA) and increased body fat percentage. However, no association was found between BF% and PAL assessed by diary in this validation study ($r=0.08$, $p=0.62$). B_{12} plays a role in maintaining the homocysteine levels (even in the presence of adequate folate) and the methylation processes associated with DNA and RNA synthesis and expression (Figure 1.3).

The relatively poor protein intake reported by Indians and/or non-red-meat-eaters is also of concern as this may result in decreased formation of muscle mass – already validated by their significant high BF%. Poor muscle mass could also affect the drive and ability to participant in physical activity. Muscle also has a vital role in insulin uptake and glucose and lipid homeostasis. Although overt hyperglycaemia was not observed in the whole group, it can not be assumed that it will not be a problem in the future.

5.1 Limitations and strengths

This study is limited in its interpretation but does provide a starting point for evidence that will support action. The small sample size, especially in ethnic groups (eg. only six participants being Indian), is the major limitation of the present study. Firstly, the small number increases the likelihood of Type II errors in determining the associations and finding differences between comparative groups, and all the results of this validation study only represent the study group, not the population. BMI cut-off and BIA equation for FFM, FM and BF% were not ethnic specific – those for European were used as most of participants belonged to this ethnicity. Intra-individual variation is not known as most measurements were made only once in a short time frame. And all the results were not adjusted by age and social economic status (SES). Secondly, time and financial constraints limited the number of subjects to be recruited and the participant burden for measurements for this intensive validation study. The number and measurements of blood biomarkers was limited by expense and also by the amount of blood required.

Reporting of dietary intake was subject to many errors including over or under-representing a portion size by participants or by the researcher when entering the data for analysis. Similarly, reporting of physical activity is likely to be over-reported as the gold standard measurement for energy expenditure - doubly labelled water – was not involved in this validation study. Moreover, Dual-energy X-ray Absorptiometry (DEXA) is more accurate and reliable for FM, FFM, BF% and fat mass distribution than BIA. Because of the high expense and inconvenience of DEXA, it was replaced by BIA in present study.

Although this validation study had these limitations above, it is the first study in New Zealand using holo-TC II as a biomarker of B_{12} status. Given changes in the recommended intake of dietary B_{12} the prevalence of B_{12} insufficiency was not as low as previously reported. New Zealand Indian (one of the fast increasing migrant groups in New Zealand) and non-red-meat-eaters were at great risk of B_{12} insufficiency. Participants' body composition and dietary intake were validated by biomarkers. It did show that self dietary report was not a good measurement of accurate dietary B_{12} or folate intake and nutrient status.

Preliminary evidence suggests there is a reasonable proportion of young women at risk and this number is likely to increase if health and nutrition education and health promotion messages are not balanced, practical and effective (Margetts, 2006). While more research is warranted, the implications for future generations clearly call for optimization of B_{12} nutrition status in Indian and/or non-red-meat-eaters before pregnancy.

5.2 Recommendations

This study has piloted a number of tools in New Zealand women of childbearing age. They were found to be acceptable and participants reported general satisfaction with the study and appreciated the information that they received in return. This has paved the way for a longitudinal study in association with a dietary supplementation or fortified food consumption intervention to target in the short term an increase in dietary B_{12} intake with validation by biomarkers such as serum B_{12} and holo-TC II concentrations. Secondary and longer term goals would be to measure changes in physical fitness, diabetes and cardiovascular risk factors, body composition, and even the health and growth of their offspring in future.

Other recommendations include:

1. General physicians and midwives should measure serum B_{12} concentration and folate status of women planning pregnancy particularly if they are Indians and/or non-red-meat-eaters.
2. Women who had B_{12} deficiency/insufficiency might be suggested to consume supplement to boost their B_{12} levels and maintained by improved dietary intake, especially before becoming pregnant.
3. Implementing the New Zealand Health Strategy 2002 (King, 2002) suggests that it is important to improve nutrition, reduce obesity and increase the level of physical activity. The health conditions and life style of women of childbearing age nowadays will relate to future health of themselves and their offspring.
4. A balanced diet is necessary for women of childbearing age. Appropriate amount of red meats or other good sources of B_{12} for non-red-meat-eaters,

such as milk and eggs, accompanying with abundant of green leafy vegetables, which contains plenty of folate.

5.3 Conclusion

Both 24HDR and 7DDD have use in the assessment of the dietary patterns of participants, but not on reporting the true quantities of nutrient status. If the accessory of 24HDR in the validation study was applied to the NNS97, 70% (1-sensitivity) of people with B_{12} insufficiency would not be identified from the nutrient analysis of 24HDR. In this case, the conclusion of "*vitamin B_{12} intake appeared adequate for the New Zealand population*" derived from their 24HDR might not hold true. A quantitative specific food frequency questionnaire (QFFQ) might be better for screening as eating patterns like eating red meat or not eating red meat had the most significant difference in B_{12} biomarkers in the presence of no significant correlation between reported dietary intake and biomarkers for B_{12}.

The present attention to folate supplementation by policy makers needs to be moderated by considerations of interactions with the status of other micronutrients. Although the vulnerable groups, such as Indians and non-red-meat-eaters who had more odds of B_{12} insufficiency than the rest of the participants in the validation study, are not the major proportion of New Zealand population, it is still meaningful and should not be ignored. B_{12} monitoring, dietary recommendations and, if necessary, intervention supplement before pregnancy (as for folate) should be considered for New Zealand women of childbearing age, especially the vulnerable groups.

It is time for serious action in this area of health so that the risk accumulated through and imbalance in nutrition and physical activity is reduced and the health of both women of childbearing age and their future offspring is improved.

REFERENCES

Abbott, M., & Young, W. (2006). Asian Health Chart Book 2006: foundation for a new health agenda in New Zealand? *N Z Med J, 119*(1244), U2278.

Albert, M. J., Mathan, V. I., & Baker, S. J. (1980). Vitamin B12 synthesis by human small intestinal bacteria. *Nature, 283*(5749), 781-782.

Allen, R. H., Stabler, S. P., & Lindenbaum, J. (1998). Relevance of vitamins, homocysteine and other metabolites in neuropsychiatric disorders. *Eur J Pediatr, 157 Suppl 2*, S122-126.

Awan, M. M., & Saggerson, E. D. (1993). Malonyl-CoA metabolism in cardiac myocytes and its relevance to the control of fatty acid oxidation. *Biochem J, 295 (Pt 1)*, 61-66.

Baines, S., Powers, J., & Brown, W. J. (2007). How does the health and well-being of young Australian vegetarian and semi-vegetarian women compare with non-vegetarians? *Public Health Nutr, 10*(5), 436-442.

Banerjee, D. K., & Chatterjea, J. B. (1963). Vitamin B12 Content of Some Articles of Indian Diets and Effect of Cooking on It. *Br J Nutr, 17*, 385-389.

Barker, D. J. (1997). Maternal nutrition, fetal nutrition, and disease in later life. *Nutrition, 13*(9), 807-813.

Bellamy, C. (1999). *The state of the world's children*. Geneva: UNICEF.

Ben-Shlomo, Y., & Kuh, D. (2002). A life course approach to chronic disease epidemiology: conceptual models, empirical challenges and interdisciplinary perspectives. *Int J Epidemiol, 31*(2), 285-293.

Berdanier, C. D., Dwyer, J., & Feldman, E. B. (2007). *Handbook of Nutrition and Food* (Second ed. Vol. 26): CRC Press.

Bhargava, S. K., Sachdev, H. S., Fall, C. H., Osmond, C., Lakshmy, R., Barker, D. J., et al. (2004). Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. *N Engl J Med, 350*(9), 865-875.

Bjorke Monsen, A. L., Ueland, P. M., Vollset, S. E., Guttormsen, A. B., Markestad, T., Solheim, E., et al. (2001). Determinants of cobalamin status in newborns. *Pediatrics, 108*(3), 624-630.

Bjorntorp, P. (1987). Classification of obese patients and complications related to the distribution of surplus fat. *Am J Clin Nutr, 45*(5 Suppl), 1120-1125.

Black, A. E. (2000). Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes Relat Metab Disord, 24*(9), 1119-1130.

Black, A. E., Bingham, S. A., Johansson, G., & Coward, W. A. (1997). Validation of dietary intakes of protein and energy against 24 hour urinary N and DLW energy expenditure in middle-aged women, retired men and post-obese subjects: comparisons with validation against presumed energy requirements. *Eur J Clin Nutr, 51*(6), 405-413.

Boden, W. E. (2000). High-density lipoprotein cholesterol as an independent risk factor in cardiovascular disease: assessing the data from Framingham to the Veterans Affairs High--Density Lipoprotein Intervention Trial. *Am J Cardiol, 86*(12A), 19L-22L.

Bor, M. V., Nexo, E., & Hvas, A. M. (2004). Holo-transcobalamin concentration and transcobalamin saturation reflect recent vitamin B12 absorption better than does serum vitamin B12. *Clin Chem, 50*(6), 1043-1049.

Bouchard, C., Tremblay, A., Leblanc, C., Lortie, G., Savard, R., & Theriault, G. (1983). A method to assess energy expenditure in children and adults. *Am J Clin Nutr, 37*(3), 461-467.

Bratteby, L. E., Sandhagen, B., Fan, H., & Samuelson, G. (1997). A 7-day activity diary for assessment of daily energy expenditure validated by the doubly labelled water method in adolescents. *Eur J Clin Nutr, 51*(9), 585-591.

Butler, C. C., Vidal-Alaball, J., Cannings-John, R., McCaddon, A., Hood, K., Papaioannou, A., et al. (2006). Oral vitamin B12 versus intramuscular vitamin B12 for vitamin B12 deficiency: a systematic review of randomized controlled trials. *Fam Pract, 23*(3), 279-285.

Buzzard, I. M., Faucett, C. L., Jeffery, R. W., McBane, L., McGovern, P., Baxter, J. S., et al. (1996). Monitoring dietary change in a low-fat diet intervention study: advantages of using 24-hour dietary recalls vs food records. *J Am Diet Assoc, 96*(6), 574-579.

Carmel, R. (2002). Measuring and interpreting holo-transcobalamin (holo-transcobalamin II). *Clin Chem, 48*(3), 407-409.

Carmel, R. (2006). Cobalamin (Vitamin B_{12}). In Shils, M. E., Shike, M., Ross, A. C., Caballero, B. & Cousins, R. J. (Eds.), *Modern Nutrition in Health and Disease* (10th ed. ed., pp. 482-497). Philadelphia: Lippincott Williams & Wilkins.

Carmel, R., Green, R., Rosenblatt, D. S., & Watkins, D. (2003). Update on cobalamin, folate, and homocysteine. *Hematology Am Soc Hematol Educ Program*, 62-81.

Carmel, R., & James, S. J. (2002). Alcohol abuse: an important cause of severe hyperhomocysteinemia. *Nutr Rev, 60*(7 Pt 1), 215-221.

Carmel, R., Mallidi, P. V., Vinarskiy, S., Brar, S., & Frouhar, Z. (2002). Hyperhomocysteinemia and cobalamin deficiency in young Asian Indians in the United States. *Am J Hematol, 70*(2), 107-114.

Chhichhia, P. P. (2007). *Indian predolescent girls: lifestyle patterns and accumulated risk factors (MHSc thesis submitted).* Auckland University of Technology, Auckland.

Crop and Food Research. (2004). The Concise New Zealand Food Composition Tables.

Dalton, M., Cameron, A. J., Zimmet, P. Z., Shaw, J. E., Jolley, D., Dunstan, D. W., et al. (2003). Waist circumference, waist-hip ratio and body mass index and their correlation with cardiovascular disease risk factors in Australian adults. *J Intern Med, 254*(6), 555-563.

Day, N., McKeown, N., Wong, M., Welch, A., & Bingham, S. (2001). Epidemiological assessment of diet: a comparison of a 7-day diary with a food frequency questionnaire using urinary markers of nitrogen, potassium and sodium. *Int J Epidemiol, 30*(2), 309-317.

Deurenberg-Yap, M., Schmidt, G., van Staveren, W. A., & Deurenberg, P. (2000). The paradox of low body mass index and high body fat percentage among Chinese, Malays and Indians in Singapore. *Int J Obes Relat Metab Disord, 24*(8), 1011-1017.

Deurenberg, P., Deurenberg-Yap, M., & Guricci, S. (2002). Asians are different from Caucasians and from each other in their body mass index/body fat per cent relationship. *Obes Rev, 3*(3), 141-146.

Doak, C. M., Adair, L. S., Bentley, M., Monteiro, C., & Popkin, B. M. (2005). The dual burden household and the nutrition transition paradox. *Int J Obes (Lond), 29*(1), 129-136.

Doak, C. M., Adair, L. S., Monteiro, C., & Popkin, B. M. (2000). Overweight and underweight coexist within households in Brazil, China and Russia. *J Nutr, 130*(12), 2965-2971.

Fowden, A. L., Giussani, D. A., & Forhead, A. J. (2006). Intrauterine programming of physiological systems: causes and consequences. *Physiology (Bethesda), 21*, 29-37.

Fraser, G. E. (1999). Associations between diet and cancer, ischemic heart disease, and all-cause mortality in non-Hispanic white California Seventh-day Adventists. *Am J Clin Nutr, 70*(3 Suppl), 532S-538S.

Gallagher, D., Visser, M., Sepulveda, D., Pierson, R. N., Harris, T., & Heymsfield, S. B. (1996). How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am J Epidemiol, 143*(3), 228-239.

Garn, S. M., Leonard, W. R., & Hawthorne, V. M. (1986). Three limitations of the body mass index. *Am J Clin Nutr, 44*(6), 996-997.

Geisel, J., Hubner, U., Bodis, M., Schorr, H., Knapp, J. P., Obeid, R., et al. (2003). The role of genetic factors in the development of hyperhomocysteinemia. *Clin Chem Lab Med, 41*(11), 1427-1434.

Gibson, R. S. (2005). *Principles of Nutritional Assessment* (Second ed. Vol. 5): Oxford University Press, Inc.

Goh, V. H., Tain, C. F., Tong, T. Y., Mok, H. P., & Wong, M. T. (2004). Are BMI and other anthropometric measures appropriate as indices for obesity? A study in an Asian population. *J Lipid Res, 45*(10), 1892-1898.

Gopalan, S. (2000). Malnutrition: causes, consequences, and solutions. *Nutrition, 16*(7-8), 556-558.

Grant, C. C., Wall, C. R., Wilson, C., & Taua, N. (2003). Risk factors for iron deficiency in a hospitalized urban New Zealand population. *J Paediatr Child Health, 39*(2), 100-106.

Guerra-Shinohara, E. M., Paiva, A. A., Rondo, P. H., Yamasaki, K., Terzi, C. A., & D'Almeida, V. (2002). Relationship between total homocysteine and folate levels in pregnant women and their newborn babies according to maternal serum levels of vitamin B12. *Bjog, 109*(7), 784-791.

Harding, A. H., Sargeant, L. A., Welch, A., Oakes, S., Luben, R. N., Bingham, S., et al. (2001). Fat consumption and HbA(1c) levels: the EPIC-Norfolk study. *Diabetes Care, 24*(11), 1911-1916.

Harman, S. K., & Parnell, W. R. (1998). The nutritional health of New Zealand vegetarian and non-vegetarian Seventh-day Adventists: selected vitamin, mineral and lipid levels. *N Z Med J, 111*(1062), 91-94.

Hawkes, C., Eckhardt, C., Ruel, M., & Minot, N. (2004). Diet quality, poverty and food policy: a new research agenda for obesity prevention in developing countries. In *United Nations System. Standing Committee on Nutrition. SCN News* (Vol. 29, pp. 20-22).

Herbert, V. (1994). Staging vitamin B-12(cobalamin) status in vegetarians. *Am J Clin Nutr, 59(suppl)*, 1213S-1222S.

Herbert, V., Fong, W., Gulle, V., & Stopler, T. (1990). Low holotranscobalamin II is the earliest serum marker for subnormal vitamin B12 (cobalamin) absorption in patients with AIDS. *Am J Hematol, 34*(2), 132-139.

Herrmann, W., Obeid, R., Schorr, H., & Geisel, J. (2005). The usefulness of holotranscobalamin in predicting vitamin B12 status in different clinical settings. *Curr Drug Metab, 6*(1), 47-53.

Herrmann, W., Schorr, H., Geisel, J., & Riegel, W. (2001). Homocysteine, cystathionine, methylmalonic acid and B-vitamins in patients with renal disease. *Clin Chem Lab Med, 39*(8), 739-746.

Herrmann, W., Schorr, H., Obeid, R., & Geisel, J. (2003a). Vitamin B-12 status, particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia in vegetarians. *Am J Clin Nutr, 78*, 131-136.

Herrmann, W., Schorr, H., Obeid, R., & Geisel, J. (2003b). Vitamin B-12 status, particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia in vegetarians. . *Am J Clin Nutr, 78*, 131-136.

Hu, F. B., Manson, J. E., Stampfer, M. J., Colditz, G., Liu, S., Solomon, C. G., et al. (2001). Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med, 345*(11), 790-797.

India 1998-99: results from the National Family Health Survey. (2001). *Stud Fam Plann, 32*(3), 270-275.

International Diabetes Federation. (2005). *The IDF consensus worldwide definition of the metabolic syndrome.*: International Diabetes Federation.

Jayanthi, V. (2001). Vegetarianism in India. *Perit Dial Int, 21 Suppl 3*, S322-325.

Joosten, E., Lesaffre, E., & Riezler, R. (1996). Are different reference intervals for methylmalonic acid and total homocysteine necessary in elderly people? *Eur J Haematol, 57*(3), 222-226.

Kennedy, E. T., Bowman, S. A., Spence, J. T., Freedman, M., & King, J. (2001). Popular diets: correlation to health, nutrition, and obesity. *J Am Diet Assoc, 101*(4), 411-420.

Key, T. J., Davey, G. K., & Appleby, P. N. (1999). Health benefits of a vegetarian diet. *Proc Nutr Soc, 58*(2), 271-275.

King, H. A. (2002). *Implementating the New Zealand Health Strategy 2002*. Wellington: Ministry of Health.

Kipnis, V., Midthune, D., Freedman, L., Bingham, S., Day, N. E., Riboli, E., et al. (2002). Bias in dietary-report instruments and its implications for nutritional epidemiology. *Public Health Nutr, 5*(6A), 915-923.

Leblanc, J. C., Yoon, H., Kombadjian, A., & Verger, P. (2000). Nutritional intakes of vegetarian populations in France. *Eur J Clin Nutr, 54*(5), 443-449.

Lindenbaum, J., Savage, D. G., Stabler, S. P., & Allen, R. H. (1990). Diagnosis of cobalamin deficiency: II. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. *Am J Hematol, 34*(2), 99-107.

Lindgren, A., Kilander, A., Bagge, E., & Nexo, E. (1999). Holotranscobalamin - a sensitive marker of cobalamin malabsorption. *Eur J Clin Invest, 29*(4), 321-329.

Livingstone, M. B., & Robson, P. J. (2000). Measurement of dietary intake in children. *Proc Nutr Soc, 59*(2), 279-293.

Lukaski, H. C. (1987). Methods for the assessment of human body composition: traditional and new. *Am J Clin Nutr, 46*(4), 537-556.

Lukaski, H. C., Bolonchuk, W. W., Hall, C. B., & Siders, W. A. (1986). Validation of tetrapolar bioelectrical impedance method to assess human body composition. *J Appl Physiol, 60*(4), 1327-1332.

Lukaski, H. C., Johnson, P. E., Bolonchuk, W. W., & Lykken, G. I. (1985). Assessment of fat-free mass using bioelectrical impedance measurements of the human body. *Am J Clin Nutr, 41*(4), 810-817.

Margetts, B. (2006). Nutrition, public health, and the new nutrition science: academic thought, professional action. *Public Health Nutr, 9*(4), 407-410.

Mason, J. B. (2003). Biomarkers of nutrient exposure and status in one-carbon (methyl) metabolism. *J Nutr, 133 Suppl 3*, 941S-947S.

Maxwell, R. A., Sweet, R. A., Mulsant, B. H., Rosen, J., Kirshner, M. A., Kastango, K. B., et al. (2002). Risperidone and 9-hydroxyrisperidone concentrations are not dependent on age or creatinine clearance among elderly subjects. *J Geriatr Psychiatry Neurol, 15*(2), 77-81.

McGarry, J. D. (1995). The mitochondrial carnitine palmitoyltransferase system: its broadening role in fuel homoeostasis and new insights into its molecular features. *Biochem Soc Trans, 23*(2), 321-324.

Metz, J. (1992). Cobalamin deficiency and the pathogenesis of nervous system disease. *Annu Rev Nutr, 12*, 59-79.

Ministry of Health. (1997). *Planning for pregnancy. Folic acid and spina bifida.* Wellington: Ministry of Health.

Ministry of Health. (2003a). *DHB Toolkit: Physical Activity*. Wellington: Ministry of Health.

Ministry of Health. (2003b). *Sexual and Reproductive Health: A resource book for New Zealand health care organisations.* . Wellington Ministry of Health.

Ministry of Health. (2005). *Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes.* Canberra: Ministry of Health.

Misra, A., & Vikram, N. K. (2003). Clinical and pathophysiological consequences of abdominal adiposity and abdominal adipose tissue depots. *Nutrition, 19*(5), 457-466.

Morris, M. C., Evans, D. A., Bienias, J. L., Tangney, C. C., Hebert, L. E., Scherr, P. A., et al. (2005). Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. *Arch Neurol, 62*(4), 641-645.

Morris, M. S., Jacques, P. F., Rosenberg, I. H., & Selhub, J. (2007). Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr, 85*(1), 193-200.

Moy, K. L. (2005). *Physical activity and fitness measures in New Zealand: a study of validation and correlation with cardiovascular risk factors.* The University of Auckland, Auckland. http://researchspace.itss.auckland.ac.nz/bitstream/2292/305/9/02whole.pdf

Narayanan, M. N., Dawson, D. W., & Lewis, M. J. (1991). Dietary deficiency of vitamin B12 is associated with low serum cobalamin levels in non-vegetarians. *Eur J Haematol, 47*(2), 115-118.

Nelson, M., Black, A. E., Morris, J. A., & Cole, T. J. (1989). Between- and within-subject variation in nutrient intake from infancy to old age: estimating the number of days required to rank dietary intakes with desired precision. *Am J Clin Nutr, 50*(1), 155-167.

New Zealand Food Safety Authority. *Folate, folic acid and fortification*. Retrieved 28/05, 2008, from http://www.nzfsa.govt.nz/consumers/chemicals-toxins-additives/folate/index.htm

Oakes, N. D., Bell, K. S., Furler, S. M., Camilleri, S., Saha, A. K., Ruderman, N. B., et al. (1997). Diet-induced muscle insulin resistance in rats is ameliorated by

acute dietary lipid withdrawal or a single bout of exercise: parallel relationship between insulin stimulation of glucose uptake and suppression of long-chain fatty acyl-CoA. *Diabetes, 46*(12), 2022-2028.

Oliver, M. F. (1997). It is more important to increase the intake of unsaturated fats than to decrease the intake of saturated fats: evidence from clinical trials relating to ischemic heart disease. *Am J Clin Nutr, 66*(4 Suppl), 980S-986S.

Pan, D. A., Lillioja, S., Kriketos, A. D., Milner, M. R., Baur, L. A., Bogardus, C., et al. (1997). Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes, 46*(6), 983-988.

Parnell, W., Scragg, R., Wilson, N., Schaaf, D., & Fitzgerald, E. (2003). *NZ Food NZ Children: Key results of the 2002 National Children's Nutrition Survey*. Wellington: Ministry of Health.

Peat, J., & Barton, B. (2005). *Medical Statistics: A Guide to Data Analysis and Critical Appraisal*: Blackwell Publishing.

Popkin, B. M. (2002). An overview on the nutrition transition and its health implications: the Bellagio meeting. *Public Health Nutr, 5*(1A), 93-103.

Popkin, B. M., & Gordon-Larsen, P. (2004). The nutrition transition: worldwide obesity dynamics and their determinants. *Int J Obes Relat Metab Disord, 28 Suppl 3*, S2-9.

Popkin, B. M., Horton, S., Kim, S., Mahal, A., & Shuigao, J. (2001). Trends in diet, nutritional status, and diet-related noncommunicable diseases in China and India: the economic costs of the nutrition transition. *Nutr Rev, 59*(12), 379-390.

Popkin, B. M., Richards, M. K., & Montiero, C. A. (1996). Stunting is associated with overweight in children of four nations that are undergoing the nutrition transition. *J Nutr, 126*(12), 3009-3016.

Public Health Commission. (1995). *Low dose folic acid tablets to prevent spina bifida.* Wellington: Public Health Commission.

Quigley, R., & Watts, C. (1997). *Food Comes First: Methodologies for the National Nutrition Survey of New Zealand*. Wellington: Ministry of Health.

Quinlivan, E. P., McPartlin, J., McNulty, H., Ward, M., Strain, J. J., Weir, D. G., et al. (2002). Importance of both folic acid and vitamin B12 in reduction of risk of vascular disease. *Lancet, 359*(9302), 227-228.

Rasmussen, K., Vyberg, B., Pedersen, K. O., & Brochner-Mortensen, J. (1990). Methylmalonic acid in renal insufficiency: evidence of accumulation and implications for diagnosis of cobalamin deficiency. *Clin Chem, 36*(8 Pt 1), 1523-1524.

Ravelli, A. C., van der Meulen, J. H., Michels, R. P., Osmond, C., Barker, D. J., Hales, C. N., et al. (1998). Glucose tolerance in adults after prenatal exposure to famine. *Lancet, 351*(9097), 173-177.

Ray, J. G., Wyatt, P. R., Thompson, M. D., Vermeulen, M. J., Meier, C., Wong, P. Y., et al. (2007). Vitamin B12 and the risk of neural tube defects in a folic-acid-fortified population. *Epidemiology, 18*(3), 362-366.

Refsum, H. (2001). Folate, vitamin B12 and homocysteine in relation to birth defects and pregnancy outcome. *British Journal of Nutrition, 85*(Suppl.2), S109-S113.

Refsum, H., Yajnik, C. S., Gadkari, M., Schneede, J., Vollset, S. E., Orning, L., et al. (2001). Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. *Am J Clin Nutr, 74*(2), 233-241.

Ruderman, N. B., Saha, A. K., Vavvas, D., & Witters, L. A. (1999). Malonyl-CoA, fuel sensing, and insulin resistance. *Am J Physiol, 276*(1 Pt 1), E1-E18.

Rush, D. (1994). Periconceptional folate and neural tube defect. *Am J Clin Nutr, 59*, 511S-516S.

Rush, E. C., Chandu, V., & Plank, L. D. (2006). Prediction of fat-free mass by bioimpedance analysis in migrant Asian Indian men and women: a cross validation study. *Int J Obes (Lond), 30*(7), 1125-1131.

Rush, E. C., Crowley, J., Freitas, I. F., & Luke, A. (2006). Validity of hand-to-foot measurement of bioimpedance: standing compared with lying position. *Obesity (Silver Spring), 14*(2), 252-257.

Rush, E. C., Plank, L. D., Laulu, M., Mitchelson, E., & Coward, W. A. (2004). Accuracy of dietary energy reporting in young New Zealand men and women: relationships to body composition, physical activity level and ethnicity. *International Journal of Body Composition Research, 2*(3), 125-130.

Rush, E. C., Plank, L. D., Laulu, M. S., & Robinson, S. M. (1997). Prediction of percentage body fat from anthropometric measurements: comparison of New Zealand European and Polynesian young women. *Am J Clin Nutr, 66*(1), 2-7.

Rush, E. C., Valencia, M., & Plank, L. D. (2008). Validation of a 7-day physical activity diary against doubly labelled water. *Annals of Human Biology, in press*.

Russell, D., Parnell, W., & Wilson, N. (1999). *NZ Food: NZ People. Key results of the 1997 National Nutrition Survey*. Wellington: Ministry of Health.

Schofield, W. N. (1985). Predicting basal metabolic rate, new standards and review of previous work. *Hum Nutr Clin Nutr, 39 Suppl 1*, 5-41.

Scott, J. M. (1999). Folate and vitmin B12. *Proceedings of the Nutrition Society, 58*, 441-448.

Seidell, J. C., Perusse, L., Despres, J. P., & Bouchard, C. (2001). Waist and hip circumferences have independent and opposite effects on cardiovascular disease risk factors: the Quebec Family Study. *Am J Clin Nutr, 74*(3), 315-321.

Smith, A. D., Kim, Y. I., & Refsum, H. (2008). Is folic acid good for everyone? *Am J Clin Nutr, 87*(3), 517-533.

Spencer, E. A., Appleby, P. N., Davey, G. K., & Key, T. J. (2003). Diet and body mass index in 38000 EPIC-Oxford meat-eaters, fish-eaters, vegetarians and vegans. *Int J Obes Relat Metab Disord, 27*(6), 728-734.

Stabler, S. P., & Allen, R. H. (2004). Vitamin B12 deficiency as a worldwide problem. *Annu Rev Nutr, 24*, 299-326.

Uauy, R., & Solomons, N. W. (2006). The role of the international community: forging a common agenda in tackling the double burden of malnutrition. In Moreira, A. D. (Ed.), *United Nations System, Standing Committee on Nutrition, SCN News* (Vol. 32, pp. 24-37): Standing Committe on Nutrition.

US Food and Drug Administration, & Health and Human. (1996). Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid (final rule-21 CFR Part 101). *Fed Reg 61*, 9791-9797.

Van den Veyver, I. B. (2002). Genetic effects of methylation diets. *Annu Rev Nutr, 22*, 255-282.

Venn, B. J., Green, T. J., Moser, R., McKenzie, J. E., Skeaff, C. M., & Mann, J. (2002). Increases in blood folate indices are similar in women of childbearing age supplemented with [6S]-5-methyltetrahydrofolate and folic acid. *J Nutr, 132*(11), 3353-3355.

Volkov, I., Rudoy, I., Machagna, M., Glezer, I., Ganel, U., Orenshtein, A., et al. (2007). Modern society and prospects of low vitamin B12 intake. *Ann Nutr Metab, 51*(5), 468-470.

Wakil, S. J., Stoops, J. K., & Joshi, V. C. (1983). Fatty acid synthesis and its regulation. *Annu Rev Biochem, 52*, 537-579.

Weinbrenner, T., Schroder, H., Escurriol, V., Fito, M., Elosua, R., Vila, J., et al. (2006). Circulating oxidized LDL is associated with increased waist circumference independent of body mass index in men and women. *Am J Clin Nutr, 83*(1), 30-35; quiz 181-182.

Weir, D. G., Keating, S., Molloy, A., McPartlin, J., Kennedy, S., Blanchflower, J., et al. (1988). Methylation deficiency causes vitamin B12-associated neuropathy in the pig. *J Neurochem, 51*(6), 1949-1952.

Westerterp, K. R., & Goris, A. H. (2002). Validity of the assessment of dietary intake: problems of misreporting. *Curr Opin Clin Nutr Metab Care, 5*(5), 489-493.

Wolfe, R. R. (2006). The underappreciated role of muscle in health and disease. *Am J Clin Nutr, 84*(3), 475-482.

Woods, M. N., Tang, A. M., Forrester, J., Jones, C., Hendricks, K., Ding, B., et al. (2003). Effect of dietary intake and protease inhibitors on serum vitamin B12 levels in a cohort of human immunodeficiency virus-positive patients. *Clin Infect Dis, 37 Suppl 2*, S124-131.

World Health Organisation. (2000). Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser, 894*, i-xii, 1-253.

World Health Organisation. (2003). Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser, 916*, i-viii, 1-149, backcover.

World Health Organisation. (2004). Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet, 363*(9403), 157-163.

Yajnik, C. S., Deshpande, S. S., Jackson, A. A., Refsum, H., Rao, S., Fisher, D. J., et al. (2008). Vitamin B(12) and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia, 51*(1), 29-38.

Yajnik, C. S., Deshpande, S. S., Lubree, H. G., Naik, S. S., Bhat, D. S., Uradey, B. S., et al. (2006). Vitamin B12 deficiency and hyperhomocysteinemia in rural and urban Indians. *J Assoc Physicians India, 54*, 775-782.

Yusuf, S., Hawken, S., Ounpuu, S., Bautista, L., Franzosi, M. G., Commerford, P., et al. (2005). Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. *Lancet, 366*(9497), 1640-1649.

APPENDICES

Appendix 1: Advertisement

Volunteers Required

40 healthy women of childbearing age (18-50 years old)

We are looking for volunteers to assist us in a study on ***Validation of reported dietary vitamin B12 in women of childbearing age.***

There is a controversial issue about whether a vitamin B12 supplement should be taken with folic acid to avoid imbalances during the perinatal period. This research will provide initial/pilot data on dietary vitamin B12 intake and blood vitamin B12 status in New Zealand women of childbearing age.

In this research we will measure your body size, blood pressure, and sample your blood to check vitamin B12 and folate, serum glucose and lipid profile, and blood count. You will be asked questions and asked to complete a one week diary about your diet and activity levels. You will be given a copy of all your blood measurements.

If you would like to have more information and/or take part in the study please phone:

Liping (Sunnie) Xin

921 9999 ext 7119

021 995516

E-mail: sunnie.xin@aut.ac.nz

Appendix 2: Ethical approval

MEMORANDUM

Auckland University of Technology Ethics Committee (AUTEC)

To: Elaine Rush
From: **Madeline Banda** Executive Secretary, AUTEC
Date: 13 July 2007
Subject: Ethics Application Number 07/65 **Validation of reported dietary vitamin B12 in child bearing age women.**

Dear Elaine

I am pleased to advise that the Auckland University of Technology Ethics Committee (AUTEC) approved your ethics application at their meeting on 9 July 2007. Your application is now approved for a period of three years until 9 July 2010.

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

- A brief annual progress report indicating compliance with the ethical approval given using form EA2, which is available online through *http://www.aut.ac.nz/about/ethics*, including when necessary a request for extension of the approval one month prior to its expiry on 9 July 2010;
- 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 9 July 2010 or on completion of the project, whichever comes sooner;

It is also a condition of approval that AUTEC is notified of any adverse events or if the research does not commence and that AUTEC approval is sought for any alteration to the research, including any alteration of or addition to the participant documents involved.

You are reminded that, as applicant, you are responsible for ensuring that any research undertaken under this approval is carried out within the parameters approved for your application. Any change to the research outside the parameters of this approval must be submitted to AUTEC for approval before that change is implemented.

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.

To enable us to provide you with efficient service, we ask that you use the application number and study title in all written and verbal correspondence with us. 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 Committee 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: Liping Xin njr5912@aut.ac.nz;

Appendix 3: Participant information sheet

Participant Information Sheet

Date Information Sheet Produced:

25 June 2007

Project Title

Validation of reported dietary vitamin B12 in women of childbearing age.

An Invitation

You are invited to participate in a study that investigates dietary vitamin B12 intake of New Zealand women of childbearing age. Your involvement in this study is voluntary, and it is your choice as to whether or not you wish to participate. An interpreter is available on your request.

What is the purpose of this research?

There is a controversial issue about whether a vitamin B12 supplement should be taken with folic acid to avoid imbalances during the perinatal period. This research will provide initial/pilot data on dietary vitamin B12 intake and blood vitamin B12 status in New Zealand women of childbearing age.

The participants will benefit by being screened for anaemia, lipid profile, blood pressure, blood glucose levels, folate and vitamin B12 status. They will also receive copies of all their measurements and clinical results.

The information from this research will be used by Liping XIN to obtain an academic qualification (Master of Philosophy) from the Auckland University of Technology.

How was I chosen for this invitation?

The study will need 40 healthy New Zealand women of childbearing age, who will be recruited within AUT University North Shore campus. Exclusion criteria are: women with chronic disease or major health conditions, malabsorption diseases, physically disabled and pregnant women. Liping (Sunnie) XIN, a Master of Philosophy student in Auckland University of Technology, is responsible for the recruitment of participants.

What will happen in this research? What are the discomforts and risks? How will these discomforts and risks be alleviated?

All measurements and the blood collection will be conducted at the AUT Akoranga Health Clinic (RoomAS104). The visit will take about 1 hour. We will arrange these visits in the mornings and you will be asked not to eat or drink anything except water for at least 8 hours before the tests. First, Liping (Sunnie) XIN will explain the outline of the study to you. And your

height and weight will be measured followed by measurements of grip strength, and your waist, hip, mid upper arm circumferences and blood pressure. To estimate the water content of your body a tiny current, which you cannot feel, will be passed between your hand and your foot for a few seconds. You will be asked questions about what you ate and drank over the previous day. You will be shown how to measure and record what you eat and drink to complete a 7 day diet and physical activity diary. A quick questionnaire about your personal information, such as age, number of children if any, ethnic group, list of food not eat, taking supplement or not, and your menstruation conditions, will be filled in by yourself.

Finally, a fasting blood sample will be taken by Liping (Sunnie) XIN, to look at your level of vitamin B12 and folate, serum glucose and lipid profile, and blood count. This will involve a needle being placed in your arm and the equivalent of two tablespoons of blood removed. The process will be brief, and will not be harmful for your health. There may be some discomfort when blood is sampled. However, this will be collected by an experienced phlebotomist. The results of these tests will be given to you with a recommendation to visit your doctor if the levels are outside the reference range.

What are the benefits?

This research may help you become more aware of your vitamin B12 and folate levels and the importance of these two nutrients to you and potential offspring when preparing for a pregnancy and during pregnancy. In addition you will benefit by being screened for anaemia, lipid profile, blood pressure, blood glucose and full blood count.

What compensation is available for injury or negligence?

Compensation is available through the Accident Compensation Corporation within its normal limitations. If you have any questions about ACC, please contact the nearest ACC office.

How will my privacy be protected?

No material that could personally identify you will be used in any reports on this study. During and following the study, records will be held in filing cabinets in locked areas of the Department of Faculty of Health and Environmental Science, Auckland University of Technology with access only by authorized investigators.

What are the costs of participating in this research?

In this research, body composition measurements, health screening results and blood tests are free to all participants. Furthermore, we will provide each participant with a book named “Eat Less, Move More” (Elaine Rush), which includes lots of information and advice about diet and activity.

What opportunity do I have to consider this invitation?

You may like to think about this for a day or two before you make a decision.

You do not have to take part in this study. Should you choose not to take part this will not affect any future care or treatment or your academic progress if you are a student. If you do agree to take part you are free to withdraw from the study at any time, without having to give a reason and this will in no way affect your future health care and / or academic progress. If you have any queries or concerns regarding your rights as a participant in this study you may wish to contact a Health and Disability Advocate, telephone 0800 555 050 for Northland to Franklin.

How do I agree to participate in this research?

Please complete the attached consent form.

Will I receive feedback on the results of this research?

A copy of the body composition result will be available to each participant immediately after the completion of the measurements. And the copy of blood test results will be given to you when they come out. The overall results of this study can be made available to you at your request.

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, Ph 921 9999 ext 8091 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?

Please feel free to contact the following researchers if you have any questions about this study.

Researcher Contact Details:

Professor Elaine Rush, Ph 921 9999 ext 8091 elaine.rush@aut.ac.nz

Patricia Lucas, Ph 921 9999 ext 7134 patricia.lucas@aut.ac.nz

Nicola Power, Ph 921 9999 ext 7319 nicola.power@aut.ac.nz

Liping (Sunnie) Xin, Ph 921 9999 ext 7119 or 021 995516 sunnie.xin@aut.ac.nz

Project Supervisor Contact Details:

Professor Elaine Rush, Ph 921 9999 ext 8091 elaine.rush@aut.ac.nz.

Approved by the Auckland University of Technology Ethics Committee on 13/07/2007, AUTEC Reference number 07/65.

Appendix 4: 24-hour diet recall

Validation of Reported B_{12} Intake in Women of Childbearing Age Study

24 HOUR RECALL

Subject No. _____

Date _____

B→MT→L→AT→D

Time	Food Description	Amount	Check(cooking,methods,brands)

Appendix 5: 7-day diet diary

Please start each day on a new page

Day Date

Time	Type of Food/Drink	Amount	Time	Type of Food/Drink	Amount

Appendix 6: 7-day physical activity diary

ACTIVITY DIARY

Name/ID number

Day **Date**

Write in the empty squares the factor which corresponds best to the main activity of each 15 min period. If not sure write in the empty squares a alphabetical code and write this code together with a description of your activity in a few words in the section for notes below.

Notes

	minutes			
Hour	**0-15**	**16-30**	**31-45**	**46-60**
midnight				
1:00 a.m.				
2:00 a.m.				
3:00 a.m.				
4:00 a.m.				
5:00 a.m.				
6:00 a.m.				
7:00 a.m.				
8:00 a.m.				
9:00 a.m.				
10:00 a.m.				
11:00 a.m.				
12:00 a.m.				
1:00 p.m.				
2:00 p.m.				
3:00 p.m.				
4:00 p.m.				
5:00 p.m.				
6:00 p.m.				
7:00 p.m.				
8:00 p.m.				
9:00 p.m.				
10:00 p.m.				
11:00 p.m.				

Activity Factor	**Examples of Activities**
1	Sleeping, resting in bed
2	Sitting, eating, writing, listening, sitting in a car or bus, watching TV etc
3	Standing, washing
4	Walking indoors, light home activities
5	Walking outdoors, light work - e.g. carrying small bag
6	Leisure activities, sports, running and relaxed movement i.e. light intensity
7	Leisure activities, sports and manual work of moderate intensity
8	Leisure activities, sports and manual work of high intensity - sweating and breathing hard
9	Sports activities and work of very high to maximal intensity, competitive running

Appendix 7: Physical characteristics excluding on outlier (n=37)

	Reference value	Total N=37	Indians N=5	Non-Indians N=32	*p value	Effect size	Non-red-meat-eaters N=11	Red-meat-eaters N=26	*p value	Effect size
Age (y)		31 ± 8 (19 - 48)	28 ± 4 (24 – 33)	31 ± 8 (19 - 48)	0.25		33 ± 5 (25 – 42)	30 ± 8 (19 – 48)	0.20	
Height (cm)		164.6 ± 7.0 (149.6 – 186.0)	157.8 ± 6.2 (149.6 – 164.9)	165.6 ± 6.6 (155.5 – 186.0)	0.04	-1.22	163.7 ± 6.2 (149.6 – 171.1)	165.0 ± 7.4 (153.8 – 186.0)	0.60	-0.19
Weight (kg)	61.0†	62.2 ± 11 (42.9 – 88.9)	60.3 ± 10.6 (48.6 – 74.9)	62.6 ± 11.2 (42.9 – 88.9)	0.68	-0.21	61.8 ± 7.5 (50.9 – 74.9)	62.4 ± 12.3 (42.9 – 88.9)	0.86	-0.06
BMI (kg/m^2)	22.0†	22.9 ± 3.6 (16.5 – 31.1)	24.2 ± 3.6 (20.4 – 27.7)	22.8 ± 3.6 (16.5 – 31.1)	0.45	0.39	23.1 ± 3.2 (19.1 – 27.7)	22.9 ± 3.8 (16.5 – 31.1)	0.81	0.06
Waist circumference (cm)	<80‡	77.4 ± 8.3 (63.2 – 92.3)	82.4 ± 10.3 (68.4 – 92.3)	76.6 ± 7.9 (63.2 – 92.0)	0.29	0.63	80.2 ± 8.8 (66.5 – 92.3)	76.2 ± 8.0 (63.2 – 92.0)	0.21	0.48
Hip circumference (cm)		100.8 ± 8.2 (85.2 – 118.4)	100.1 ± 7.2 (90.9 – 107.5)	100.9 ± 8.4 (85.2 – 118.4)	0.82	-0.10	101.5 ± 6.5 (91.6 – 113.0)	100.5 ± 8.9 (85.2 – 118.4)	0.70	0.13
WHR	<0.85ˆ	0.77 ± 0.05 (0.68 – 0.87)	0.82 ± 0.05 (0.75 – 0.87)	0.76 ± 0.04 (0.68 – 0.84)	0.04	1.33	0.79 ± 0.05 (0.68 – 0.87)	0.76 ± 0.04 (0.69 – 0.84)	0.12	0.66
MUAC (cm)		28.9 ± 3.3 (20.2 – 35.2).	30.2 ± 3.1 (26.5 – 33.5)	28.7 ± 3.3 (20.2 – 35.2)	0.37	0.47	29.5 ± 2.6 (25.0 – 33.5)	28.7 ± 3.5 (20.2 – 35.2)	0.42	0.26
FM (kg)		17.6 ± 7.4 (6.3 – 34.9)	22.6 ± 6.9 (14.1 – 30.0)	16.8 ± 7.2 (6.3 – 34.9)	0.14	0.82	19.7 ± 6.9 (10.1 – 30.0)	16.7 ± 7.5 (6.3 – 34.9)	0.24	0.42
FFM (kg)		44.7 ± 6.9 (30.7 – 64.3)	37.7 ± 5.9 (31.0 – 44.9)	45.8 ± 6.4 (30.7 – 64.3)	0.03	-1.32	42.1 ± 4.1 (33.1 – 46.8)	45.7 ± 7.5 (30.7 – 64.3)	0.07	-0.60
BF%	<35%°	27.5 ± 8.3 (12.2 – 46.7)	37.0 ± 7.1 (27.5 – 46.7)	26.0 ± 7.5 (12.2 – 44.2)	0.02	1.51	31.3 ± 8.3 (19.8 – 46.7)	25.9 ± 7.9 (12.2 – 44.2)	0.09	0.67

Mean ± SD; Range in parenthesis; *p value calculated using independent unequal variance t test; BMI, Body mass index; WHR, waist to hip ratio; MUAC, mid upper arm circumference; FM, Fat mass; FFM, Fat free mass; BF%, Body fat percentage; †Reference from Ministry of Health (2005); ‡Reference range from International Diabetes Federation (2005); ˆReference from WHO (2000), °Reference value obtained from Goh et al. (2004).

Appendix 8: Associations among measurements of participants

Table 8.1: Selected significant correlations of BMI

Parameter	r* (95% CI)	p value
Waist circumference (cm)	0.94 (0.89, 0.97)	<0.001
Hip circumference (cm)	0.92 (0.85, 0.96)	<0.001
WHR	0.55 (0.28, 0.74)	<0.001
MUAC (cm)	0.96 (0.92, 0.98)	<0.001
FFM (kg)	0.64 (0.40, 0.80)	<0.001
FM (kg)	0.94 (0.89, 0.97)	<0.001
BF%	0.73 (0.54, 0.85)	<0.001
Diastolic blood pressure (mmHg)	0.34 (0.02,0.60)	0.04
Serum B_{12} (pmol/L)	-0.34 (-0.60, -0.02)	0.04
HDL cholesterol (mmol/L)	-0.40 (-0.64, -0.09)	0.01
Total/HDL ratio	0.43 (0.13, 0.66)	0.01

*r, Pearson's correlation co-efficient calculated using bivariate correlation, followed by 95% confidence intervals in bracket; correlations significant at $p<0.05$; BMI, Body mass index; WHR, Waist to Hip Ratio; MUAC, Mid Upper Arm Circumference; FFM, fat free mass; FM, fat mass; BF%, body fat percentage; HDL cholesterol, high-density lipoprotein cholesterol.

Table 8.2: Selected significant correlations of grip strength

Parameter	r* (95% CI)	p value
Left hand (kg)		
Height (cm)	0.38 (0.07, 0.62)	0.02
WHR	-0.54 (-0.73, -0.27)	<0.001
FM (kg)	-0.34 (-0.60, -0.02)	0.04
BF%	-0.49 (-0.70, -0.20)	0.02
Pulse (bpm)	-0.34 (-0.60, -0.02)	0.04
RBC (10E12/L)	-0.34 (-0.60, -0.02)	0.04
Right Hand (kg)		
Height (cm)	0.41 (0.10, 0.65)	0.01
WHR	-0.48 (-0.69, -0.19)	<0.005
FFM (kg)	0.38 (0.07, 0.62)	0.02
BF%	-0.52 (-0.72, -0.24)	<0.001
Pulse (bpm)	-0.48 (-0.69, -0.19)	<0.005

*r, Pearson's correlation co-efficient calculated using bivariate correlation, followed by 95% confidence intervals in bracket; correlations significant at p<0.05; WHR, Waist to Hip Ratio; FM, fat mass; BF%, body fat percentage; RBC, Red Blood Cell Count; FFM, fat free mass.

Table 8.3: Selected significant correlations of WHR

Parameter	r* (95% CI)	p value
MUAC (cm)	0.55 (0.28, 0.74)	<0.001
FM (kg)	0.62 (0.38, 0.78)	<0.001
BF%	0.68 (0.46, 0.82)	<0.001
Diastolic blood pressure (mmHg)	0.35 (0.03, 0.60)	0.03
Pulse (bpm)	0.44 (0.14, 0.67)	0.01
HDL cholesterol (mmol/L)	-0.48 (-0.69, -0.19)	<0.005
LDL cholesterol (mmol/L)	0.34 (0.02, 0.60)	0.04
Serum triglyceride (mmol/L)	0.33 (0.01, 0.59)	0.05
Total/HDL ratio	0.46 (0.17, 0.68)	<0.005

*r, Pearson's correlation co-efficient calculated using bivariate correlation, followed by 95% confidence intervals in bracket; correlations significant at p<0.05; WHR, Waist to Hip Ratio; MUAC, Mid Upper Arm Circumference; FM, fat mass; BF%, body fat percentage; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol.

Table 8.4: Selected significant correlations among other anthropometric variables

Parameter 1	Parameter 2	r* (95% CI)	p value
Weight (kg)	Diastolic blood pressure (mmHg)	0.41 (0.10, 0.65)	0.01
Height (cm)	Systolic blood pressure (mmHg)	0.34 (0.02, 0.60)	0.04
Waist circumference (cm)	Diastolic blood pressure (mmHg)	0.43 (0.13, 0.66)	0.01
Waist circumference (cm)	Pulse (bpm)	0.38 (0.07, 0.62)	0.02
Hip circumference (cm)	Diastolic blood pressure (mmHg)	0.36 (0.05, 0.61)	0.03
MUAC (cm)	BF%	0.70 (0.49, 0.83)	<0.001
MUAC (cm)	Diastolic blood pressure (mmHg)	0.40 (0.09, 0.64)	0.01
FFM (kg)	Systolic blood pressure (mmHg)	0.38 (0.07, 0.62)	0.02
FFM (kg)	Diastolic blood pressure (mmHg)	0.35 (0.03, 0.60)	0.03
FM (kg)	Diastolic blood pressure (mmHg)	0.37 (0.06, 0.62)	0.02
FM (kg)	Pulse (bpm)	0.39 (0.08, 0.63)	0.02
BF%	Pulse (bpm)	0.49 (0.20, 0.70)	<0.005

*r, Pearson's correlation co-efficient calculated using bivariate correlation, followed by 95% confidence intervals in bracket; correlations significant at $p<0.05$; MUAC, Mid Upper Arm Circumference; FFM, fat free mass; FM, fat mass; BF%, body fat percentage.

Table 8.5: Selected significant correlations of biomarkers

Parameter 1	Parameter 2	r* (95% CI)	p value
HDL cholesterol (mmol/L)	Weight (kg)	-0.35 (-0.60, -0.03)	0.03
HDL cholesterol (mmol/L)	Waist circumference (cm)	-0.44 (-0.67, -0.14)	0.01
HDL cholesterol (mmol/L)	MUAC (cm)	-0.37 (-0.62, -0.06)	0.02
HDL cholesterol (mmol/L)	FM (kg)	-0.45 (-0.67, -0.15)	<0.005
HDL cholesterol (mmol/L)	BF%	-0.47 (-0.69, -0.18)	<0.005
Haemoglobin (g/L)	LDL cholesterol (mmol/L)	0.37 (0.06, 0.62)	0.02
Haemoglobin (g/L)	Total/HDL ratio	0.36 (0.05, 0.61)	0.03
LDL cholesterol (mmol/L)	BF%	0.42 (0.12, 0.65)	0.01
Serum triglyceride (mmol/L)	Waist circumference (cm)	0.33 (0.01, 0.59)	0.04
Serum triglyceride (mmol/L)	MUAC (cm)	0.33 (0.01, 0.59)	0.04
Serum triglyceride (mmol/L)	BF%	0.36 (0.05, 0.61)	0.03
Serum triglyceride (mmol/L)	Serum folate (nmol/L)	-0.33 (-0.59, -0.01)	0.05
Total/HDL ratio	Weight (kg)	0.34 (0.02, 0.60)	0.04
Total/HDL ratio	Waist circumference (cm)	0.47 (0.18, 0.69)	<0.005
Total/HDL ratio	Hip circumference (cm)	0.35 (0.03, 0.60)	0.03
Total/HDL ratio	MUAC (cm)	0.44 (0.14, 0.67)	0.01
Total/HDL ratio	FM (kg)	0.49 (0.20, 0.70)	<0.005
Total/HDL ratio	BF%	0.57 (0.31, 0.75)	<0.001
Total/HDL ratio	Serum triglyceride (mmol/L)	0.43 (0.13, 0.66)	0.01
Total cholesterol (mmol/L)	Fasting glucose (mmol/L)	0.33 (0.01, 0.59)	0.04
Holo-TC II (pmol/L)	Fasting glucose (mmol/L)	0.35 (0.03, 0.60)	0.03

*r, Pearson's correlation co-efficient calculated using bivariate correlation, followed by 95% confidence intervals in bracket; correlations significant at $p<0.05$; HDL cholesterol, high-density lipoprotein cholesterol; MUAC, Mid Upper Arm Circumference; FM, fat mass; BF%, body fat percentage; LDL cholesterol, low-density lipoprotein cholesterol; Holo-TC II, holotranscobalamin II.

Table 8.6: Selected significant correlations of serum B_{12} concentration

Parameter	r* (95% CI)	p value
BMI	-0.34 (-0.60, -0.02)	0.04
Waist circumference (cm)	-0.38 (-0.62, -0.07)	0.02
WHR	-0.36 (-0.61, -0.05)	0.03
MUAC (cm)	-0.33 (-0.59, -0.01)	0.04
FM (kg)	-0.33 (-0.59, -0.01)	0.04
BF%	-0.35 (-0.60, -0.03)	0.03
Holo-TC II (pmol/L)	0.77 (0.60, 0.87)	<0.001

*r, Pearson's correlation co-efficient calculated using bivariate correlation, followed by 95% confidence intervals in bracket; correlations significant at p<0.05; BMI, Body Mass Index; WHR, Waist to Hip Ratio; MUAC, Mid Upper Arm Circumference; FM, fat mass; BF%, body fat percentage; Holo-TC II, holotranscobalamin II.

Table 8.7: Selected significant correlations of dietary analysis

Parameter 1	Parameter 2	r* (95% CI)	p value
%Energy from protein	Hip circumference (cm)	-0.33 (-0.59, -0.01)	0.05
Daily folate intake (µg)	Serum triglyceride (mmol/L)	0.37 (0.05, 0.62)	0.03
%Energy from fat	Haemoglobin (g/L)	0.38 (0.06, 0.63)	0.02
Daily B_{12} intake (µg)	%Energy from fat	0.36 (0.04, 0.61)	0.03
Daily B_{12} intake (µg)	%Energy from carbohydrate	-0.41 (-0.65, -0.10)	0.01
%Energy from saturated fat	Holo-TC II (pmol/L)	0.52 (0.24, 0.72)	<0.001

*r, Pearson's correlation co-efficient calculated using bivariate correlation, followed by 95% confidence intervals in bracket; correlations significant at p<0.05; Holo-TC II, holotranscobalamin II.