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Acknowledgements

This series of studies were only possible with the extensive contributions of many colleagues in the Chinese University of Hong Kong and many other people over a period of five years. Firstly, I would like to thank Professor Jean Woo, Department of Medicine & Therapeutics, the Chinese University of Hong Kong. She contributed greatly in the planning of the study and provided historical data for analysis. She also gave very valuable advice in the presentation of the thesis.

Professor Edith Lau and Professor Suzanne Ho, Department of Family and Community Medicine, the Chinese University of Hong Kong, advised on the planning of the main study. Edith also released her research assistants to assist in data collection, and provided historical data for analysis.

Professor Calvin Pang, formerly in the Chemical Pathology Department, developed the assays for serum and urinary Methylmalonic acid (MMA), with the help of his MPhil student, Lai Wai Kai. Mr Lai should be commended for his time and effort in developing and performing the MMA assays over a two-year period.

Professor Catherine Tang, the Department of Psychology, the Chinese University of Hong Kong, advised on the choice of neuropsychological tests, and provided three excellent psychology graduates in the administration of these tests.

Professor Woo Kam Sang, Department of Medicine & Therapeutics, contributed in the homocysteine study by providing homocysteine, vitamin B12 and folate assays, and providing normative data for comparison. Professor Thomas Chan, Department of Medicine & Therapeutics, gave valuable advice on the presentation of the thesis.
The following research assistants participated the study: Eliza Leung, Allie Chow, Martin Li, Ricky Lai, Mandy Kwan. Aprille Sham in the Department of Family & Community Medicine and Ashley Yu in the Clinical Epidemiology and Research Centre gave invaluable statistical advice. The vitamin B12 supplementation trial was supported by the Direct Grant from the Chinese University of Hong Kong.

Dr Charles Yu, Dental Officer in United Christian Hospital, Miss Hui Wai Hing in the Community Dietetic Centre performed dental examination and conducted dietary survey respectively in one of the studies.

I would like to thank my wife, Beckie, for all her support and encouragement. Last but not least, I would like to thank all the subjects who participated the studies. A special thank is due to the staff in Sin Tin Dao old age home, Shatin. They played a crucial part in the recruitment of subjects both within the home and in the community. I should also like to thank the staff in the following old age homes which participated the studies: Chi Lin Hostel and Care & attention home, Po Ching Hostel and Care & attention home (Fanling), Tung Wah Sam Hospital group care & attention home (Tsuen Wan), Cheuk Lam Temple (Tsuen Wan, Salvation Army Lun Hang old age Hostel (TaiWai), Li Chan Yuk Sim old age hostel (Shatin), Ho Shing old age hostel (Shatin), Lo Mun Huen old age hostel (Shatin), and Ho’s old age hostel (Shatin).
Summary of Thesis

"Health Status of Older Chinese Vegetarians"

Timothy Chi Yui Kwok

Introduction

Vegetarian diets are generally thought to be beneficial to health. But the Chinese vegetarian foods in Hong Kong are significantly different from those in Western countries and dietary problems are generally commoner in old age. There is therefore a need to examine the influence of the Chinese vegetarian diet on the health status of older people in Hong Kong.

Objectives

1. Assess the adequacy of nutritional intake of the older Chinese vegetarians in the community and in the old age homes, and their relationship with dental status.
2. Assess the nutritional status of the older vegetarians, as compared with local norms.
3. Compare the prevalence of a variety of chronic diseases, and bone mineral density of older vegetarians with those of local non-vegetarians of similar age.
4. To ascertain the prevalence of vitamin B12 deficiency among older vegetarians and to investigate its clinical significance.
Subjects and Methods

The main study examined multidimensional health status of 113 older Hong Kong Chinese women who adhered to a strict vegetarian diet for more than ten years for religious reasons (Taoism and Buddhism). The measurements included body mass index, triceps and biceps skinfold thicknesses and mid arm circumference, supine blood pressures, electrocardiograph (ECG), WHO cardiovascular questionnaire, 24-hour dietary recall, and bone densities by Dual energy X ray absorptiometry. Fasting blood samples were analyzed for methylmalonic acid (MMA), vitamin B12 and complete blood count, glucose, renal function test, cholesterol, triglyceride, total iron binding capacity, and albumin. Fasting urine samples were analyzed for sodium, potassium, creatinine and MMA. Omnivorous subjects in published local studies were used for comparison when available.

Two other cross sectional studies were subsequently performed. The first one examined the relationship between dental status of vegetarian old age home residents, as assessed by a registered dentist, and their dietary intakes as assessed by 24-hour food-weighing records made by research assistants. In the second, the plasma homocysteine concentrations of thirty-two young and middle-aged vegetarian women were compared with those of 32 age sex matched local omnivorous subjects.

In addition, residents in three other vegetarian old age homes were screened for metabolic vitamin B12 deficiency (serum MMA > or = 0.4 μmol/L) by urinary MMA in order to compare its prevalence with that in 133 omnivores in old age
The optimal cutoff value of urinary MMA to predict metabolic vitamin B12 deficiency was established by examining the serum and urinary MMA in vegetarian subjects who had normal renal function tests.

50 older subjects with low serum vitamin B12 concentration (< 120 pmol/L) were recruited from the vegetarian study subjects and medical patients completed a randomized controlled trial of the effect of parenteral vitamin B12 supplementation on their performance in a battery of neuropsychological tests: Mini Mental State Examination, Digit Span, Wechsler Memory Scale (revised)- Logical Memory and Visual Reproduction, Wechsler Adult Intelligence Scale (revised) - Similarities, Block Design, Motor function scale of the Adult Luria-Nebraska Neuropsychological Battery, and General Health Questionnaire.

Results

The older Chinese vegetarians had lower intakes of calories, fat, protein and B vitamins than omnivores. Their fibre intakes were higher, but were much lower than those in Western vegetarians. The vegetarian old age home residents had higher intakes of calcium and potassium than community dwelling vegetarians and omnivores, probably because greater consumption of milk based supplements. When compared with omnivorous old age home residents, vegetarian old age home residents were at greater risk of inadequate intakes of calories, protein, B vitamins, and were equally at risk of low calcium intake.

Old vegetarians were significantly leaner than omnivores (33.2 ±6.9 versus 34.9 ±7.0 % body fat). But 45.4% of them had body mass index greater than the
recommended 23 kg/m² for Chinese. Although they had similar anthropometric measurements of lean mass, i.e. arm muscle circumference and corrected arm muscle area, their average serum albumin was significantly lower than that of historical omnivorous control subjects (37.5±2.6 versus 42.0 ±3.0 g/L, p< 0.001, Student t test).

Over two thirds of older vegetarian women had osteoporosis. But older vegetarians and omnivores had comparable average bone densities at hips and lumbar spine, with or without adjustment by fat percentages, age, old age home residence, dairy foods consumption and ever smoking. Among the older vegetarians, on stepwise linear regression, urinary sodium/creatinine and body fat percentages had explained variance of 29% for lumbar spinal bone density.

The older vegetarians had a lower prevalence of definite ischaemic heart disease than omnivores (10% versus 31%, p<0.005), as defined by WHO cardiovascular questionnaire, history and ECG. However, 19 out of 89 (21.3%) of the older vegetarians had possible ischaemic heart disease, defined by minor ECG changes suggestive of myocardial ischaemia, and this was associated with history of hypertension.

The fasting cholesterol concentrations of older vegetarians were lower than those of omnivores (4.8± 1.1 versus 5.4±0.9 mmol/L, p<0.001, Student t test), but their triglyceride concentrations were similar (1.6 ±1.4 versus 1.7 ± 1.1 mmol/L).

The prevalence of hypertension (blood pressure greater than 140/90 mmHg and or history of hypertension) among older vegetarians was 64%. This was comparable to that found in a 60-74 yrs old subgroup of a local community survey
of cardiovascular risk factors. When compared with the older subjects in another local study, the blood pressures of older vegetarians were not significantly different despite having a significantly greater urinary excretion of sodium (29.8 versus 17.5 mmol/mmol creatinine, \( p < 0.001 \), Student t test). The prevalence of diabetes mellitus (fasting glucose greater than 7.8 mmol/L and or history of diabetes mellitus) was 14.6%, which was also similar to that observed in two local studies.

Among older vegetarians, hypertension was associated with high urinary excretion of sodium, and lower dietary calcium intake. On stepwise linear regression, the combination of urinary sodium/creatinine ratio and total daily calcium intake accounted for 26% of variance in systolic blood pressure in older vegetarians not on antihypertensive medications. Urinary sodium/creatinine ratio also correlated with diastolic blood pressures (R 0.29) and had an explained variance of 9%.

Fasting urinary MMA/creatinine ratio correlated linearly with serum MMA. The optimal cut-off value for fasting urinary MMA/creatinine to detect raised serum MMA (\( > 0.4 \mu\text{mol/L} \)) was found to be 2 \( \mu\text{mol/mmol creatinine} \). Sixty four out of 68 subjects (94%) with fasting urinary MMA/creatinine greater than 2 \( \mu\text{mol/mmol creatinine} \) had serum vitamin B12 concentration <300 pmol/L, and 47% < 150 pmol/L.

The prevalence of raised urinary MMA was 59.7%, 60.2% and 36.1% in vegans (N 62), lactovegetarians (N 83), and omnivores (N 133) respectively (\( p < 0.05 \), Chisquare test). The prevalence of definite vitamin B12 deficiency (defined by serum vitamin B12 <150 pmol/L and serum MMA \( \geq 0.4 \mu\text{mol/L} \)) in vegans,
lactovegetarians and omnivores (N 26) was 57%, 29% and 8% respectively
(Chisquare test, p<0.000).

One hundred and nineteen older vegetarians had completed blood count. The
prevalence of subnormal iron status (total iron capacity <15%) was 11/110 (10%).
After excluding the iron deficient and renal impairment subjects, serum MMA but
not vitamin B12 correlated with haemoglobin concentrations (correlation
coefficient -0.34, p 0.001), MCV (0.31, p 0.003). Moderate metabolic vitamin B12
deficiency (serum MMA >1.0μmol/L) was associated with anaemia, but
macrocytosis was only observed in 4 out of the 25 subjects.

Fifty older subjects with serum vitamin B12 < 120 pmol/L with or without
dementia were randomized to have an average of four month course of parenteral
vitamin B_{12} supplementation. The supplemented group (N 23) improved in
performance IQ (74.9 ± sd 13.1 improved to 80.7±12.0, p< <0.005, Student t test),
but the change was not significantly greater than that of control subjects (N 27).
The supplemented subjects, on the other hand, deteriorated significantly more than
control subjects in three of the five subscores in the motor function scales:
kinesthesia (sense of position and movement) 2.6 ± 2.4 worsened to 4.2 ± 2.8; fine
motor 8.6± 3.4 to 9.8 ±3.0; oral motor 0.6 ±1.3 to 0.6± 1.3. The group differences
in the changes of these parameters were significant (p<0.05, Student t test).

Seventy-six vegetarian old age home residents had dental examination.
Sixty- three of them were edentulous, and 53% had less than six functional teeth
units. The latter was associated with significantly less fibre intake, but other dietary
intakes were comparable to those with more functional units. The commonest
reasons for chewing difficulties were related to ill fitting or no dentures (91%).

Moreover, in a separate study of 32 younger Chinese vegetarian women (average age 45.6±7.7 yrs old), their plasma homocysteine concentrations were found to be significantly higher than age matched omnivorous control subjects (9.4±sd 2.5 versus 6.8±1.6 μmol/L, p<0.001, Student t test). Their high prevalence of vitamin B12 deficiency (47% had vitamin B12 concentration < 150 pmol/L) was most likely to be responsible, as the serum folate concentrations of vegetarians were very high, when compared with those of omnivores (67.9±23.5 versus 15.1±4.4 nmol/L, p<0.001, Student t test)

Conclusion

There was evidence of dietary inadequacies in older Chinese vegetarians in the community as well as in old age homes. In the old age home setting, poor dental status was associated with decreased fibre intake, but other nutritional intakes were maintained by soft diets. Although they were leaner than omnivores, nearly half of them were overweight. On the other hand, their significantly lower serum albumin concentrations, however, was evidence of protein under nutrition.

Hypertension and diabetes mellitus were as prevalent in older vegetarians as in omnivores. Although the serum cholesterol concentrations of vegetarians were lower than the omnivores, their triglyceride concentrations were not reduced, and the study on younger vegetarians showed increased concentrations of plasma homocysteine despite much higher serum folate concentrations.
High salt intake in older vegetarians was associated with higher systolic and diastolic blood pressure, and low calcium intakes had an additive effect on systolic blood pressure. High salt intake together with low fat percentages were associated with lower lumbar bone density among older vegetarians, but their bone densities were not significantly different from those of omnivores.

Vitamin B12 deficiency was very prevalent among Chinese vegetarians, vegans in particular. It was associated with predominantly normocytic anaemia. A randomized controlled trial of vitamin B12 supplementation in older subjects with subnormal serum vitamin B12 concentrations did not demonstrate any significant benefit in cognitive function.

A more balanced diet, aided by better dental care and vitamin B12 fortified foods or oral vitamin B12 supplement, may confer significant health benefits to older Chinese vegetarians.
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Publications from this Thesis

Full papers in peer-reviewed journals


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Chapter 1  Background and Objectives

1.1 The Need to Study Older Chinese Vegetarians

Vegetarian diets are generally recognized to be beneficial to health. Data is strong that vegetarians are at lesser risk for obesity, hyperlipidaemia, and atonic constipation. Evidence is good that risks for hypertension, coronary artery disease, type II diabetes are lower (Dwyer, 1988). But vegetarianism may have deleterious effects, e.g. vitamin B12 deficiency. Most vegetarian studies were based on adults on Western vegetarian diets which are primarily lacto-ovo-vegetarian. There is a scarcity of data on the more restrictive vegetarian diets, in older people, and in non-Caucasians.

The Chinese vegetarian diets are different from the Western ones in a number of ways. Firstly, dairy products are not popular among Chinese, especially the older generation. Lactose intolerance, which is common among Chinese people, could be contributory. Secondly, the Chinese vegetarian diet is heavily based on soya foods. Over the years, many forms of soya based foods and recipes have been developed (see photographs in Appendix C). In the past, when resources were scarce, preserved ToFu (a soya food) and vegetables were commonly consumed. These foods are high in salt content and may be carcinogenic (Lee et al., 1994). Nowadays in Hong Kong, fresh vegetables and fruit are abundant and affordable, but older people may have problems eating them because of poor dental health.

There are also differences in the lifestyle between Western and Chinese vegetarians. While the Western vegetarians tend to be health conscious middle
class people who are well informed about the risk and benefits of vegetarian diet, the older vegetarians in Hong Kong are not well-educated working class people, with little knowledge about nutritional or health issues.

There are grounds for concern regarding the adequacy of vegetarian diets in older Chinese vegetarians. Older people generally have a more restricted diet for a variety of reasons: social and economic deprivation, cultural beliefs about foods, poor dentition, and chronic diseases resulting in poor appetite dietary restriction.

Moreover, the older vegetarians in old age homes may be more at risk of nutritional inadequacy, as old age home residence itself imposes further dietary restrictions, and some residents of these homes are not even vegetarians beforehand.

It is well documented that vegetarians, especially vegans, are at great risk of vitamin B12 deficient. Although vitamin B12 deficiency has been an established cause of megaloblastic anaemia and a variety of neurological diseases, it does not invariably have clinical manifestations. The most controversial issue is the effect of vitamin B12 deficiency on cognitive function. While some authorities advocate screening and correction of vitamin B12 deficiency which is prevalent in the general older population, the evidence of the link between vitamin B12 deficiency and the incidence of dementia is lacking.

With the rapid socioeconomic development in the last few decades in Hong Kong, obesity, hypertension, diabetes mellitus, ischaemic heart disease and osteoporosis are becoming common medical conditions of old age. A similar trend
has also emerged across more prosperous areas of China. It would be interesting
to examine the relationship between vegetarian diet and the prevalence of these
diseases in the Chinese setting.
1.2 Population of Vegetarianism in Hong Kong

The Hong Kong Vegetarian Society conducted telephone surveys about vegetarian habits every two years since 1995. The proportion of vegetarians was 1.3% in 1995, 0.9% in 1997, 1.6% in 1999, and 1.5% in 2001. The average prevalence of vegetarians in Hong Kong is therefore approximately 1.3%, which is equivalent to about 90,000 people. In the 2001 survey, questionnaire was successfully administered to subjects above the age of 9 years old in 519 out of 1459 telephone calls. 5.6% of surveyed subjects reported vegetarian diets in one quarter or more of the time in a month. One fifth of these were Buddhists. Out of the 8 subjects who were vegetarians, 6 and 2 did it for health and religious reasons respectively.

Although the proportion of vegetarians was low, nearly a quarter of respondents reported avoidance of red and white meat (22% in 2001, 16% in 1999). Female sex, age above 50 years, housewives and Buddhism were associated with meat avoidance.
1.3 Older Vegetarians in Hong Kong

In 1991, an age-sex stratified random sample of 2030 people aged 70 years or over was surveyed to ascertain the social and health profile of older people in Hong Kong (Ho & Woo, 1994). Dietary practice was examined by a questionnaire enquiring about the frequency of the consumption of common food categories in the previous week. 32 out of 1033 (3.1%) female subjects, and 8 out of 999 (0.8%) male subjects aged 70 years and over reported no intake of red or white meat, fish or liver (Personal communication). They were likely to be vegetarians. Eight female (0.5%) and no male subjects reported no intake of milk in addition. They were probably vegans.

Apart from a quest for health, religion is an important reason for vegetarian diet among older people in Hong Kong. Vegetarianism is a long established tradition of Taoism and Buddhism in China. Taoists are vegans while milk and eggs are allowed in Buddhism. In Hong Kong, Buddhism is more popular than Taoism, and is only second to Christianity. The Buddhists and the Taoists have been active in community services, and have obtained government funding to run old age homes. There are currently a total of 7 old age homes with 1,200 beds run by Buddhist and Taoist organizations. In these homes, animal food products are prohibited. In this way, some of the older people joining these homes become vegetarian for the first time.
1.4 Old Age Homes in Hong Kong

In year 1996, 11% of the Hong Kong 6 million population were above 65 years old. There were approximately 40,000 old age home (OAH) beds, two thirds of which were run by private enterprises. The quality of care in private old age homes was generally unsatisfactory. Regulatory controls were only introduced in 1999. One third of OAH beds were mainly subsidized by the government. Most of these subvented old age homes were Care & Attention (C&A) homes for the disabled, and nursing care was available. The staffing ratio was about 1 nurse and 3 care attendants for 15 residents. Typically 6-8 residents shared a room of 200 square feet. Because of their low costs and relatively high quality of care, the waiting time could be as long as four years. Three meals were provided in all subvented OAH, usually from their own kitchens. The nursing staff usually set the menu. Residents did not have a choice on foods. Afternoon and bedtime snacks were available. There were no resident doctors in any of the subvented OAH.

In addition, the Hong Kong government also funded old age hostels which were designed for non-disabled older people who had a housing problem. The older people were expected to look after themselves, including managing their own cooking. But communal meals as in the Care and Attention homes could also be provided if necessary. There was usually only one nurse who is available at normal working hours. In the last few years, these hostels have been gradually replaced by sheltered housing.
1.5 Health Survey of Older Chinese Vegetarians

In 1995, residents of three old age homes (OAH) for vegetarians – Sin tin Dao, Po Ching and Chi Lin were invited to have a comprehensive health survey in a research clinic in the Prince of Wales Hospital, a regional teaching hospital of Hong Kong. The first OAH was a self financed home run by a Taoist group called Sin Tin Dao. The other two subvented homes were run by Buddhists. The majority of the residents in these homes had been vegetarians for years for religious reasons before becoming institutionalized. A minority of them were nuns. Through the contact with the Sin Tin Dao OAH, Taoists living in the community were also invited to participate in the health survey (see photographs in Appendix C).

Sin Tin Dao is a branch of the Taoist faith. Its followers are strict vegans and this is regarded as a very important element of its religion. For more than a century, there has been a movement of its followers, mostly unmarried women, to lead a subsistent communal life in small groups. They sustained themselves financially by sharing their incomes from light manual work or agriculture. This movement has been dying down in Hong Kong in recent years, as there have been few young recruits. When these women became old, needing some attention, they could choose to be admitted to Sin Tin Dao OAH, which was the only OAH for this denomination in Hong Kong (see photographs in Appendix C).

Sin Tin Dao OAH was a non-profit making, self sufficient, OAH with 90 residents (see photographs in appendix C). The accommodation and quality of care was similar to subvented OAH. Chi Lin was a subvented OAH with 200
residents. Because it had the benefit of generous donations from the followers of its temple, its accommodation and quality of care was better than the other two homes. All residents were given daily oral vitamin and mineral supplements for free. Po Ching OAH was a subvented old age hostel with 100 residents. Both Sin Tin Dao and Chi Lin OAH's had a hostel section. All the OAH subjects who participated in the survey in the research clinic were hostel residents.

1.5.1 Objectives of the Health Survey of Older Chinese Vegetarians

1. Compare the dietary intake and food quality of the older Chinese vegetarians in the community with those in old age homes, and with those of local historical non-vegetarian older subjects.

2. Compare the nutritional status of the vegetarians with local historical non-vegetarian older subjects.

3. Compare the prevalence of hypertension, diabetes mellitus and ischaemic heart disease, and bone mineral density of vegetarians with those of local historical non-vegetarian older subjects.

4. To identify dietary factors related to blood pressure and bone density among older vegetarians.

5. To ascertain the prevalence of vitamin B12 deficiency among older vegetarians, and its relationship with haematological status.
1.6 Other Related Studies

Many older vegetarians in the health survey were found to be vitamin B12 deficient. In order to examine the effect of the vitamin B12 deficiency on their cognitive function, a randomized controlled trial of parenteral vitamin B12 supplementation was performed. A minority of the subjects were hospital patients who were found to be vitamin B12 deficient when they presented with dementia or mild anaemia. The hypothesis was that vitamin B12 deficiency caused cognitive impairment in older people and that the impairment was reversible.

Subsequently, two additional cross sectional studies were conducted. The first one examined the relationship between dental status and dietary intake in a vegan old age home. It came from the concern that the dental status of old age home residents had been reported to be poor (Yu, 1997) and this might further restrict the food choice of vegetarian old age home residents, thereby compromising dietary intakes.

The second one compared the plasma homocysteine concentrations of younger female vegetarians with those of age matched historical female non-vegetarian subjects. The high prevalence of vitamin B12 deficiency is likely to cause elevated plasma homocysteine concentrations in vegetarians. But the question was whether high folate intake in Chinese vegetarians might prevent this rise in plasma homocysteine concentrations.
1.7 Summary

This thesis provides insight into the influence of the Chinese vegetarian diet on the nutritional status and the common health problems of older people, both in the community and in the old age home setting. The influence of vitamin B12 deficiency on cognitive function and haematological status was also examined.
Chapter 2 Risks and Benefits of Vegetarian Diets- a Literature Review

2.1 Definition

The vegetarian diets are heterogeneous and so are their health effects. It is therefore important to define the type of vegetarian diet before evaluating its effects. Vegans do not consume any food of animal origin. Lactovegetarians are similar except that they consume dairy products. Lacto-ovo-vegetarians consume dairy products and eggs. More recently, there has been development of special vegetarian groups who believe in even more restricted diets, e.g. Macrobiotics – natural unprocessed foods, consisting of large amount of brown rice with small amounts of fruits; vegan-fruitarian diet – raw fruits, nuts and berries.
2.2 Vegetarianism in the West & in China

It is important to realize that vegetarian diet is only part of the vegetarian lifestyle. Before the 19th century in the Western world, Vegetarianism was justified for moral and meta-physical reasons (Whorton, 1994). Only in the last century, as more scientific evidence gathered, vegetarian diets were increasingly seen to be a healthy alternative diet by some health conscious individuals. Therefore vegetarianism is frequently associated with either puritan or health conscious lifestyles, e.g. abstinence from alcohol, smoking, caffeine, and increased physical exercise etc. These factors have to be controlled for in evaluating the health effects of vegetarian diets in these highly selected groups.

In China, vegetarianism is primarily for religious reasons. Buddhism is against slaughtering animals for food, but allows its followers to drink milk and tolerates eating eggs to a limited extent. However the more dedicated followers would have more restricted diets and become vegans.

Another popular religion is Taoism. It advocates a puritan life-style for the benefit of the soul and body. Any food which is of animal origin is seen as unclean. The maintenance of a vegan diet is seen as a vital part of its religion.
2.3 Vegetarian Studies

The great majority of scientific studies on vegetarian diets were conducted from 1960's onwards. The most studied group of vegetarians has been the religious group, Seventh-day Adventists. This evangelical Christian Church originated in the New England states of the United States in the 19th century. The Church advocated vegetarianism, abstinence from drugs, alcohol, tobacco, and caffeine, and encouraged physical exercises and good education. Their advantage as study subjects was their excellent compliance to vegetarian diet. They were initially vegans, but they have become lactovegetarians in recent years. The uniqueness of this vegetarian group is that they share similar lifestyle, yet they adopt a variety of diets. The confounding influence of lifestyle apart from diet may then be controlled for.

In comparing vegetarians with omnivores, it is important firstly to determine the nature of the vegetarian diet in question. As fore mentioned, there are different types of vegetarians. Even among vegetarians, the diet can vary greatly in different countries because of cultural and geographical differences. The nature of omnivorous diets also varies greatly from populations to populations. Instead of focusing on vegetarians, researchers in recent years have investigated the preventive roles of fruit and vegetables, and their constituents, in chronic disease.
2.4 Mortality

The longest longitudinal study involved 27,530 vegetarian and nonvegetarian Seven-Day Adventists (SDA) in California in 1960. They were followed up for 21 years. All cause mortality was decreased in those who were more vegetarian diet (consumed salads at least three times a week), after adjustment for age, sex, smoking, history of major chronic disease and age of exposure to teachings of SDA (Kahn, 1984). However the SDA lifestyle itself was associated with lower total mortality, especially cardiovascular mortality, in men and in converts at a younger age (Fonnebo, 1994). Another study recruited 11,000 Welsh vegetarians and nonvegetarian health food-store customers and followed them up for 7 years. No group difference in all-cause mortality was found (Burr & Sweetnam, 1982). In a more recent cohort study of more than 10,000 health conscious subjects recruited from health food shops, vegetarian societies and magazines, consuming fruit daily and not vegetarianism per se was associated with reduction in all-cause mortality, cardiovascular and cerebrovascular mortality (Key et al., 1996).

A recent meta-analysis of five large vegetarian cohort studies in US, UK and Germany reaffirmed that the overall and ischaemic heart disease related mortality rates were significantly decreased in vegetarians compared with non-vegetarians of similar lifestyles (Key et al., 1999).

Overall, Western vegetarians have lower mortality, primarily because of lower cardiovascular mortality. This can be attributed to their healthy lifestyles. But whether the benefit was derived from vegetarian diet per se remains uncertain.
2.5 Vegetarian Diet and Ischaemic Heart Disease

Studies have consistently demonstrated reduced mortality from ischaemic heart disease in middle-aged vegetarians (Thorogood et al., 1994; Key et al., 1996; Burr & Sweetnam, 1982; Phillips et al., 1978). But in some of these studies, the difference in cardiovascular mortality was not significant in older people and in women (Phillips et al., 1978; Burr & Sweetnam, 1982). The most likely explanation is that younger men consumed more meat than older people and women, and would therefore benefit more from vegetarian diets. It is noteworthy that in all of these studies, the subjects were all health conscious people and had lower cardiovascular risks than the general population. In a recent meta analysis of 5 major cohort studies of vegetarians in US, UK and Germany, involving a total of 76172 men and women, aged 16-89 years, and incidence of 8330 deaths over an average of 10 years, mortality from ischaemic heart disease was 24% lower in vegetarians than in nonvegetarians with similar lifestyles (Key et al., 1999). It should however be pointed out that the benefit was more marked at younger ages and restricted to those on vegetarian diet for more than 5 years. Moreover there were at most 1146 vegans (1.5%), involving 165 deaths in this sample. There was no significant reduction in cardiovascular death in this subgroup.

While there is little dispute that vegetarian diets are beneficial to cardiovascular health, there remains uncertainty about the individual components of the diet which are most beneficial. A study suggested that fruit was most important (Key et al., 1996). A recent large-scale cohort study of 34192 California
Seventh-day Adventists, which examined the dietary habit of vegetarians and non-vegetarians in more detail, showed that nut consumption >5 times a week had an odds ratio of 0.5 for fatal ischaemic heart disease and 30% reduction in lifetime risk in developing the disease. It remains uncertain whether the apparent benefit can be attributed to the avoidance of meat.

2.5.1 Benefits of Fruit & Vegetables

Many constituents of fruits and vegetables are known to have protective effects against coronary artery disease. But solid evidence of benefit only emerged recently (Joshipura et al. 2001, Liu et al. 2000, Bazzano et al. 2002). In a large epidemiological study which combined the Nurses’ Health Study and the Health Professionals’ Follow-up Study (Joshipura et al. 2001), involving 84,251 women aged 34 to 59 years and 42,148 men aged 40 to 75 years who were followed up for 14 years and 8 years respectively, persons in the highest quintile of fruit of vegetable intake had a relative risk for coronary artery disease of 0.80 (95% CI 0.69 to 0.93). A particular strength of this study is that the semi-quantitative food frequency questionnaire included 15 fruits and 28 vegetable items, so that specific types of fruit and vegetable could be examined separately. In subgroup analysis, green leafy vegetables and vitamin C-rich fruits and vegetables were found to have contributed most to the protective effect. In contrast, increased potato consumption did not confer any benefit.

In the same study, the subjects in the highest quintile of fruit and vegetable intake when compared with those in the lowest quintile also had a lower incidence
of ischaemic stroke (relative risk 0.69, 95% CI 0.52 – 0.92). Cruciferous vegetables, green leafy vegetables, citrus fruit contributed most to the protective effect. Legumes and potatoes were not contributory (Joshipura et al 1999).

2.5.2 Low Serum Cholesterol Concentration in Vegetarians

Vegetarians have been shown to have lower serum cholesterol concentrations in cross sectional studies (Thorogood et al., 1990; Resnicow et al., 1991; Knuiman & West, 1982). A 6-week cross over trial of a lacto-ovo vegetarian diet also showed a significant reduction in serum cholesterol concentration in men but not in women (Masarei et al., 1984). There was no significant change in triglyceride concentrations. It was thought that high fibre, low saturated fat and cholesterol intakes of vegetarians were responsible for the apparent reduction in cholesterol concentrations. However it is possible to achieve the same reduction in cholesterol concentrations by replacing saturated fats by unsaturated fats (Thorogood, 1994). Moreover, a recent meta-analysis of 38 human studies showed that consumption of soy protein resulted in significant decrease in total cholesterol concentration (Anderson et al., 1995). It was estimated that the ingestion of 25 or 50 g of soy protein per day could decrease serum cholesterol by 9%. Similarly leguminous seeds, e.g. chick peas (Bingwen et al., 1981), nuts (Abbey et al., 1994), soluble fibres abundant in fruits, oat cereals, legumes etc (Ripsin et al., 1992) can reduce serum cholesterol concentrations.

Vegetarians have low cholesterol intake. But dietary cholesterol is not associated with serum cholesterol concentration (Dawber et al. 1982) and is not
associated with incidence of coronary heart disease (Gordon et al. 1981). It is rather the types of dietary fat which determines the serum cholesterol concentrations. Ahren et al. (1975) showed that the degree of saturation of fats is the key determinant of serum cholesterol concentrations. This was further confirmed by McNamara et al. (1987). Human volunteers were given diets containing saturated or polyunsaturated fat, together with high or low cholesterol content. It was found that serum cholesterol concentration was significantly increased by saturated fat diet but not by high cholesterol diet. Keys et al. (1965) and Hegsted et al. (1965) independently formulated predictive equations for serum cholesterol concentrations based on the levels of saturation of dietary fat.

When saturated fat is replaced by polyunsaturated fat, LDL cholesterol decreases and high density lipoprotein (HDL) cholesterol changes only slightly. The latter is an independent protective factor against atherosclerosis (Gotto 2001). This dietary change may also have beneficial effects on insulin sensitivity (Summers 2002) and type 2 diabetes (Salmeron et al. 2001).

However when saturated fat is replaced by carbohydrate, which is often the case in vegetarians (Haddad et al., 1999; Hunt et al., 1988), both LDL and HDL cholesterol decrease, the LDL:HDL ratio remaining unchanged and the triglyceride concentrations rising (Parks and Hellerstein 2000). At the same time, there may also be an associated increase in insulin resistance (Jeppesen et al. 1998). The overall cardiovascular benefit of this dietary change is therefore uncertain.
2.5.3 Dietary Fat, Serum Cholesterol Concentration and Cardiovascular Risk

Serum cholesterol concentration has been known to be associated with atherosclerosis since the 1950's. The Framingham study showed conclusively that low-density lipoprotein cholesterol primarily accounts for this association (Wilson et al. 1980). Intervention trials consistently showed that reduction of LDL cholesterol decreases the incidence of mortality and coronary artery disease events (Pedersen et al. 1998).

In the Nurses’ Health Study which was a prospective study of 80,082 middle-aged nurses for fourteen years, saturated fat instead of total fat intake was associated with coronary heart disease outcomes (Hu et al. 1997). On multivariate analysis, when compared with equivalent energy from carbohydrate, an increment of five percent of energy from polyunsaturated fat was associated with a lower risk of developing myocardial infarction or cardiovascular related mortality (relative risk 0.62, 95% confidence intervals 0.46-0.85, p = 0.002), whereas an increment of five percent of energy from saturated fat, was only associated with a non-significant increase in risk (relative risk 1.17, 95% confidence interval 0.97 to 1.41, p=0.10). Therefore this study confirmed the theoretical advantage of unsaturated fat over carbohydrate in cardiovascular risk reduction.

2.5.4 Antioxidant and Atherosclerosis

Plant foods are abundant in antioxidants, e.g. vitamin E, vitamin C and beta carotene. There have been a lot of research interests in the preventive roles of
antioxidants in atherosclerosis. There is evidence that oxidation of low-density lipoprotein (LDL) is linked to atherosclerosis (Witztum and Steinberg 1991). In vitro studies showed that oxidative systems like metal ions (Ray 1954), lipoxygenase (Sparrow et al. 1988), myeloperoxidase (Savenkova et al. 1994), and peroxynitrite (Darley-Usmar et al. 1992) may be involved. Antibodies against oxidized-LDL react with components of atherosclerotic lesions, but not with normal artery (Palinski et al 1989). Patients with carotid atherosclerosis have higher level of autoantibodies to oxidized-LDL than control subjects (Salonen et al. 1992). Antioxidants may therefore prevent atherosclerosis.

Prospective studies showed an inverse relationship between coronary artery event and vitamin E intake in female American nurses (Stampfer et al. 1993) and male health professionals (Rimm et al. 1993). The latter study showed an inverse relationship between coronary artery event and beta carotene intake (Rimm et al. 1993). Similarly, the EPIC-Norfolk prospective study involving 19,496 people aged 45-79 years recruited from general medical practice and followed for four years showed a continuous inverse relationship between cardiovascular death and plasma ascorbic acid concentrations (Khaw et al., 2001).

However randomized placebo controlled trials of vitamin E supplement have been largely disappointing, except the Cambridge heart oxidant study which randomized 2002 British men and women with known coronary artery disease (Stephens et al. 1996). The vitamin E supplement group had a 77% reduction in nonfatal heart attack, but with no change in mortality. However a more recent and substantial trial involving 8500 men and women at risk of coronary artery disease
showed no benefit of vitamin E supplement in the incidence of coronary artery event after 4 1/2 years (Yusuf et al. 2000). The Alpha Tocopherol Beta Carotene on the incidence of lung cancer prevention study group (1994) randomized 29,000 male smokers and found no benefit of either vitamin supplement in coronary artery events.

The reasons for the negative results of these trials are unclear. One possible explanation is that the subjects had adequate intake of antioxidants, and would therefore not benefit from supplementation. The second possibility is that the vitamins examined were not effective in preventing the oxidative processes which are most relevant to atherosclerosis. There are arguments to experiment with different combinations of antioxidants, as they can act synergistically. For example, alpha-tocopherol and ascorbic acid synergistically inhibit oxidation of liposomal membranes and LDL (Noguchi and Niki 1998). On the other hand, antioxidants may interact with each other and act as pro-oxidants (Halliwell 2000).

Recent interest has turned to non-nutritional oxidative compounds found in plant foods, e.g. flavonol. In examining the antioxidant activities of apples, Eberhardt et al. showed that apple extracts had far greater antioxidant activities than their vitamin C content would suggest (2000). Phytochemicals e.g. flavanol were thought to be responsible for the “unexplained” antioxidant activities. In the Seven Countries Study, the average population intake of flavonol, rather than that of vitamin E, beta carotene or vitamin C, was related to 25-year coronary heart mortality rates (Kromhout et al. 1996, Hertog et al. 1995). Another novel strategy is to block more up-stream oxidases, e.g. the use of Angiotensin II inhibitor to
block the production of hydrogen peroxide by inhibiting NADPH oxidase (Munzel and Keaney 2001).

2.5.5 Homocysteine

Homocysteine is formed upon the demethylation of methionine, and is eliminated by two vitamin dependent pathways involving folate, vitamin B12 and vitamin B6 (Hajjar et al. 2001). Elevated plasma homocysteine concentration-hyperhomocysteinaemia is increasingly recognized to be an independent risk factor for stroke, myocardial infarction and cardiovascular mortality (Malinow et al. 1999). Because vegetarians are at risk of vitamin B12 deficiency, the consequential elevation of plasma homocysteine concentrations may put them at increased vascular risk.

Experimental studies showed that hyperhomocysteinaemia induces endothelial dysfunction (Tawakol et al. 1997), vascular inflammation (Hofmann et al. 2001), and lipid peroxidation (Loscalzo 1996). There have been many prospective cohort studies to examine the relationship between hyperhomocysteinaemia and cardiovascular disease. Most of them showed a positive relationship (Nygard et al. 1997, Stampfer et al. 1992), but some were inconclusive (Evans et al., 1997, Ridker et al. 2000). Despite these observations, there remains controversy about the causative role of homocysteine in vascular diseases (Christen et al. 2000). The problems lie in the interactions between hyperhomocysteinaemia and most other cardiovascular risk factors e.g. age, sex, hypertension, diabetes mellitus, renal impairment, systemic inflammation,
smoking, lipid profile etc. (Christen 2000, Van Guldener et al. 2001). Unless all these confounders are adjusted for, no conclusion can be made from prospective studies.

The reason why there has been some much research interest in hyperhomocysteinaemia is that it is common and that it is modifiable by vitamin supplementation. In a cross sectional study of 600 elderly hospital inpatients (Ventura et al., 2000), the prevalence of hyperhomocysteinaemia (fasting homocysteine $\geq 15\mu\text{mol/L}$ or 4 hours after methionine load $\geq 35\mu\text{mol/L}$) was as high as 61%. Folate supplementation is very effective in lowering homocysteine concentrations (Homocysteine Lowering Trialists' Collaboration 1998), and was shown to improve endothelial function in middle aged hyperhomocysteinaemic subjects (Woo et al. 1999). Vitamin B12 (Araki et al. 1993) and or vitamin B6 (McKinley et al., 2001) can also further lower homocysteine concentrations. A randomized placebo controlled trial showed that oral supplements of folate, vitamin B$_{12}$ and vitamin B$_{6}$ (folic acid 1 mg/day, cyanocobalamin 400 mcg/day, vitamin B$_{6}$ 10 mg/day) significantly lowered plasma homocysteine concentration and decreased the incidence of a combined cardiovascular endpoint at one year after coronary angioplasty (Schnyder et al. 2002). However these positive trial results could not establish the causative role of hyperhomocysteinaemia in cardiovascular diseases, as B vitamins may have their independent roles in vascular health. Further trials of folate supplementation in the United States may be more difficult to interpret because of the fortification of flour with folic acid to prevent neural tube defect in neonates (Bostom et al. 2001).
2.6 Vegetarian Diet and Obesity

Many cross sectional studies have shown that vegetarians, especially vegans, are leaner than omnivores and the prevalence of obesity is low in vegetarians (Key & Gwyneth, 1996, Appleby et al., 1998). The total energy intakes of vegetarians are similar to omnivores, but the intakes of total fat, saturated fat, protein are lower, and the intakes of carbohydrates, fibre are higher (Haddad et al., 1999; Appleby et al., 1998; Hunt et al., 1988). Some studies also showed a reduction in protein intake in vegetarians (Ball & Bartlett, 1999; Janelle & Barr, 1995; Levin et al., 1986).

A lactovegetarian diet was effective in weight reduction in moderately overweight people, but it was no more effective than a well balanced diet (Hakala & Karvetti, 1989). It is intriguing that vegetarians should be leaner despite having normal or even above normal energy intakes (Levin et al., 1986). There was a small cross sectional study showing that resting metabolic rate was increased in vegetarian men (Toth & Poehlman, 1994). It has been hypothesized that animal protein can potentiate the insulin response to co-ingested carbohydrate, whilst legume protein is more slowly absorbed and soy protein provokes a greater release of glucagon, an enhancer of fat oxidation (McCarty, 2000). Sofar the lower body mass index in vegetarians has been attributed to reduced fat mass, and there has not been any study on lean mass in vegetarians. A recent small sized randomised trial of a lactovegetarian diet showed a smaller increase in muscle mass in response to high resistance exercise (Campbell et al., 1999).
2.7 Vegetarian Diet and Hypertension

The most convincing study of the effect of vegetarian diets on blood pressure compared blood pressures of 98 Seventh-day Adventist (SDA) vegetarians with those of 82 SDA omnivores and 113 Mormon omnivores. The influence of religious lifestyle was minimized. After adjustment for age, height and weight, mean blood pressures of SDA vegetarians were significantly lower than those of the other two groups in both sexes (Rouse et al., 1983a). When compared with the omnivorous diets, the vegetarian diet was higher in fibre, polyunsaturated fat, magnesium, potassium and lower in saturated fat and cholesterol. The association of vegetarian diet and lower blood pressure was also observed in white and black older American adults (Melby et al., 1993).

The effect of vegetarian diets on blood pressure has been subject to randomised controlled trials. In an earlier trial, 59 healthy omnivorous adults aged 25-63 were randomised into control, and two experimental groups. The experimental groups subjects had a vegetarian diet similar to that of the lacto-ovo-vegetarian diet of the SDA for six weeks either in the first or second half of the trial period. There was a significant fall of 5mm Hg systolic and 2-3 mm Hg diastolic pressures. This was not mediated by changes in sodium and potassium intakes (Rouse et al., 1983b). Similar magnitude of fall in blood pressure was observed in mild hypertensive subjects when they were randomised to have an ovolactovegetarian diet for six weeks (Margetts et al., 1986).

In a more recent larger scale trial, 459 Americans with no or mild hypertension were randomised into control diet, a diet rich in fruits and vegetables,
and a combination diet rich in fruits, vegetables, low fat dairy products and reduced saturated and total fat. When compared with control diet, the combination diet lowered blood pressures more than the fruit and vegetable diet, and it lowered blood pressure significantly more in the hypertensive subjects (11.4 mm Hg systolic, 5.5 mm Hg diastolic in hypertensives, versus 5.5 and 3.0 mm Hg respectively in normotensives) (Appel et al., 1997).

It remains unclear as to how vegetarian diets lower blood pressure. Increased proportion of unsaturated fatty acids relative to saturated fatty acids did not lower blood pressure in normotensive subjects (Margetts et al., 1984) or hypertensive subjects (Sacks, 1987). Dietary fibre supplement was also shown to be ineffective in lowering blood pressure in normotensives (Margetts et al., 1987). In a trial of complex dietary changes in 95 hypertensive subjects, low fat and high fibre diet did not have additive effect on salt restriction (Sciarrone et al., 1992).

It can be concluded that a complex diet involving low fat with a high proportion of unsaturated fats, and high contents of fibre, fruits and vegetables can lower blood pressure. This effect is independent of sodium intake. Although most vegetarian diets fit the characteristics of this complex diet, the latter does not necessarily exclude meat. Finally, the blood pressure lowering effect cannot be attributed to a single dietary component.
2.8 Vegetarian Diet and Diabetes Mellitus

Vegetarian diets are high in fibre and complex carbohydrates which have been shown to improve diabetic control by delaying glucose absorption, and increasing insulin sensitivity (Simpson et al., 1981; Jenkins et al., 1976; Hjollund et al., 1983). Saturated fats taken by meat eaters may increase insulin secretion, and possibly lead to insulin insensitivity (Collier & O’Dea, 1983).

However there have been few studies to compare prevalence of diabetes mellitus between vegetarians and non-vegetarians. In an epidemiological study of 25,698 adult White Seventh day Adventists, meat consumption was positively associated with self-reported prevalence of diabetes mellitus in both sexes. On reviewing the death certificates during the 21 years of follow-up, meat consumption was positively associated with the diagnosis of diabetes mellitus in male but not in female, after adjusting for age and body fatness (Snowdon & Phillips, 1985). An earlier study showed a positive association between animal fat consumption and blood glucose (Gear et al., 1980).
2.9 Vegetarian Diet and Cancers

2.9.1 Colonic Cancer

There are theoretical reasons why colonic cancer can be prevented by vegetarian diets. Firstly cooking meat may form carcinogenic substances e.g. polycyclic aromatic hydrocarbons and heterocyclic amines (Jagersatd et al., 1991; Bogovski, 1983). Moreover ingestion of meat increases faecal content of potentially carcinogenic N-nitroso compounds (Bingham et al., 1996). On the other hand, high dietary fibre protects against colonic cancer (Bingham, 1987; Anderson et al., 1994), though the exact mechanism is unclear.

In two large cohort studies: the Nurses' health study (88,764 women) and the Health Professionals' Follow-up Study (47,325 men), involving 1,743,645 person years and 937 cases of colonic cancer, no significant association between fruit and vegetables intakes, and colonic cancer was found (Michels et al. 2000).

The mortality rate and incidence of colonic cancer have been reported to be lower in vegetarians than in the general population (Phillips, 1980; Frentzel-Beyme & Chang-Claude, 1994). But the confounding influence of lifestyles was not adjusted for. In a cohort study of 34,198 California Seventh-day Adventists, cancer incidence was checked by a yearly surveillance programme consisting of postal questionnaire, computer linkage cancer registries and death registries. The odds ratio of developing cancer was significantly raised at 1.88 in nonvegetarians, when compared with vegetarians (Fraser, 1999). But some prospective studies have shown negative results (Key et al, 1982; Thun et al, 1992). In the meta analysis of these five large scale cohort studies, the mortality rate attributed to...
colonic cancer was not reduced in vegetarians when compared with nonvegetarians of similar lifestyles (Key et al., 1999).

2.9.2 Breast and Prostate Cancers

Earlier small scale studies suggested that vegetarians had lower plasma concentrations of the oestrogen and androgens in female and male respectively (Goldin et al., 1981; Belanger et al., 1989). These led to the postulation that vegetarian diets might prevent breast cancer in female and prostate cancer in male. Two prospective Seventh-day Adventist studies showed that vegetarian men had lower mortality and incidence of prostatic cancer, than non-vegetarians (Snowdon et al. 1984; Fraser 1999). There is no epidemiological evidence that the amount of meat consumption is related to risk of breast cancer (Mills et al., 1988; Mills et al., 1989). In two large-scale prospective studies, no association between vegetarian diet and prostatic cancer and breast cancer was observed (Allen et al., 2000; Thomas et al., 1999).

2.9.3 Antioxidants and Cancers

Many compounds in vegetables and fruit prevent cancer in animal models (Steinmetz and Potter, 1991). Apple extracts were shown to inhibit colon cancer cell and liver tumour cell lines in vitro (Eberhardt et al., 2000). Burr et al. demonstrated prospectively that daily fruit consumption is also associated with lower cancer mortality (Burr et al., 2001). It was widely thought that antioxidant vitamins, e.g. vitamin C, E and beta carotene, were responsible (Block, 1993).
Plasma Vitamin C concentration had an inverse relationship with cancer mortality in men but not in women in the PIC-Norfolk prospective study as mentioned above (Khaw et al., 2001). The benefit was more likely to be related to fruit intake rather than vitamin C per se (Lightsey et al., 2001).

Greenberg et al. randomized 864 patients who had a recent history of colonic adenoma to receive placebo only, beta carotene (25 mg) plus placebo, vitamin C (1g) and E (400 mg) plus placebo, or beta carotene plus vitamin C and E. The primary end point was the occurrence of new adenomas at year one and at year 4 on colonoscopy. The benefit in any of the supplements was observed. Furthermore, the Alpha-Tocopherol, beta carotene cancer prevention study group (1994) randomized 29,133 male smokers aged 50-69 years in Finland to have alpha-tocopheral (50 mg daily) alone, beta carotene (20 mg daily) alone, both vitamins or placebo. After 5-8 years follow-up an incidence of 876 lung cancer, no significant change in risk of lung cancer was observed for alpha-tocopherol, but unexpectedly, the risk was increased by 18% (95% confidence interval 3-36%) for betacarotene supplements. This may be explained by the dual actions of free radicals. They may be involved in the carcinogenesis, but they are also involved in the elimination of cancer cells (Horrobin, 2001). Antioxidants may therefore promote the growth of pre clinical cancers.

In conclusion, epidemiological and experimental studies suggested that fruit and vegetables prevent cancer. It is theoretically plausible that antioxidants prevent cancers, but antioxidant vitamins in the form of supplements are not effective in preventing cancers, and may even be harmful.
2.10 Vegetarian Diet and Dementia

2.10.1 Incidence of Dementia in Vegetarians

There have very few studies to compare vegetarians and omnivores in the incidence of dementia. A substudy of 2,984 Seventh-day Adventists (SDA) showed no difference between vegetarians and nonvegetarians in incidence of dementia assessed by reviewing hospital medical records over five years, though a matched substudy of 272 SDA subjects, including 68 vegans, showed a borderline trend for more heavy meat eaters than vegetarians to develop dementia (Giem et al., 1993). Because of the small number of demented cases, these findings should be regarded as preliminary. On the other hand, there have been studies showing an inverse relationship between saturated fat intake and dementia.

2.10.2 Antioxidant and Dementia

The brain is a good substrate for oxidation as it is a large consumer of oxygen, and polyunsaturated fatty acids which are major constituents of cell membranes are susceptible to lipid peroxidation (Coyle and Puttfarcken, 1993). In addition, oxidative stress can lead to protein inactivation, DNA oxidation, and atherosclerosis (Kristal and Yu, 1992).

There is neuropathological evidence that lipid peroxidation (Subbarao et al., 1990), protein oxidation (Markesbery, 1997), and DNA oxidation (Mecocci et al., 1994) are more prevalent in Alzheimer’s diseased brain when compared with controls.
Antioxidants in foods or in the supplement form may prevent the development of Alzheimer's disease (AD). By virtue of their potential beneficial effects on atherosclerosis, vascular dementia may also be prevented too. A cross-sectional study involving 5182 non-demented men and women in Rotherdam showed an association between beta carotene intake as estimated by a validated food frequency questionnaire and cognitive impairment (mini mental state examination score, MMSE <25/30, even after controlling for age, smoking and education (Jama et al., 1996). After six years of follow-up with the incidence of 177 cases of dementia and 149 cases of AD, dietary intake of vitamin E was found to be associated with reduced risk of AD and dementia. So were vitamin C and vegetable intakes (Engelhart et al., 2000).

A special problem of interpretation of longitudinal studies of such kind is that the dietary assessment is demanding on memory. The "non-demented" subjects at baseline might have had a mild degree of cognitive impairment, therefore introducing bias towards a positive association between poor diet factors and dementia.

The most quoted clinical trial of antioxidants in Alzheimer's disease was performed by the Alzheimer's Disease Cooperative Study Group (Sano et al., 1997). In the trial, 341 patients with moderate to severe AD were randomized to have high dose vitamin E, Selegiline or both, or a placebo for two years. The MMSE scores were not well matched at baseline. There was no significant group difference in adverse outcome as defined by death, institutionalization, loss of 2/3 basic activities of daily living or progression to severe dementia. However, after
adjusting for baseline MMSE, there was a significant delay in the time for the
treated subjects to reach the adverse outcome. This trial has to be interpreted with
cautions as the positive result was only apparent after statistical adjustment, and
the primary endpoint was a combination of several unrelated outcomes. As the
dose of vitamin E supplement used was so much higher than dietary intake and
the subjects were already significantly demented, the trial shed no light on the
relationship between dietary antioxidant and the development of dementia.

2.10.3 Vitamin B12 Deficiency and Dementia

Vegetarians are at high risk of vitamin B12 deficiency. It is therefore
possible that older vegetarians may be at risk of dementia. Vitamin B\textsubscript{12} is a very
important vitamin for well-being of the brain. It holds a key position in the
synthesis of DNA and S-adenosylmethionine (SAM) (Regland and Gottfries,
1992). In the brain, SAM-dependent methylations are extensive and required for
the production of neurotransmitters, phospholipids and myelin (Selhub, 2000).
Retarded myelination of the brain on MRI imaging was reported in a fourteen
month old infant with dietary vitamin B12 deficiency. The brain atrophy and
myelination improved significantly after 5 months of vitamin B12
supplementation (Lovblad et al., 1997).

Yet vitamin B\textsubscript{12} deficiency is not uncommon even in non-vegetarian older
people, especially among the demented (Bernard et al., 1998; Garry et al. 1984)
because of poor diet, malabsorption, and drugs. There is also a tendency of
decreasing plasma vitamin B12 concentrations with increasing age (Haller et al.,
The crucial question is whether vitamin B₁₂ deficiency contributes to brain dysfunction or merely co-exist with dementia.

There is cross sectional evidence that vitamin B₁₂ deficiency is associated with cognitive impairment (Bernard et al., 1998) and Alzheimer disease (Clarke et al., 1998; McCaddon and Kelly, 1994) in older people. The Bronx Longitudinal Aging Study (Crystal et al., 1994) followed up 410 non-demented and healthy people aged 75-85 years for five years. The subjects had a yearly laboratory tests, including serum vitamin B₁₂ concentration and neuropsychological tests which included Blessed Test of Information, Memory and Concentration (BIMC), WAIS subtests, and others. If there was an increase of 4 or points on the BIMC test, the subjects were seen by a neurologist and had a full workup for dementia. In the five years, 3 out of the 22 subjects (13.6%) with low serum vitamin B₁₂ concentrations developed dementia, while 57 of the 388 subjects (14.7%) with normal vitamin B₁₂ concentrations did. The sample size of low serum vitamin B₁₂ concentrations was too small for any conclusive answer. Based on incidence rate of dementia in the normal serum vitamin B₁₂ concentration group, this sample size had only sufficient power to detect a relative risk of 4 for low serum vitamin B₁₂ concentration subjects to develop dementia. Moreover the report did not mention whether the subjects with low serum vitamin B₁₂ concentrations received supplementation from their physicians.

In another prospective study in Sweden, 370 non-demented people aged 75 years and older were followed up for three years (Wang et al. 2001). In order to recruit more subjects with borderline low cognitive status, about half of the...
initially screened subjects had mini-mental state examination (MMSE) score of <24/30. The primary outcome was Alzheimer’s disease or dementia diagnosed by two physicians at the 3 year follow-up. 86 subjects died in the interim period. Hospital records were reviewed, and 8 of them were diagnosed to be demented. Vitamin B12 and folate concentrations were checked at baseline and deficient subjects were left untreated. After three years, 78 subjects (21.1%) were diagnosed to have dementia and 60 (16.2%) the Alzheimer’s type. The results showed that subjects with either low vitamin B12 or folate concentrations when compared with those with normal concentrations had a relative risk of 1.8 and 2.1 in dementia and Alzheimer’s disease respectively. Interestingly subgroup analysis showed that the increase in risk was only significant in those with MMSE >26/30. The interpretation of this study is limited by the small sample size and the uncertain cognitive status of a significant proportion (23.2%) of subjects who died within the three-year study period.

A bigger prospective study of older Europeans by the SENECA investigators (Eussen et al., 2002) also showed no correlation between plasma vitamin B12 and folate concentrations with the changes in scores of mini mental state examination scores and geriatric depression scale. But only 586 out of the original 1099 subjects examined 5 years previously participated in the follow-up study.
2.10.3.1 Effect of Vitamin B12 Supplementation in Dementia

Although there were case reports of confusional states reversed by vitamin B$_{12}$ supplementation (MacDonald, 1956), the clinical response of dementia to vitamin B$_{12}$ supplementation in deficient subjects have not been encouraging. In a case series of 26 demented patients with subnormal serum vitamin B$_{12}$ concentrations in a memory clinic, vitamin B12 replacement over six months did not result in improvement in cognitive function, disability, and caregiver burden (Teunisse et al., 1996). In another cases series of demented geriatric outpatients with serum vitamin B12 concentrations $<$200 pmol/L (Cunha et al., 1995), treatment outcome was available in 19 out of 46 patients. Three of the 19 patients improved clinically and MMSE returned to normal values. In two of these, the response was seen at three months. All these three patients had early dementia of less than two years duration. In 24 demented patients with vitamin B12 deficiency and symptoms of delirium, 15 patients with mild to moderate dementia improved clinically, and in regional cerebral blood flow studies. The remaining 9 severely demented patients did not improve either clinically or in cerebral blood flow.

Martin et al. (1992) reported the response of a six month course of parenteral vitamin B12 supplementation in 18 patients with cognitive dysfunction and low serum cobalamin ($<$150 pmol/L). 11 out of 18 patients showed cognitive improvement. Subjects with cognitive symptoms for less than 12 months gained an average of 20 points on the Mattis Dementia Rating Scale (maximum score 144) ($p<0.01$, paired t test). Those with longer duration of symptom did not improve. In the largest reported case series in a UK memory clinic (Eastley et al.,
2000), out of 125 patients with low serum vitamin B12, 66 patients with dementia and 22 patients with cognitive impairment returned to follow-up after vitamin B12 supplementation. The demented patients showed no improvement and no less deterioration, in neuropsychological function, when compared with age matched demented patients with normal serum vitamin B12 concentration. However a significant treatment effect was observed in patients with cognitive impairment. Based on these case series of vitamin B12 supplementation, one can conclude that established dementia does not usually respond to correction of vitamin B12 deficiency, while those with cognitive impairment, early dementia and those with delirium symptoms may improve as early as three months after supplementation.

To date, there has not been any randomized trial of vitamin B\textsubscript{12} supplementation in demented or cognitively impaired people, probably because of ethical considerations. In a small randomized trial, thirty-nine elderly community dwelling subjects with vitamin B\textsubscript{12} deficiency received either cobalamin or placebo intra-muscular injections for one month. Improvement in subjective ratings of psychiatric and general well being was observed in both groups (Hughes et al. 1970), probably because of placebo effect.

2.10.4 Hyperhomocysteinaemia and Dementia

Deficiency of vitamin B\textsubscript{12} leads to elevation of plasma homocysteine, which has recently emerged as an important independent risk factor of cerebrovascular disease and Alzheimer’s disease both in cross sectional and longitudinal studies (Seshadri et al. 2002, Vermeer et al. 2002, McCaddon et al.)
In a series of 33 demented patients, vitamin B12 and folate supplementation resulted in improvement in MMSE and in memory and attention in those with mild to moderate dementia, and raised plasma homocysteine concentrations (Nilsson et al., 2001). Vitamin B12 supplementation may therefore have a role in mildly demented patients with hyperhomocysteinaemia, even though the serum vitamin B12 concentrations are normal.
2.11 Vegetarian Diet and Osteoporosis

There have been few studies comparing bone density of vegetarians with omnivores. There was no difference in bone density between lactovegetarians and omnivores in Caucasian studies either cross-sectionally or longitudinally (Tesar, 1992; Reed, 1994). But in a study of 258 Taiwan Buddhists postmenopausal nuns and female followers, long-term vegan practice was associated with increased risk of osteoporosis (Chiu et al., 1997).

There are several possible mechanisms whereby vegetarian diets may affect bone density. Firstly although lactovegetarians have adequate calcium intake from dairy products (Weaver & Plawecki 1994), vegans may have low calcium intakes, because plant foods are not rich in calcium, and the phytates and fibre contents of plant foods inhibit calcium absorption (Nordin et al., 1987). The relationship between low calcium intake and osteoporosis has been a contentious issue. Earlier Caucasian studies failed to confirm the association (US Department of Health and Human Services, 1984). But recent studies confirmed the importance of adequate calcium intake in bone health of older people. In a cross sectional study of 1765 older Australians, daily calcium at the lowest tertile (<465 mg daily) was associated with lower femoral bone density (Nguyen et al., 2000). Another cross sectional study involving over a thousand subjects over 50 years old in South Asian countries, showed that the risk of hip fracture was significantly increased in those with calcium intake of less than 498 mg daily in both sexes (Lau et al., 2001). A longitudinal study followed up 134 predominantly postmenopausal women for 3.9 years. High calcium intake was found to be
associated with less reduction in femoral bone density (Uusi-Rasi et al., 2001). An earlier randomized controlled trial conducted on Hong Kong older Chinese women who had lower calcium intakes than Caucasians showed that calcium supplement of 800 mg daily resulted in a significant increase in bone density which was additive to the effect of load bearing exercise for ten months (Lau et al., 1992).

Secondly, the reduced fat mass of older vegetarian women may predispose them to osteoporosis (Gillette-Guyonnet et al., 2000; Marone et al., 1997; Lau et al., 1996). The association between fat mass and bone mass or density is however not significant in older men (Baumgartner et al., 1996, Coin et al., 2000). In postmenopausal women, adipose tissue may have an important endocrine function in preserving bone mass. Firstly, it converts androgens to oestrogen. Secondly, it secretes leptin which may enhance osteoblast differentiation (Thomas et al., 1999).

Thirdly, vegetable and animal protein may have different effects on bone metabolism. Animal foods provide predominantly acid precursors, whereas protein in vegetable foods is accompanied by base precursors not found in animal foods. Imbalance between dietary acid and base precursors leads to a chronic net dietary acid load that may have adverse consequences on bone. However the current data in this area is conflicting. Sellmeyer et al. (2001) found that a lower ratio of animal protein was associated with less bone loss at femoral neck and hip fractures in a seven-year follow-up study of 1037 older white women. On the other hand, in a community-dwelling cohort of 572 women and 388 men aged 55-92 years in California (Promislow et al., 2002) showed a positive association
between animal protein consumption, assessed by food frequency questionnaires in 1988-1992, and bone mineral density, measured 4 years later. This association was statistically significant in women. Conversely, a negative association between vegetable protein and bone mineral density was observed in both sexes.

Fourthly, vegetarian women may have lower serum oestradiol concentrations because of increased faecal excretion (Goldin et al., 1981; Goldin et al., 1982; Armstrong et al., 1981). On the other hand, plant foods, especially soya foods are abundant in phytoestrogens which may preserve bone mass (Potter et al., 1998; Arjmandi et al., 1998; Tham et al., 1998). This will be discussed in more detail in section 2.12.1.

Lastly, vitamin B12 deficiency which is prevalent among vegetarians may have adverse effects on bone health. In a small sized study of vegetarians and omnivores, vitamin B12 intake correlated positively with bone density (Barr et al., 1998). A more recent study showed that serum vitamin B12 concentration was inversely associated with bone density in frail older women (Dhonukshe-Rutten et al., 2003).

2.11.1 Recommended Calcium intake for Older people

There has been considerable controversy about the recommended intake of calcium in general and in older people in particular. This is reflected by the magnitude of differences of recommended intakes among countries, and over time. The RDA in the US in 1989 for calcium was 800 mg daily for older people (National Research Council 1989), which was similar to that in the UK.
(Department of Health, 1991). However, in 1994, the optimal calcium intake for older Americans was recommended to be 1,500 mg daily (National Institute of Health 1994). In 1997, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition board, Institute of Medicine (1997) in the US recommended an adequate intake of 1200 mg daily. The most updated RDA in the UK for calcium intake in men and women aged 19-50 years was 700 mg daily, and the lower limit of adequate calcium intake was set at 400 mg daily.

The main source of contention is about the relationship between calcium intake and bone mineral density. There is general agreement that calcium is an important nutrient for bone mineral density. But calcium intake interacts with other powerful determinants of bone mass e.g. vitamin D receptor genotype (Krall et al. 1995), physical exercise (Specker 1996) and oestrogen (Prestwood et al., 1999).

With the reduction in body mass and therefore body calcium store with ageing, the nutritional requirement for calcium should be lower in old age, because the obligatory loss is reduced. But many randomized controlled trials have shown that very high calcium intakes mostly achieved by oral supplements can reduce bone loss with ageing (Heaney, 2000), However, high calcium intake (>1200 mg daily) as suggested by the Americans is very difficult to achieve. Even in modern Australia, the mean calcium intake in older women was 646 mg daily, and 14 % had calcium intake less than 300 mg daily (Pasco et al., 2000).
2.12 Benefits of Soya Foods

Soy bean products are popular foods among Chinese. According to a large scale health survey of older people in Hong Kong, conducted in 1991, 64% of the 2032 subjects reported intake of soya bean products at least once a week (Ho and Woo, 1994). Understandably, soya bean products are particularly popular among Chinese vegetarians.

Soya bean is nutrient dense, fibre rich and is high-quality source of protein (Anderson 1999). Moreover they are a very good source of isoflavones or phyto-oestrogen which have multiple biological effects (Wagner 2001).

However the benefit of soya products in mortality is not convincing. A cohort study of 29,000 subjects in a defined geographical location in Japan for seven years showed that high soy protein intake was associated with a marginally reduced risk of mortality (95% confidence intervals of hazard ratio 0.69, 1.01; p for trend 0.07) after adjustment for total energy intake and non-dietary covariates (Nagata 2002). In another study of a cohort of 6000 randomly selected Japanese household in 1980-85, age standardized mortality rates for stomach cancer in both sexes, and ischaemic heart disease in women at the year 1995 were inversely correlated with soy protein intake (r=-0.31, -0.31, p = 0.04, 0.045 respectively) (Nagata 2000). But the mortality rate for colorectal cancer was positively correlated with soy product intake (r=0.32, p 0.03 in men, and r=0.44, p =0.001 in women).

There has been much controversy on the effect of soy protein on serum lipids. Although the cholesterol lowering effect of soy protein was well
documented in animal studies (Chait 1993), human studies were inconclusive (Carroll 1991). A meta analysis of 38 studies (mostly random assignment with cross over design) concluded that soy protein consumption is associated with significant decrease in serum cholesterol (95% confidence interval of decrease 0.35 to 0.85 mmol/L), LDL cholesterol (0.30 to 0.82 mmol/L), and Triglyceride concentrations (0.003 to 0.29 mmol/L). The decrease is more significant in those with high initial serum cholesterol concentrations (Anderson 1995).

2.12.1 Soy Proteins versus Isoflavones in Soya Foods

More recent studies attempted to separate out the effect of soy protein from that of isoflavones on serum lipids. Lichtenstein (2002) studied the effect of four different diets lasting for six weeks in a random order on forty two subjects with LDL cholesterol $\geq 3.36$ mmol/L. Diets contained a minimum of 25 g animal protein or isolated soy protein, with each containing trace amounts or 50 mg of isoflavones. Soy protein modestly lowered total and LDL cholesterol and triglyceride concentrations in subjects with LDL cholesterol $\geq 4.14$ mmol/L only (-4%, -5% and -15% respectively). Soy-derived isoflavones had no significant effect on serum lipids. Potter (1998) randomly assigned sixty six hypercholesterolemic postmenopausal women to have a 6 month diet with no soy protein, 40 g soy protein with 1.39 mg isoflavone/g protein per day, or 40 g soy protein with 2.25 mg isoflavone/g protein per day. All diets were low in fat and cholesterol. Soy protein intake significantly decreased non HDL cholesterol. The concentration of isoflavone did not have an independent effect. Therefore soy
protein rather than isoflavone appeared to have a modest lowering effect on LDL cholesterol in hypercholesterolaemic subjects. The apparent benefit of soya food intakes may be mostly attributed to the displacement of fatty foods (Lichtenstein 2002).

There is evidence that the isoflavones in soya foods can preserve bone density in peri menopausal and postmenopausal women. Alekel (2000) randomly assigned sixty nine peri menopausal women to have 24 weeks of isoflavone rich (80.4 mg/day) soy protein, isoflavone-poor soy protein or whey protein. The whey protein group lost an average of 1.28% in spinal bone mineral density, but the soy protein group had no significant change. Analysis of variance showed that isoflavones rather than soy protein exerted the bone preserving effect. In the forementioned trial by Potter (1998), soy protein with 90 mg isoflavones, but not the one with 56 mg isoflavones had a significant spinal bone preserving effect in post menopausal women.

These studies were short term and examined spinal bone only. Trials of longer duration are difficult to perform. But the long-term effect of soya foods on bone health can be deduced from observational studies in countries where soya foods are popular. Indeed, cross sectional studies of postmenopausal women did confirm that soy intake positively correlated with bone density (Horiuchi et al. 2000, Somekawa et al. 2001). In contrast with oestrogen replacement, high soya food consumption is associated with lower breast cancer risk in cross sectional studies in Chinese and Japanese populations (Anderson et al. 1999). Although soy supplementation (45 mg of isoflavones) in eighty four premenopausal subjects
had a weak oestrogenic effect on the breast, it did not lead to epithelial cell proliferation (Hargreaves et al. 1999). Some oestrogen-receptor-positive breast cancer cell lines proliferate in response to soy isoflavone genistein at low concentration, but are inhibited at higher concentrations (Hsieh et al. 1998). Moreover oestrogen-receptor-negative breast cancer cells are inhibited by soy isoflavones (Wang and Kurzer 1997).
2.13 Nutritional Deficiency

It is the stated position of the American Dietetic Association that appropriately planned vegetarian diets should be healthy and nutritionally adequate (Messina & Burke 1997). But the nutritional adequacy of the vegetarian diet remains a concern. Comparative studies between vegetarians and non-vegetarians showed that the vegetarian diet was lower in protein, total fat, saturated fatty acids, cholesterol, but at an adequate level. (Hunt et al., 1988; Ball & Bartlett 1999). However, some studies showed subnormal intake of vitamin B6 and iron (Shultz & Leklem, 1983; Millet et al., 1989). There have been few studies on the dietary intakes of older vegetarians. Two cross sectional studies of healthy older lactovegetarians showed satisfactory dietary intakes (Brants et al., 1990; Nieman et al., 1989).

Most of these studies involved small numbers of health conscious lactovegetarians. A more recent French study examined the nutritional intakes of 145 vegetarians of all ages and included Hindu and macrobiotic vegetarians. It was found that the vegetarians were generally more aware of nutritional requirements than the general public. But the more restrictive the vegetarian diet, the more likely were there deficiencies in minerals and vitamins. Macrobiotic diet and women were particularly at risk (Leblanc et al., 2000).

Overall there is no evidence of inadequate macronutrients in vegetarians. But intakes of some micronutrients require attention. Vegans and those with chronic diseases are likely to be more at risk of such nutritional deficiencies. The
common nutritional deficiencies associated with vegetarian diet will be discussed below.

2.13.1 Vitamin B12 Deficiency in Vegetarians

Vitamin B12 is provided almost exclusively from animal sources. Bacteria in contaminated or fermented vegetarian foods may provide a small amount, but this source becomes less available as hygiene improves with better living conditions. Some algae contain analogues of vitamin B12 which can increase the apparent vitamin B12 concentration measured by competitive protein binding assay but may not be bioactive (Dagnelie & van Staveren 1994). In Western countries, many processed foods including breakfast cereals are fortified by vitamin B12, and most vegetarians consume dairy products and eggs which contain vitamin B12. Asian and Chinese vegetarians have more restricted diets, and tend not to eat breakfast cereals. They may consequently be more at risk of vitamin B12 deficiency. Nutritional megaloblastic anaemia has been reported to be a common problem amongst Hindu Asian vegans in the UK (Chanarin et al. 1985). Other studies of vegans showed that the prevalence of vitamin B12 deficiency was over 50% (Miller et al., 1991; Bar-Sella et al., 1990)

2.13.1.1 Diagnosis of Vitamin B12 Deficiency

Before the development of radioimmunoassay, microbiologic assays overestimated serum vitamin B12 concentrations by detecting other corrinoids which not active in humans (Herbert, 1988). Even with radioimmunoassay, the
sensitivity and specificity of serum vitamin B12 in detecting clinical vitamin B12 deficiency has been questioned (Magnus, 1986, Stabler et al., 1990). Falsely high or low serum vitamin B12 concentrations can be caused by changes in binding proteins (Norman et al., 1982).

Total serum vitamin B12 comprises of that on transport protein – transcobalamin II, and that on storage protein – haptocorrin. Herbert described four stages of vitamin B12 deficiency (Herbert, 1994). In stage one, there is a negative balance of vitamin B12, resulting in a decrease in saturation of transcobalamin. The serum vitamin B12 concentration is not significantly affected, because most vitamin B12 is on haptocorrin. In stage two, the cellular store of vitamin B12 concentration is depleted resulting in a decrease in haptocorrin vitamin B12 and total serum vitamin B12 concentration. However different cell lines may experience different degrees of depletion, depending on the initial amount of storage and the utilization rate. For example the bone marrow and nervous tissues have lower storage than the liver cells. Despite depleted stores, vegetarians can maintain adequate vitamin B12 status for years by upgrading the mechanisms to re-absorb vitamin B12 excreted in bile (Kanazawa and Herbert, 1983) and to absorb the traces of vitamin B12 from bacteria in the small intestine (Albert et al., 1980). In stage three, the depletion of vitamin B12 at the cellular level results in deranged metabolism. In stage four, clinical manifestations of vitamin B12 deficiency appear.

In the search for biochemical markers for deranged metabolism resulting from vitamin B12 depletion, methylmalonic acid (MMA) and homocysteine were
found to be most useful. Vitamin B12 is a coenzyme of L-methylmalonyl-CoA mutase and methionine synthetase. Vitamin B12 deficiency leads to the accumulation of methylmalonic acid (MMA) and homocysteine respectively. Because folic acid is a substrate for methionine synthetase which metabolizes homocysteine into methionine, serum homocysteine concentration is also elevated in folate deficiency (Kang et al., 1987). MMA is therefore a more specific marker of vitamin B12 deficiency. But because clinically vitamin B12 deficient patients occasionally did not have elevated serum MMA concentrations, the combination of serum MMA and homocysteine concentrations are recommended to rule out vitamin B12 deficiency (Savage et al., 1994).

Both serum MMA and homocysteine are elevated by renal impairment (Savage et al., 1994; Friedman et al., 2001). It is therefore recommended that serum folate and creatinine should be checked when MMA and homocysteine are used to screen for vitamin B12 deficiency in older people (Herrmann et al., 2000). Measuring MMA in the urine adjusted by urinary creatinine may obviate this problem (Norman et al., 1982). But urinary excretion of MMA may be increased after food intake (Rasmussen, 1989). Lifestyle factors e.g. smoking, alcohol, caffeine, dietary vitamin B6, riboflavin can also elevate serum homocysteine concentrations (Jacques et al, 2001). Rare inborn errors of metabolism of vitamin B12 can lead to elevated serum MMA and homocysteine, but are usually lethal in infancy (Mahoney and Rosenberg, 1975). Polymorphisms of the methylenetetrahydrofolate reductase gene (Lievers et al., 2001) can cause mild elevation of serum homocysteine. The 677-missense mutation was found to be
present in as many as 35% in older people. But it was not a significant
determinant of serum homocysteine concentrations among older people (Lievers
et al., 1999).

In the older patients, a low serum vitamin B12 concentration (<150 pmol/L)
is usually associated with raised metabolites, indicating tissue deficiency. But
macrocytosis, hypersegmented neutrophils, and raised serum homocysteine
started to appear with serum vitamin B12 <250 pmol/L (Metz et al., 1996). But in
a larger community sample of 548 older Framingham Study cohort, in those with
serum vitamin B12 concentration < 148 pmol/L, the percentage of raised serum
MMA was only around 40%. Similar percentage of raised serum MMA was
observed in those with borderline low serum vitamin B12 concentration (148-185
pmol/L), and the percentage remained significant at concentrations between 185-
258 pmol/L (Lindenbaum et al., 1994). This suggests that tissue vitamin
deficiency can occur in the older people at serum vitamin B12 concentration much
higher than the adult normal range. This could be explained by the very low
concentration of vitamin B12 bound to transcobalamin II – the transport protein.
Marcus et al. (1987) showed that the 17% of total serum vitamin B12 were carried
in transcobalamin in younger people, in comparison to 4% in older people,
irrespective of serum vitamin B12 concentrations. As the older people had normal
concentration of transcobalamin, the defect could be in the delivery of vitamin
B12 to the tissues.
2.13.1.2 Clinical Manifestations of Vitamin B12 Deficiency

Vitamin B12 is a well-established cause of megaloblastic anaemia. The response of megaloblastic anaemia to vitamin B12 supplementation is invariably prompt, provided that there is no co-existing haematological problem. The casual relationship between a variety of neurological disorders and vitamin B12 deficiency is however far from clear.

Healton et al. (1991) reported the largest retrospective study of clinical neurological disorders attributed to vitamin B12 deficiency on the basis of significant clinical response to vitamin B12 supplementation and subnormal serum vitamin B12 concentrations. Over a period of 17 years in two New York hospitals, 153 episodes of vitamin B12 related neurological disorders were carefully described. Most of these patients had pernicious anaemia. The commonest neurological symptom was parasthesia (70%), followed by ataxia (29.8%). The commonest neurological sign was diminished vibration sense (69.9%), followed by proprioceptive loss (47.1%). These suggested that myelopathy was more common than peripheral neuropathy. Mental impairment was only observed in 18 episodes (11.8%), but patients thought to have Alzheimer’s disease were excluded. Most subjects had signs of improvement by three months of vitamin B12 supplementation. Shorter duration of symptoms and milder severity of impairment were associated with better recovery.

The most striking observation of this study was that the haematocrit was proportionally correlated with the severity of neurological deficits and that 16.3% of the episodes had normal haematocrit and mean corpuscular volume. This
suggests that the neurological manifestations of vitamin B12 deficiency may occur before the deficiency is sufficiently severe to affect the bone marrow. This is consistent with the observation that acute exposure to nitrous oxide during anaesthesia inactivates vitamin B12 and can lead to cytopenias (Ames et al., 1978). But chronic low dose exposure to nitrous oxide for months can lead to neurological impairment without anaemia (Layzer, 1978).

It is commonly believed that folate supplementation can precipitate neurological damage in the presence of vitamin B12 deficiency. It may be that folate protects against haematological manifestations while having no effect on neurological manifestations per se (Herbert, 1994). In the Healton study, four patients were given folate supplements inadvertently before vitamin B12 deficiency was diagnosed. Two of them appeared to have deteriorated after folic supplementation. But there was no overall correlation with serum folate concentration and the severity of neurological impairment (Healton, 1991). Vegetarians can be expected to have better folate status and worse vitamin B12 status than the general population. Whether this combination put them on increased risk of neurological complications from vitamin B12 deficiency is an important question to consider.

It is now recognized that there are many older people with evidence of metabolic vitamin B12 deficiency may have no or very little symptoms or signs. In a cross sectional study of 303 ambulatory older American veterans, a broader definition of deficiency (serum vitamin B12 < 258 pmol/L and MMA or homocysteine elevated by more than two standard deviation) did not show any
correlation with cognitive impairment and general health as measured by the RAND 36-item health survey questionnaire. Only the 19 subjects with low serum vitamin B12 (<150 pmol/L) reported more bodily pain and were more likely to have cognitive impairment as defined by subnormal mini-mental state examination scores (Bernard et al., 1998).

In a follow-up study of older patients with raised plasma MMA at 1.0 to 3.9 years after being investigated for suspected vitamin B12 deficiency (Havas et al., 2001a), the 432 responders showed high variation in plasma MMA concentrations from baseline (coefficient of variation 34%), and plasma MMA concentrations did not significantly correlate with neurological disability score (a summed score of muscle strength, reflexes, and sensory loss) and neurological symptoms. There was a weak but significant correlation between follow-up plasma MMA and haemoglobin concentration. Those with plasma MMA increased by 20% or more were not significantly different from those without the increase in neurological disability score, neurological symptoms or haemoglobin concentration. The limitation of this study is that the response rate was only (51%) and the responders were significantly older and had higher baseline plasma MMA concentrations. Consequently, the responders only had marginally high plasma MMA concentrations (median 0.33, range 0.29-0.36µmol/L). The cutoff value of serum MMA for clinical vitamin B12 deficiency is 0.4 µmol/L ((Savage et al., 1994). The fact that these subjects did not receive vitamin B12 supplementation suggested that the attending physician did not think that the deficiency was significant or clinically relevant. The design of the study was therefore biased
towards a negative result.

The same group randomized 140 subjects with plasma MMA between 0.4 and 2.0 μmol/L (Havas et al, 2001b). Those with anaemia, macrocytosis and plasma creatinine >120 μmol/L were excluded. The subjects had one weekly injection of 1 mg vitamin B12 or normal saline for 4 weeks. At three months follow-up, despite a significant reduction in plasma MMA in the treatment group, there was no group difference in changes in neurological symptoms, neurological disability score, haemoglobin concentration and mean red cell volume. However, the plasma vitamin B12 concentrations of these subjects were not low (median 278, 254 pmol/L in treatment and placebo groups respectively), suggesting that the vitamin B12 deficiency was not severe. The sample size might not be adequate because neurological disability score was based on clinical examination which is not sensitive to change, and neurological symptoms are prevalent and variable among older people. The vitamin B12 supplementation regime was also probably not adequate for neurological improvement.

The overall impression of the results of these studies is that the clinical manifestations of metabolic vitamin B12 deficiency in older people are observable only when the deficiency is moderately severe, and these manifestations are subtle. The vitamin B12 status probably fluctuates significantly in borderline cases of deficiency. The long-term health hazards of asymptomatic metabolic vitamin B12 deficiency, particularly in cognitive function in older people, is yet to be determined.
2.13.2 Iron Deficiency in Vegetarians

Iron is present in plant foods in the non-haem form which is less easily absorbed than haem iron from animal sources. Moreover, the bioavailability of non-haem is affected by a number of factors. For example, ascorbic acid enhances iron absorption while tea (Gillooly et al. 1983) and phytates (Cook & Monsen 1976) inhibit its absorption. Soybean is rich in iron, but it has been shown to inhibit iron absorption. Its phytate content may be responsible as iron absorption was shown to increase substantially when phytates were removed (Hurrell et al. 1992). But its protein also plays a major role, as food processing modifies the availability of iron. For example, fermented soy product showed enhanced iron absorption when compared with soy flour (Macfarlane et al. 1990).

The low bioavailability of non-haem iron of vegetarian diets was demonstrated in a cross sectional study showing that despite adequate intake of iron in 50 mostly nonvegan vegetarians, 38% of them were iron deficient according to serum ferritin concentrations (Alexander et al. 1994). Similar finding was obtained in a cross sectional study of young Chinese Buddhist vegetarians and medical students in Taiwan (Shaw et al. 1995).

Western vegetarians usually have adequate iron intake from fortified, cereals, brown bread and milk. They are also likely to have good intake of vitamin C and take vitamin supplements. But Asian vegetarians, especially those in poorer regions, are vulnerable to iron deficiency because their staple foods, e.g. rice are very low in iron content, and soya products and tea are often consumed. A cross sectional study in the UK showed that iron deficiency was prevalent in both
Caucasian and Asian young healthy female vegetarians, but the latter tended to be more deficient and had lower haemoglobin concentrations and mean corpuscular volumes (Reddy and Sanders 1990).

2.13.3 Other Minerals in Vegetarians

Vegetarian diets often have low content of zinc and selenium. Moreover their absorption is impaired by phytates and fiber (Gibson, 1994). But there is no evidence that their status in western lactovegetarians is compromised (Gibson 1994; Hunt et al. 1998).
2.14 Immunity of Vegetarians

There are some suggestions that strict vegetarianism is associated with increased risk of tuberculosis. In a survey of 1187 Indians in London, vegetarians had a 2.8 fold increase in history of tuberculosis when compared with Indians on mixed diets (Chanarin & Stephenson, 1988). A case control study compared the characteristics of 56 Asian patients with history of tuberculosis in the previous ten years with 100 age sex matched Asian controls recruited by postal invitation and from hospital outpatient clinics. It was found that lactovegetarians had an 8.5 fold risk of tuberculosis when compared with daily meat/fish eaters. However, the study had methodological problems and failed to control for other significant factors like diabetes mellitus (Hayward et al., 1995), and vitamin D deficiency (Wilkinson et al., 2000). Moreover, a cross sectional study showed that vegans did not differ from non-vegetarians in functional immunocompetence as measured by mitogen stimulation or natural killer cell cytotoxic activity (Haddad et al. 1999). In additional, a cross-over trial of a 6 week lactovegetarian diet in 8 atheletic men showed no change in in-vitro immunity functions (Richter et al. 1991).
2.15 Conclusion

There is strong evidence that vegetarian diets can prevent ischaemic heart disease, obesity, lower serum cholesterol and blood pressure, and reduce risk of colonic and prostatic cancers. Fruit and vegetables, particularly soya foods which are very popular among Chinese vegetarians, are beneficial to cardiovascular health and cancer risk. There is evidence that heavy intakes of meat increase cardiovascular and colonic cancer risks. But there is no evidence that the absolute exclusion of meat and fish is beneficial.

On the other hand, vegetarians are at great risk of vitamin B12 deficiency. Its neurological and haematological manifestations are independent of each other. Milder deficiency is often asymptomatic, but the associated rise in plasma homocysteine concentrations may be detrimental to cardiovascular health. Vegans have additional risks in deficiency in calcium and iron. They may also be at risk of osteoporosis and impaired immunity. As most vegetarian studies were conducted in Western countries where most vegetarians consume dairy products, there is scarcity of data on vegans, older vegetarians, and vegetarians in countries outside the Western World.
2.16 Key Points

1. There is evidence that vegetarian diets prevent ischaemic heart disease and reduce the risk of some cancers. These benefits may be attributed to antioxidants, isoflavones contents of fruit, vegetables and soya foods, and lower intake of saturated fat.

2. There is no evidence that these benefits are derived from exclusion of meat intake.

3. More restrictive vegetarian diets are associated with increased risk of dietary deficiencies, especially vitamin B12 deficiency.

4. Vitamin B12 deficiency is often subclinical. Its effect on cognitive function of older people is uncertain. The associated hyperhomocysteinaemia is an important cardiovascular risk factor.
Chapter 3  Research Outline

3.1  Health Survey of Older Vegetarians (Main Study)

Vegetarians in three old age homes (OAH) for vegetarians – Sin Tin Dao, Po Ching and Chi Lin and Sin Tin Dao Taoist followers in the community were invited to have a comprehensive health survey in a research clinic in the Prince of Wales Hospital (PWH), a regional teaching hospital in Hong Kong in 1995. The background of these religious groups and old age homes was described in Chapter One (see 1.2.1 and 1.2.2). The inclusion criteria were age greater than 55 years and exclusive vegetarian diet for ten years or more. The study was approved by the Clinical Research Ethics Committee, the Chinese University of Hong Kong. The study was supported by the collaborating colleagues of the author, Professor Jean Woo, Department of Medicine, Professor Edith Lau, Department of Community and Family Medicine and Professor Calvin Pang, Department of Chemical Pathology, without any external funding support. There was no conflict of interest about individuals conducting the study.
3.2 Subjects of Main study

One hundred seventeen female subjects attended the research clinic. No male subject was recruited. Four of them had been vegetarian for less than ten years. They were included as supplementary subjects in studies related to vitamin B12 status only. All the other subjects were self ambulatory except two who required personal assistance on walking. These two subjects were regarded as disabled, and were excluded from all studies which involved comparison with historical control subjects, as all the control subjects were community dwelling and independent. But the disabled vegetarian subjects were included in studies related to vitamin B12 status.

Therefore a total of one hundred and eleven female independent long term (ten years or more) vegetarian subjects were recruited for comparison with omnivorous control subjects. Their average age was 78.0 ± sd 7.3 years old (range 56-94 years). 66 (59.5%) of them were vegans, the rest being lactovegetarians. 57 (51.4%) of them were OAH residents (13, 26, 18 in Sin Tin Dao, Po Ching, and Chi Lin OAH’s respectively). The remaining 54 subjects were community dwelling Sin Tin Dao Taoist followers, except one Buddhist.

Written consent was obtained. All the following measurements were performed by two research nurses. Venesection was performed by author, and laboratory tests were performed in the Chemical Pathology and Haemtaology Department of PWH.
3.3 Measurements of Main Study

3.3.1 Anthropometry

1. Body mass index

Body weight (Wt, kg) was measured by a hospital beam balance with subjects wearing a light hospital gown. Standing height without shoes (Ht, cm) was measured by a height measure attached to beam balance. Body mass index was calculated by the equation: \( \frac{Wt}{Ht^2} \) kg/m².

2. Upper limb anthropometry

(a) Skinfold thickness was measured to the nearest 0.1 mm by a pair of Holtain calipers (Holtain Ltd., Crymych, Dyfed) in duplicate in the following sites of the extended and relaxed left arm: 1. Triceps (TSF), midpoint between acromion process and the olecranon process; 2. Biceps (BSF), at the same level as TSF, directly above the center of the cubital fossa.

(b) Mid-arm circumference (MAC) was measured to the nearest 0.1 cm at the same level as TSF with a soft tape measure. The average of two readings was taken.

Upper arm anthropometric measurements are subject to interobserver and intraobserver variation. The main source of variation comes from the location of skinfold. Variation can be minimized by training (Sullivan et al., 1989). The two research assistants were experienced in performing skinfold thickness measurements. Their reproducibility in these measurements was assessed by repeated measurements in six normal adult subjects. The inter-
observer coefficients of variation for BSF, TSF and MAC were 20%, 15% and 2% respectively.

(c). Arm muscle circumference (AMC) was derived from the equation: AMC (cm) = MAC (cm) – (π x TSF (mm)/10)

(d). Corrected arm muscle area (CAMA) was calculated by the equation: CAMA (cm²) = ((AMC)²/4 π) – 6.5

This is used as an indicator of protein nutritional status. It has been shown to be predictive of mortality of geriatric inpatients (Muhlethaler et al., 1995). A local study of community dwelling older people showed that CAMA correlated moderately well with lean mass estimated by Dual energy X ray absorptiometry (correlation coefficient 0.57 in female, 0.65 in male; Kwok et al., 2001a).

(e). Body fat percentages (Fat%) was calculated by the following equation for female, which was locally validated against Dual energy X ray absorptiometry (Kwok et al., 2001a): Fat% = 12.274 +1.12 x BMI + 17.308 x log (TSF + BSF) –27.149.

This equation was derived from 533 apparently well Chinese people aged 70-81 years in Hong Kong. Standard error of estimation was 4.1% weight, which was comparable to similar predictive equations for fat percentages in adults. There was a significant trend of under-estimation in fat subjects in female. In medical outpatients, the under-estimation was more significant, probably because of central obesity (Kwok et al., 2001a).
3.3.2 **Clinical Measurements**

1. Supine blood pressures were taken by a trained nurse using a standard mercury sphygmomanometer, after the subject lay on the couch for five minutes. Korotkoff phase V was taken as the diastolic pressure. Blood pressures greater than 140/90 were considered to be raised.

2. A resting electrocardiograph (ECG) was performed by a trained nurse and read by a physician (T Kwok). The Minnesota Coding (Rose & Blackburn, 1968) was used to classify ECG. The Whitehall Criteria (Rose et al., 1977) were used to classify the ECG into probable (codes 11,1.2) or possible (codes 1.3, 4.1-4.4, 5.1-5.3, 7.1) ischaemic heart disease.

3.3.3 **Questionnaire (see appendix A)**

1. Mental test score- the information / orientation section of the Clifton assessment procedure for the elderly (Pattie & Gilleard, 1976). This test has 12 brief questions, primarily about orientation. Because of its conciseness, it has been used in population studies. It has high sensitivity and specificity when compared with more detailed tests such as CAMDEX (Brayne and Calloway 1989, Clarke et al. 1991). This questionnaire has been translated into Chinese, but has not been validated. Nevertheless this questionnaire is relatively less dependent on educational level and cultural background. Scores below 8 were considered to indicate cognitive impairment (Victor, 1991). In a local study involving a randomly selected sample of 2,011 people above 70 years old, 22% of women had
scores below 8, which was comparable to that in Mainland China and Western countries (Woo, 1994).

Nineteen subjects (16.8%) and ten subjects (8.8%) of the vegetarian subjects had mental test score less than 8 and 7 respectively. In view of the fact that the cutoff value of mini mental state examination was shown to be lower than that in Western countries because of lower educational level of our local older population (Chiu et al., 1994), and the number of available vegetarian subjects was limited, I decided to use a lower cutoff value of 7, instead of 8, in excluding subjects from analysis of questionnaire data and 24 hour recall dietary data (see below).

2. Cardiovascular questionnaire- WHO Cardiovascular Questionnaire (Rose et al., 1982) translated into Chinese was administered. It is widely used in population surveys. It had been compared with doctor’s diagnosis in older people aged 65-95 years, and the sensitivity was 79%, specificity 98% and predictive value 88% (Dewhurst et al., 1991). To ensure reliability, 10 subjects who had mental test score less than 7/12 were excluded from analysis.

3. Enquiry on general health, physical activity, smoking and alcohol intake. Subjects were asked if they performed regular physical exercise, and its duration. They were only regarded to perform regular exercise if the duration was more than 20 minutes.

4. Past history of ischaemic heart disease, hypertension, diabetes mellitus, strokes, cancer etc and medication were specifically enquired about and noted.
This questionnaire was identical to part of the one used in a population survey conducted in Hong Kong in 1991 (Ho & Woo, 1994).

3.3.4 Dietary Assessment

1. 24-hour recall method

   The research assistants were trained to perform the assessment. The subjects were asked to recall the actual food and drink consumed in the previous 24 hours. In order to facilitate estimation of quantity, subjects were shown a bowl, a plate, a spoon, and a cup, and asked to quantify as portions of these common household measures. These measures were converted into weight at time of coding. The food composition analysis was performed by computer software based on food composition table for South East Asia (US Department of Health, Education and Welfare, 1972). To ensure reliability, 10 subjects who had mental test score less than 7/12 were excluded from the dietary recall.

   24 hour recall method of dietary assessment is not as accurate as weighed food records. It may be more problematic in older people because of memory impairment. It also does not take into account of day to day variations. It is however useful for group comparison.

2. Use of vitamin or mineral supplements, dietary supplements, milk and eggs were enquired.
3.3.5 Bone Density

Bone densities at lumbar spine L1-L4 in anteroposterior projection, and at proximal femur were measured by Dual energy X ray absorptiometry (DXA, Hologic QDR-2000 bone densitometer, Software: Enhanced Array Whole Body V5.67A). Calibration was performed daily on a lumbar spine phantom, and the coefficients of variation for the spine, total hip were 0.65% and 0.78% respectively.

3.3.6 Laboratory Tests

1. Blood – 15 ml of fasting blood was taken. A small portion of whole blood was taken for complete blood count including erythrocytes, leukocytes, platelets, haemoglobin, and mean cell volume, on a Coulter autoanalyzer (Coulter Electronics, Oakville, Ontario, Canada). Serum was obtained from the rest of the sample. One mL was stored at -20 °C for analysis of methylmalonic acid (MMA) within 2 months. The remaining serum sample was analysed for cobalamin and folate by solid-phase no-boil Dualcount radioimmunoassay with radioisotopes $^{57}$Co and $^{125}$I purchased from Diagnostic Products Corporation (Los Angeles, CA, USA), and biochemistry tests of glucose, renal function test, cholesterol, triglyceride, iron, transferrin, total iron binding capacity, and albumin on a Dimension AR chemistry analyzer (Dupont, Willmington, DE, USA).

2. Urine – 20-50 mL of morning fasting urine sample was analyzed for creatinine, sodium and potassium. Urinary creatinine was determined by the Jaffe’s reaction (kinetic) without deproteinization on a Hitachi 911 autoanalyser.
(Boehringer Mannheim, Mannheim, Germany). Part of urine sample was frozen at –20°C and analyzed for MMA within two months.

3.3.7 Serum and Urine Methylmalonic acid (MMA) assay

The method of Gas Chromatography-Mass Spectrometry, described by Marcell et al. in 1985, was used with some modification. 2-Methyl-propanedioic acid (MMA) was purchased from Sigma (St Louis, MO, USA), d3-MMA, 99.946%-d3, was obtained from C/D/N Isotopes Inc (Vaudreuil, Quebec, Canada). Perfluorotributylamine (PFTBA) from Hewlett Packard was used as the calibrator for mass spectrometry. N-methyl-N-(tert-butylidimethylsilyl) trifluoroacetamide (MTBSTFA) and dimethylformamide (DMF) were of silylation grade purchased from Pierce (Rockford, IL, USA). All the assays were performed for not more than two months after sample collection by a MSc research student who was also a medical technologist in the Department of Chemical Pathology, Prince of Wales Hospital.

Extraction of MMA To 0.5 mL of serum in a screw-capped centrifuge tube, 30 μL of d3-MMA (300 ng) as internal standard, 1 mL of deionised water, 20 μL of concentrated hydrochloric acid (37%) and 5 mL of diethyl ether were added. After vigorous vortex for 5 minutes, the organic phase was collected after centrifugation. The aqueous layer was extracted with another 5 mL of diethyl ether. The collected ether layer was transferred to a conical centrifuge tube for dryness. After drying by evaporation in nitrogen, the residue was redissolved in 1 mL of distilled water, which was acidified again with 20 μL of concentrated
hydrochloric acid (37%). After being saturated with sodium chloride, the mixture was extracted with 3 mL of ethyl acetate for 3 times. All the extract was pooled into the same vial and blown to dryness under a stream of nitrogen. These ether extraction steps were not required for urine samples. 50 μL of freshly prepared derivatizing agent (equal parts of MTBSTFA and DMF) was added and whirled gently. The derivatization reaction was performed in an oven at 80°C for 1 hour. After derivatization reaction, the mixture was transferred to a mini-vial for GC-MS analysis.

Gas Chromatograph-Mass Spectrometry analysis - A Hewlett Packard (Palo Alto, CA, USA) 5890 II gas chromatograph equipped with a HP 7636 A auto-sampler was interfaced to a HP 5970A mass spectrometer through a HP-1 100% methylsilicon capillary fused silica column (30 m length, 0.1 mm ID, 0.33 μm film thickness). Helium was the carrier gas at a column pressure of 160 kPa and a column flow rate of 1.13 mL/min. The injector temperature was 250 °C and the split ratio of inlet to column gas flow was 15:1. The MMA derivatives were separated by a 1-ramp GC temperature program: initial temperature at 130°C increased at 8°C/min to 260°C, solvent delay 4 min. The GC analysis was completed in 16.25 min. The mass spectrometry was carried out in electron ionisation mode and both ions with m/z 403 and 406 were monitored. Quantification was based on the ratio of the peak areas of ions with m/z 403 to those of ions with m/z 406.

The imprecision (CV) of the method was estimated by analysis of two serum-based (0.25 and 0.42 μmol/L) and two urine-based (1.13 and 8.23 umol/L)
QC samples within one month. The measured value for each sample was read off a calibration curve obtained in the same run. The imprecision of the whole procedure was as follows: within-run CV for serum MMA were 9.6% and 3.4% respectively and for urinary MMA, 6.4% and 3.1% respectively; and inter-assay CV for serum MMA, 17.4% and 12.8% respectively and for urinary MMA, 7.9% and 7.0% respectively. The lowest detection limit, defined at the minimum concentration of QC sample with a CV < 15% in a run, was 0.127 μmol/L, which was comparable to other chromatographic techniques (Rasmussen et al., 1990).
3.4 Control Subjects in Main Study

Local historical non-vegetarian older subjects were used to compare with older vegetarians in dietary intakes, nutritional status, blood pressures, urinary sodium and potassium: creatinine ratios and prevalence of ischaemic heart disease. These studies were shown in the following sections.

3.4.1 Multidimensional Health Assessment of the Elderly living in Sheltered Housing in Hong Kong (Woo, 1988)

The sheltered housing complex consisted of flats shared by up to six elderly people, and was located near Prince of Wales Hospital. The residents were supervised by staff of the Social welfare Department. They should be capable of self care and had a housing problem. They had to cook for themselves. The response rate was 96%. 176 men and 250 women were recruited. The mean age of the older women was 70.7 (sd 4.7) years.

They attended a research clinic in PWH. They had height and weight measured, blood pressure taken at sitting position by a standard sphygmomanometer. The average of two readings was taken. A casual urine sample was collected for urinary electrolytes.

24 hour dietary recall was administered by a trained nurse. The quantity of nutrients was calculated using food composition tables for South East Asia (US Department of Health, Education and Welfare, 1972). There was no difference between the above 70 years olds and the below 70 years olds in dietary intakes.
The dietary intakes were comparable to another local cohort study of 1,000 older subjects living with relatives and friends (Ho et al., 1988).

The female subjects of this study were used to compare with the older vegetarians in dietary intakes, blood pressure, urinary sodium and potassium: creatinine ratios.

3.4.2 Health Survey of Older People in Hong Kong (Ho & Woo, 1994)

A health survey was carried out on a group of older Chinese in Hong Kong, aged 70 and over, selected from a registered list of all recipients of Old age and disability allowances. The old age allowance list covered 90% of the elderly age 70 years and over, independent of income. Subjects were stratified by age and sex, such that there would be 300 subjects in the 70-74 and 75-79 age groups in each sex, and 150 subjects in the 80-84, 85-89 and 90+ age groups in each sex. The response rate was 60% (997 men and 1035 women). A physical health questionnaire was administered.

Of these subjects, all those living in the region of PWH were invited to attend a research clinic in PWH. About 60% of them did so. They had the following measurements: resting ECG, WHO Cardiovascular Questionnaire (Rose et al., 1982), Information/orientation section of the Clifton Assessment procedure for the elderly, weight and height, non-fasting blood taken for cholesterol and triglyceride. Information was also obtained on past medical history of ischaemic heart disease, diabetes, hypertension, smoking habit, use of alcohol, and exercise habit etc. Full data was available in 96 men and 101 women.
The female subjects of this study were used to compare with the older vegetarian women in the prevalence of ischaemic heart disease.

3.4.3 One-year Follow-up Study of Vertebral Fracture in Older Women in Hong Kong (Lau et al., 1996)

481 women aged 71-80 years, community dwelling and functionally independent, were initially enrolled an epidemiological study of vertebral fracture in Hong Kong. 400 of these returned for the one-year follow-up. They had bone mineral density (BMD) of spine and hip measured by Dual energy X ray absorptiometry (DXA, Hologic QDR-2000 bone densitometer, Software: Enhanced Array Whole Body V5.67A). In addition, height without shoes, weight with light hospital gown, armspan, triceps and biceps skinfold thicknesses (TSF, BSF), and mid-arm circumference were measured. Exercise habit, smoking, alcohol consumption, intake of dairy products, history of backpain in the previous 12 months were enquired by a structured questionnaire.

These subjects were used to compare with the older vegetarian women in bone density and nutritional status.

3.4.4 Recruited Control Subjects

Two groups of omnivorous control subjects were specifically recruited for comparison with the older vegetarians because of the lack of local data.

1. Comparison of vitamin B12 status of vegetarians and omnivores
Twenty six subjects above 55 years old non-vegetarian residents in accommodation provided by a Taoist temple – Cheuk Lam temple were screened for vitamin B12 deficiency by fasting urinary MMA, serum MMA, and vitamin B12 concentration. These subjects shared the same lifestyle of the vegetarians in the temple. No formal dietary assessment was performed.

To ensure that the above control subjects had similar vitamin B12 status as normal older people in Hong Kong, their urinary MMA concentrations were compared with those of 107 subjects (71.1% female) above 55 years old in an old age hostel.

2. Dietary intakes of omnivorous old age home residents

40 female omnivorous old age hostel residents in two homes volunteered for 24-hour dietary recall. All were functionally independent. They were assumed to be mentally alert and no mental score was performed.
3.5 Supplementary Vegetarian Subjects for Vitamin B12 Studies

In order to recruit more vegetarian subjects for vitamin B12 related studies, additional subjects were recruited from four vegetarian old age homes for serum vitamin B12, MMA and urinary MMA (Chi Lin 13, Sin Tin Dao 12, Cheuklam 9, Tung Wah 6). The 13 subjects in Chi Lin OAH also had blood analyzed for complete blood count and biochemical indices as in the Main study. These additional subjects from Chi Lin and Sin Tin Dao OAH’s tended to be frailer than the ones in the main study, because they did not volunteer to attend the research clinic in the first instance.

Cheuklam was a Taoist temple. As a charity, it provided sheltered housing for approximately 50 older people. The residents looked after themselves and were allowed to eat their own foods. It was regarded as an old age hostel. Tung Wah OAH was a 100 bed subvented OAH for older vegetarian men, situated next to Cheuklam Temple. Residents were not obliged to be exclusive vegetarians.
3.6 Outline of Other Studies

Subsequent to the main study, three further studies were conducted. Two of which were related to the clinical significance of vitamin B12 deficiency which was found to be very prevalent among older Chinese vegetarians. The methodology of each study would be described in detailed in its corresponding chapter. However, the studies are outlined below according to the time sequence.

3.6.1 Randomized Trial of Supplementation on the Cognitive Function of Older People with Subnormal Cobalamin Concentrations (Chapter 11)

Between 1995 and 1996, fifty Chinese subjects more than 60 years old with serum cobalamin concentration < 120 pmol/L were randomised into supplement and control groups. Thirty-seven of these subjects (74%) were subjects of the main health survey. Five other subjects were recruited after screening in vegetarian old age homes, and eight subjects were medical patients who were found to be vitamin B12 deficient because of dementia (5) and mild anaemia (3).

Fasting serum methylmalonic acid concentrations (MMA) were measured. A battery of neuropsychological tests (see below), administered by three master level clinical psychology trainees. Supplement group received intramuscular cyanocobalamin injections, while control group received no intervention. They were followed up at an average of four months.

The study was approved by the Clinical Research Ethics Committee, the Chinese University of Hong Kong. The study was supported by the Direct Grant
from the Chinese University of Hong Kong. There was no conflict of interest about individuals conducting the study.

3.6.1.1 Neuropsychological Tests

All subjects of the trial had a battery of neuropsychological tests (see appendix B): Mini mental State Examination (Chiu et al., 1994), General Health Questionnaire (Chi & Boey, 1994, Chi, 1995), Wechsler Memory scale (Wechsler, 1987), Wechler Adult Intelligence test – revised (Wechsler, 1981) and Luria Nebraska Neuropsychological Battery (Golden, 1981). Because of the limitation in time and attention span of older people, items from the last three test batteries were selected to examine verbal and performance IQ, visual and verbal memory, and motor functions. The selection was based of feasibility in older people and the scope of cognitive function covered.

The tests were administered by three master level clinical psychology trainees and took about one hour to complete. Because of the time availability of the research assistants, it was not possible to arrange for the same assistant to perform the baseline and follow-up tests. No reproducibility study was undertaken. As in other neuropsychological tests, a practice effect could be expected (Larrabee and Crook 1988). But this was unlikely to last as long as four months.

The details of the neuropsychological tests administered are described as follows:

1. Mini-mental state examination
This test has 11 items, covering various cognitive functions e.g. language, memory, attention, and copying design. The overall score is useful in screening for dementia. It has been translated and validated in older people in Hong Kong. The cutoff value of 20 out of 30 was found to have a sensitivity of 97.5% and specificity of 93.7% in distinguishing patients with dementia from normal subjects (Chiu et al., 1994).

2. General Health questionnaire

This is a generic questionnaire consisting of 30 questions to assess the general mood and anxiety of adults. To make it easier for older people to answer, the original four graded answers were reduced to yes and no answers. It has been translated in to Chinese and validated in Hong Kong (Chi & Boey, 1994, Chi, 1995).

3. Wechsler Memory scale (Wechsler, 1987)

(a). Visual memory – It consisted of four items, of which the first three contained a single figure, and on the fourth card had two figures. The cards were shown to the subject for five seconds, and the subject was asked to draw the figure by memory. Thirty minutes later, subjects were asked to reproduce the four figures again. Scoring guidelines were available in the manual. The maximum combined score was 82. Uneducated people tended to do badly in this test.

(b). Verbal memory - A contemporary story was told by the examiner. The subject then recalled the story. The story contained 23 memory units. The subject gained one point for each idea recalled. Thirty minutes later, the subject was asked to
retell the story. The maximum combined score was therefore 46. This test was subject to the interpretation of the examiner, and the education level of the subject.


This is a widely used test battery to examine intelligence of all ages. There is no local validation or normative data.

(a). Similarities –

It was designed to assess abstract thinking. It asked the client to identify how 14 successive pairs of nouns were alike. Testing began with the first item for all clients and proceeded throughout the series. The clients’ responses were scored 2, 1, 0 points for satisfactory, partially satisfactory and unsatisfactory answers. Subjective scoring by examiners was required, though general criteria and examples were available in the manual. The maximum score was 28.

(b). Block design –

It was a measure of general intelligence, and required a significant degree of visual-spatial ability. It was considered to culturally free and relatively less affected by educational levels.

The client was presented with nine cards depicting geometric designs in white and red, and with a number of identical plastic cubes each having two red, two white, and two red and white sides split on the diagonal. The task was to reproduce the design on the card with the cubes. The client’s performance was timed; after the second item, additional points were awarded for rapid, errorless performance.
The first design was copied by the client from a model constructed by the examiner. In the second design, the examiner demonstrated how to construct model by copying a stimulus figure on the card. A second demonstration and trial was allowed for initial failure, and half credit awarded if successful. The first five designs were constructed with four blocks and one minute was allowed. From design 6 onward, the designs were constructed with nine blocks, and two minutes were allowed. In the final design, the figure was presented after being rotated by 45 degrees by the examiner.

Scores from Similarities were used to estimate the Verbal IQ, while scores of Block design were used to estimate Performance IQ, by referring to published Tables for conversion (Brooker & Cyr, 1986).

(c). Digit span test

This is a subset of the revised Wescher Adult Intelligence test designed to examine attention. It consisted of two different tasks – 1. immediate recall for the repetition of increasing numbers of digits, 2. repeat backwards an increasing series of numbers. The scoring is simple: the item is passed if repeated or reversed exactly correct. Two trials are given for each digit length and both count toward the raw score. Digits forward begins with a three-digit number and proceed until both trials of a series are failed. Digits backwards begins with two three-digit practice items and proceed with a two-digit series and continues until both trials of a series are failed. Digits forward had maximum span of 8 digits, and digit backward had maximum span of 7 digits. The maximum total score was 24.
Lezak suggested that span of six forward was normal, five marginal, and three
defective (Lezak 1983, p. 268).

5. **Luria Nebraska Neuropsychological Battery**

The examination techniques were first developed by AR Luria as qualitative tests of the brain damaged adults. They were collected and organized by AL Christensen. Golden and his colleagues (Golden, 1981) later selected items from Christensen’s manual to form a battery so that Luria’s test procedures can be administered and evaluated in a standardized manner.

There are 11 clinical scales: Motor functions, Rhythm, Tactile functions, Visual functions, Receptive speech, Expressive speech, Writing, Reading, Arithmetic, Memory, and Intellectual processes, involving 269 items. Performance on each item is evaluated on a three-point scale, from 0 for no impairment to 2 for severely impaired.

The Motor function was the only clinical scale used. It consisted of 51 items. In 15 items, there was a time limit of 10-20 seconds. In 6 items, quality of drawing was scored. The total score is the simple summation of the scores in all items. The scale can be divided into 5 subscores: Kinesthesia, Drawing, Fine motor, Spatial movement, and Oral movement. This clinical scale took about 20 minutes to administer.
3.6.2 The Association between Dental Status and Dietary Intakes among Chinese Vegetarian Old Age Home Residents (Chapter 12)

In 1999, a cross sectional study was conducted to examine the relationship between dental status as determined by a fully trained dentist and dietary intakes as assessed by 24 food weighing records. The study was conducted in Sin Tin Dao OAH, one of the homes involved in the main survey in 1995. Complete data was available in 68 out of 86 residents. The study was approved by the Clinical Research Ethics Committee, the Chinese University of Hong Kong. The study received no external funding. There was no conflict of interest about individuals conducting the study.

24-hour food weighing records are more accurate than 24 hour dietary recall, because it is not dependent on memory. Ideally it should be performed over 3 days or more. However we only managed to do it on one day. The dietary intakes of the subjects were measured by a 24 hours food record by two postgraduate students in dietetics at weekdays. Food intake at breakfast, lunch and dinner was estimated by firstly weighing and averaging individual items of three standard portions, and then the food left over by individual residents was directly observed and recorded by research assistants. Snacks consumed during the day were also recorded by the researchers. But the bedtime snacks consumed the previous night were recorded and samples of the snacks were presented to research assistants by the nursing home staffs. Most subjects consumed half a glass of oral nutritional supplement (Ensure) daily. The nutritional composition of the consumed foods was calculated by Food tables from multiple sources – UK
and two Mainland Chinese institutions (Holland et al., 1992; Tsang and Fung, 1992; Institute of Health, Chinese Medical Sciences Institute, 1992). This database could only calculate crude fibre content.

The dental status of all the residents of the same vegetarian old age home was examined by a fully trained dental surgeon. All residents underwent a standardized oral examination procedure with the diagnostic criteria based on the WHO standard. Data included the Decayed-Missing-Filled-Teeth value (DMFT), number of functional teeth unit(s) (see 3.4.3.1), denture status, subjective view on masticatory function and reasons for difficulty in eating were recorded.

3.6.2.1 Decayed-Missing-Filled-Teeth value and Functional Teeth Units

1. DMFT: According to the Department of Noncommunicable Diseases Surveillance/Oral Health (WHO Collaborating Centre, Malmö University, Sweden), DMFT describes the prevalence of dental caries in an individual. It is a way to numerically express the caries prevalence and is obtained by calculating the number of Decayed (D), Missing (M) and Filled (F) Teeth (T). The maximum value DMFT is 32 when all teeth have been affected.

2. Functional Teeth Unit(s): One function teeth unit is defined as two teeth (either natural or denture tooth) occluding to each other for masticatory function. If any tooth is symptomatic due to pain or mobility or it is an unfit denture tooth, that unit will not be counted. The maximum number of teeth units is 16.
3.6.3 Plasma Homocysteine Concentrations of Chinese Vegetarian Women

(Chapter 13)

In 2000, thirty-eight younger Taoist vegetarians participated the study. The inclusion criteria were as follows: 1. aged 18 to 70 years; 2. community dwelling; 3. vegetarian for five years or more; 4. no known major disease, e.g. vascular disease, renal disease, diabetes mellitus, hypertension, hyperlipidaemia; 5. not on regular medication, including vitamin supplements containing vitamin B. Because there were only three male subjects, and there is reported sex difference in homocysteine concentration, they were excluded from analysis.

They all attended the research clinic in Prince of Wales Hospital, and had fasting blood samples taken for analysis of plasma homocysteine (3.4.4.1), vitamin B12, folate and renal function tests. Younger vegetarian subjects were selected for this study because renal impairment and co-existing diseases which are common in old age can have major influences on plasma homocysteine concentrations.

The control subjects were normal middle-aged adults who participated in a screening programme for hyperhomocysteinaemia in Hong Kong and Macau, which was run by my colleague, Professor KS Woo. Those found to have hyperhomocysteinaemia (Woo et al., 1999). 29 female subjects in Hong Kong matched within one year of the vegetarian subjects were arbitrarily selected to be control subjects. The same inclusion and exclusion criteria were applied, and the same research assistant in the same laboratory undertook all the biochemical assays. The study was approved by the Clinical Research Ethics Committee, the
The study received no external funding. There was no conflict of interest about individuals conducting the study.

3.6.3.1 Homocysteine Assay

The IMx homocysteine assay is based on the Fluorescence Polarization Immunoassay (FPIA) technology. Bound homocysteine (oxidized form) is reduced to free homocysteine (HCY) and the free HCY is enzymatically converted to S-adenosyl-L-homocysteine (SAH) as outlined below:

The probe assembly delivers the sample, pretreatment solution (dithiothreitol and adenosine), Enzyme (S-adenosyl-L-homocysteine hydrolase (bovine), and FPIA #1 diluent buffer to the predilution well of the sample cartridge. The pretreatment solution reduces HCY and mixed disulfide and protein bound forms of HCY present in plasma or serum into one chemical form HCY. The enzyme then converts HCY to SAH.

An aliquot of the predilution mixture, antibody, and FPIA#1 diluent buffer are delivered to the cuvette and a background measurement is made by the FPIA optical assembly. Tracer, FPIA#1 diluent buffer, and a second aliquot of the predilution mixture are then transferred to the cuvette. SAH and labeled Fluorescein Tracer compete for the sites on the monoclonal antibody molecule. The intensity of polarized fluorescent light is measured by the FPIA optical assembly.
3.7 Summary of Outline

The main study compared the dietary intakes, nutritional status, bone density, blood pressure, urinary sodium and potassium:creatinine ratios, proportion of subjects with ischaemic heart disease with historical data of local non-vegetarian women. Every effort was made to ensure that the two groups were matched in age/sex, and important confounders. Adjustment for confounders was facilitated by access to the raw data sets, and the use of same questionnaires and measurement methods. These were made possible by the fact that the persons who collected the data – Professor Jean Woo, Prof. Edith Lau and Prof. Suzanne Ho in the Department of Family and Community Medicine of the Chinese University of Hong Kong were part of the research team at the inception of the project. Additional older vegetarian subjects from old age homes were recruited to investigate the relationship between urinary MMA and serum MMA, the prevalence of vitamin B12 deficiency and its influence on haematological status. In order to be consistent with the characteristics of the historical control subjects, the vegetarian subjects were selected in different ways as shown in Table 3a.
Table 3a Selection of Vegetarian Subjects of Main Study for Comparison with Control subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vegetarian</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inclusion criteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary intake</td>
<td>MS&gt;6</td>
<td>H1</td>
</tr>
<tr>
<td>Anthropometry</td>
<td>108</td>
<td>H3</td>
</tr>
<tr>
<td>Bone density</td>
<td>67-82 yrs</td>
<td>H3</td>
</tr>
<tr>
<td>Ischaemic Heart Disease</td>
<td>&gt;=70 yrs, MS&gt;6</td>
<td>H2</td>
</tr>
<tr>
<td>Hypertension</td>
<td>111</td>
<td>H1</td>
</tr>
</tbody>
</table>

Abbreviations: MS- mental score (Information/orientation section of Clifton assessment procedure for the elderly, max. score 12); H1 – historical control 1 (Woo, 1988, see section 3.3.1); H2- historical control 2 (Ho&Woo, 1994, see section 3.3.2); H3- historical control 3 (Lau et. al, 1996, see section 3.3.3).

The vegetarian subjects thus found to be vitamin B12 deficient, together with those cases of vitamin B12 deficiency the author found in clinical practice, were then invited to participate a randomized controlled trial of parenteral vitamin B12 supplementation. The objective was to examine the effect of vitamin B12 supplementation on their cognitive function.

Since then, two more cross sectional studies were performed. The first one examined the association between dental status and dietary intake in a vegetarian OAH (Sin Tin Dao). In collaboration with one of my colleagues, Professor KS
Woo, the final study compared the plasma homocysteine concentration of younger vegetarian women with age matched historical control subjects. The vegetarian subjects used in all the above studies were summarized in Figure 3a.
Figure 3a A Diagramatic Summary of the Vegetarian Subjects involved in all the Studies in this Thesis

1995 Main study

Research Clinic (N 117) Old age homes (N 40)

Chapter 4 Dietary intake and Anthropometry
Chapter 5 Bone Density
Chapter 6 Ischaemic heart disease
Chapter 7 Hypertension and Diabetes Mellitus

Chapter 8 Urine and Serum Methylamalonic acid
Chapter 9 Vit.B12 status
Chapter 10 Vit.B12 and haemoglobin

1995/6 Randomized controlled trial of vitamin B12 supplementation in deficient older people (Chapter 11)

1999 The association between dental status and dietary intakes among Chinese vegetarian old age home residents (Chapter 12)

2000 Plasma Homocysteine levels of Chinese vegetarian women (Chapter 13)
3.7.1 Statistical Methods

1. Presentation of group data

In most circumstances, group data was represented by the mean and standard deviation. To facilitate multiple group comparison, 95% confidence intervals of the mean were presented. For skewed and small sized group data, median with the range of minimum and maximum was presented.

2. Group comparison

Student t test was used for group comparison if data of both groups was normal in distribution. ANOVA was used when there were more than two groups to compare. If the data was skewed, Mann Whitney test was used for comparison of two groups, Kruskal Wallis test for comparison of more than two groups. Chisquare test was used to compare groups of categorical data. When changes within the same subjects over time were examined for significance, Paired t test was used.

3. Association between two variables

Correlation analysis was used to examine the association between two variables. Pearson correlation coefficients were determined when the association was linear data was normal in distribution. Spearman coefficients of rank correlation were determined when the data was skewed.

When a variable is potentially or known to be dependent on a variety of factors, and these factors interact with each other, in order to examine if an associated factor determined by correlation analysis had an independent influence on the dependent variable, analysis of covariates of variance (ANCOVA) was
performed. In this analysis, one variable has to be defined as the dependent variable, and the other one as independent variable. The other variables which significantly correlated with the dependent variables \( (p<0.05) \) were entered as covariates. With this adjustment, if the variable in question could still explain a significant proportion of variance of the dependent variable, it could be regarded to be an independent predictor.

4. **Regression**

When two variables were associated, the dependent variable could be predicted by the independent variable by linear regression. The explained variance was the proportion of variance of the dependent variable which could be explained by the independent variable.

If the dependent variable was categorical, logistic regression was performed. The strength of the predicting variable was determined by the odd ratio, and the 95% confidence interval of the odd ratio. If the 95% CI of the odd ratio was narrow and far from unity, the variable should be regarded as a strong predictor.

If there were several factors which could predict the dependent variable, the strongest and most independent variables could be elicited by stepwise regression, i.e. eliminating variables which did not contribute to explained variance or odd ratio, and adding in the ones which did contribute. At the end the model with the least variables which could explain the most variance or accurate categorization of the dependent variable could be determined.
5. Receiver Operating Characteristic (ROC) curve

ROC curve (Griner et al., 1987) for urinary MMA/creatinine ratio to predict raised serum MMA was drawn to determine the optimal cutoff points associated with the highest sensitivity and specificity. The area under the ROC curve indicates the predictive value of the urinary MMA/creatinine for raised serum MMA. The maximal area is 1.

SPSS version 10 was used for all statistical analysis. Medcalc MedCalc for Windows was used to draw the ROC graph.

3.7.2 Sample Size Calculation

Based the data of the historical control populations, the numbers of vegetarian subjects required for each parameter to be compared in the main study were shown in Table 3b. A sample size of 75 vegetarians should be adequate. But the historical control subjects were all community dwelling apparently well older people in Hong Kong, and the source of recruitment of vegetarians in the main study was predominantly from old age hostels and old age homes, it was necessary to increase the total sample size and to adjust statistically for the potential differences between institutionalized and community dwelling vegetarians.

The sample size calculation for the cross sectional study on plasma homocysteine in chapter 13 was shown in Table 3b.
Table 3b Sample Size Calculation for Comparison with Historical Control

Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Control subjects</th>
<th>Mean</th>
<th>SD</th>
<th>Expected Difference</th>
<th>No. of Vegetarians required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index kg/m²</td>
<td>320</td>
<td>23.5</td>
<td>3.9</td>
<td>10%</td>
<td>44</td>
</tr>
<tr>
<td>Fat percentage</td>
<td>320</td>
<td>34.9</td>
<td>7</td>
<td>10%</td>
<td>64</td>
</tr>
<tr>
<td>Energy intake Kcal/day</td>
<td>250</td>
<td>1557</td>
<td>510</td>
<td>20%</td>
<td>42</td>
</tr>
<tr>
<td>Protein intake g/day</td>
<td>250</td>
<td>60</td>
<td>22</td>
<td>20%</td>
<td>53</td>
</tr>
<tr>
<td>Vitamin C mg/kcal/day</td>
<td>250</td>
<td>84</td>
<td>54</td>
<td>30%</td>
<td>73</td>
</tr>
<tr>
<td>Bone density</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar Spine g/cm²</td>
<td>388</td>
<td>0.71</td>
<td>0.13</td>
<td>10%</td>
<td>53</td>
</tr>
<tr>
<td>Femoral Neck g/cm²</td>
<td>388</td>
<td>0.53</td>
<td>0.09</td>
<td>10%</td>
<td>46</td>
</tr>
<tr>
<td>Inter trochanteric g/cm²</td>
<td>388</td>
<td>0.72</td>
<td>0.14</td>
<td>10%</td>
<td>60</td>
</tr>
<tr>
<td>Total Hip g/cm²</td>
<td>388</td>
<td>0.6</td>
<td>0.11</td>
<td>10%</td>
<td>53</td>
</tr>
<tr>
<td>Systolic BP mmHg</td>
<td>168</td>
<td>143</td>
<td>24</td>
<td>10%</td>
<td>45</td>
</tr>
<tr>
<td>Diastolic BP mmHg</td>
<td>168</td>
<td>79</td>
<td>12</td>
<td>10%</td>
<td>37</td>
</tr>
<tr>
<td>Urinary Na/creatinine mmol/mmol</td>
<td>166</td>
<td>17.5</td>
<td>4.3</td>
<td>20%</td>
<td>24</td>
</tr>
<tr>
<td>Urinary K/creatinine mmol/mmol</td>
<td>166</td>
<td>4.3</td>
<td>4.5</td>
<td>50%</td>
<td>69</td>
</tr>
<tr>
<td>Homocysteine µmol/L</td>
<td>30</td>
<td>6.8</td>
<td>1.7</td>
<td>20%</td>
<td>25</td>
</tr>
</tbody>
</table>

* Sample size required to have 80% power to detect the anticipated percentage difference at p value of <0.05.
4.1 Introduction

Literature review in Chapter 2 showed evidence of benefits and risks of the vegetarian diet. It also revealed a lack of data on the more restrictive vegetarian diets, and on the older people. The concern over the adequacy of the vegetarian diet of the older Chinese vegetarians in Hong Kong has been discussed in Chapter One. This study was therefore undertaken to examine the adequacy of the diet of the older Chinese vegetarians in the community and in old age homes, and to compare their nutritional status with local omnivores.
4.2 Subjects and Methods

The vegetarian subjects in the main study were the subjects of this study. They were described in Chapter 3. Dietary recall data was available in 104 vegetarian subjects, 3 subjects having refused and data on 4 subjects went missing. Nine subjects were excluded because of subnormal Clifton mental test score (<7/12). Data of 95 vegetarian subjects was therefore available for computerized dietary analysis which was based on food composition tables for South East Asia (US Department of Health, Education and Welfare, 1972). The contribution of vitamin or mineral supplements was not included, because one is more interested in the quality of food taken than the absolute amount of intake.

For the study of nutritional status, one current smoker was excluded. The following measurements were compared with historical control subjects: Body mass index (BMI), Arm muscle circumference (AMC), Corrected arm muscle area (CAMA), body fat percentages calculated by BMI and biceps and triceps skinfold thicknesses (Fat%), and serum albumin concentration (g/L).

The control subjects for the comparison of dietary intakes and serum albumin concentrations consisted of 250 women aged 60 years, and over, living in a private housing estate close to Prince of Wales Hospital. The survey was conducted in 1980’s (Woo, 1988). The response rate was 96%. The mean age was 70.7 (sd 4.7) years. There was no difference between the above 70 years olds and the below 70 years olds in dietary intakes.
Forty apparently well and ambulatory non-vegetarian old age hostel residents were recruited for 24-hour dietary recall for comparison with the vegetarian old age home residents.

For comparison of anthropometric data, 352 community-dwelling, apparently well older Chinese women who were subjects of a local community survey of osteoporotic vertebral fracture (Lau et al., 1996) were used as historical control subjects. Two current smokers were excluded.
4.3 Results

4.3.1 Dietary Intake

The average ages of vegetarian and omnivorous subjects were shown in Table 4a. The community dwelling vegetarians were significantly younger than the historical controls and vegetarian OAH residents. On further analysis, there was no correlation between age and energy intake among them, and in the historical control subjects, there was no significant difference in dietary intake between the under 70 year olds and the over 70 year olds. The vegetarian OAH subjects were comparable to the omnivore OAH subjects in age. Because of the way the subjects were recruited, almost all the community dwelling vegetarians (97%) were vegans, and 35% of vegetarian OAH subjects were lactovegetarians.

The dietary intakes of the vegetarians in community and those in old age homes were compared with historical control group and omnivorous old age hostel residents respectively in Table 4a. The dietary intake of vegetarian subjects was compared with the recommended intake by the Department of Health in Taiwan for the 70 year-olds in 1993. The percentages of vegetarian subjects with intake below two thirds of the recommended intake were shown in Table 4b.

Overall, when vegetarians were compared with omnivorous in the equivalent resident setting, the vegetarian diet was lower in calories, protein, fat, and the B vitamins, but the vitamin C and dietary fibre contents were higher. The vegetarian diet in OAH had higher content of calcium and potassium than that in non-vegetarian OAH. But this was not the case in the diet of community dwelling vegetarians who were predominantly vegans. When the total dietary intakes were
considered, vegetarians were more likely to have subnormal intake of calories, protein, and the B vitamins than non-vegetarian OAH residents.
Table 4a  Comparison of Vegetarian Diets with Omnivorous Diets in Community and Old Age Homes

<table>
<thead>
<tr>
<th></th>
<th>Community Veg N=38</th>
<th>OAH Veg N=57</th>
<th>Community Omnivore¹ N=250</th>
<th>OAH Omnivore N=40</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD  95% CI</td>
<td>Mean±SD  95% CI</td>
<td>Mean±SD  95% CI</td>
<td>Mean±SD  95% CI</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>74.4±7.4 72.1,76.7</td>
<td>79.3±6.5 77.6,81.0</td>
<td>70.7±4.7 70.1,71.3</td>
<td>82±6.4 80.0,84.0</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>1224±338 1118,1330</td>
<td>1146±374 1050,1242</td>
<td>1557±510 1494,1620</td>
<td>1336±367 1222,1450</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>33±14 29,37</td>
<td>35±18 30,40</td>
<td>60±22 57,63</td>
<td>47±20 45,49</td>
</tr>
<tr>
<td>% Calorie from</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>76±9 73, 79</td>
<td>75±9 73, 77</td>
<td>64±12 63, 65</td>
<td>62±12 58, 66</td>
</tr>
<tr>
<td>Protein</td>
<td>11±3 10, 12</td>
<td>12±3 11, 13</td>
<td>16±5 15, 17</td>
<td>14±4 13, 15</td>
</tr>
<tr>
<td>Fat</td>
<td>15±10 12, 18</td>
<td>14±9 12, 16</td>
<td>19±12 18, 20</td>
<td>23±10 20, 26</td>
</tr>
<tr>
<td>Per 1000 kcal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>268±127 228,308</td>
<td>366±203 314,418</td>
<td>221±168 200,242</td>
<td>220±134 178,262</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>764±346 655,873</td>
<td>1068±415 961,1175</td>
<td>783±449 727,839</td>
<td>691±674 482,890</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>8.6±2.2 7.9, 9.3</td>
<td>8.6±2.6 1.9, 9.3</td>
<td>8.3±4.5 7.7, 8.9</td>
<td>8.1±3.6 7.0, 9.2</td>
</tr>
<tr>
<td>Retinol eq. (ug)</td>
<td>402±321 301,503</td>
<td>482±274 411,553</td>
<td>407±33 403,411</td>
<td>439±785 196,682</td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>0.4±0.2 0.3, 0.5</td>
<td>0.4±0.2 0.4, 0.5</td>
<td>0.6±0.3 0.6, 0.6</td>
<td>0.5±0.2 0.4, 0.6</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.4±0.2 0.3, 0.5</td>
<td>0.4±0.2 0.4, 0.5</td>
<td>0.5±0.2 0.5, 0.5</td>
<td>0.4±0.2 0.3, 0.5</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>5.0±3.0 4.0, 6.0</td>
<td>4.7±1.5 4.3, 5.1</td>
<td>7.4±3.3 7.0, 7.8</td>
<td>7.7±7 5.5, 9.9</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>112±60 93, 131</td>
<td>108±66 91, 125</td>
<td>84±54 77, 91</td>
<td>95±86 68, 122</td>
</tr>
<tr>
<td>Dietary Fibre (g)</td>
<td>7.7±3.4 6.6, 8.8</td>
<td>8.6±2.6 7.9, 9.3</td>
<td>NA NA</td>
<td>5.6±2.7 4.8, 6.4</td>
</tr>
</tbody>
</table>

* p <0.05; ** p<0.005; *** p< 0.0005, Significant difference from omnivores in corresponding setting

# p<0.05; ## p<0.005, Significant difference from vegetarians in OAH

¹ Historical control (Woo, 1988); Veg – vegetarian, OAH – old age home, CI - Confidence Interval
### Table 4b  The Proportion of Older Vegetarians with Inadequate Dietary Intakes

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>RDA (Taiwan)</th>
<th>Vegetarian Community (n=38)</th>
<th>Vegetarian OAH (n=57)</th>
<th>Omnivore OAH (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>1600</td>
<td>13(34%)##</td>
<td>27(47%)**</td>
<td>10(25%)</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>55</td>
<td>27(71%)</td>
<td>38(67%)**</td>
<td>11(28%)</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>600</td>
<td>28(74%)</td>
<td>34(60%)</td>
<td>31(78%)</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>600</td>
<td>2(5%)</td>
<td>2(4%)</td>
<td>5(13%)</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>10</td>
<td>7(18%)#</td>
<td>16(28%)</td>
<td>10(25%)</td>
</tr>
<tr>
<td>Retinol equivalent (mg)</td>
<td>500</td>
<td>15(40%)</td>
<td>18(32%)</td>
<td>23(58%)</td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>0.8</td>
<td>28(74%)</td>
<td>44(77%)**</td>
<td>20(50%)</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>1.4</td>
<td>36(95%)</td>
<td>54 (95%)</td>
<td>36(90%)</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>11</td>
<td>28(74%)</td>
<td>46(81%)**</td>
<td>18(45%)</td>
</tr>
<tr>
<td>Ascorbic acid (mg)</td>
<td>50</td>
<td>2(5%)</td>
<td>6(11%)</td>
<td>5(13%)</td>
</tr>
<tr>
<td>Fat calories$^2$</td>
<td>&lt;30%</td>
<td>4(11%)</td>
<td>3(5%)**</td>
<td>13(33%)</td>
</tr>
</tbody>
</table>

1 Number (%) of subjects with intake below two thirds of RDA
2 Number (%) of subjects with intake above RDA

*p<0.05; ** p<0.005 Significant difference from omnivores in OAH
# p<0.05; ## p<0.001 Significant difference from vegetarians in OAH

RDA – Recommended Daily dietary allowance by Department of Health in Taiwan in 1993

OAH – Old age home
4.3.2 Nutritional Status

One hundred and eight vegetarian subjects of the main study were included, 5 vegetarian subjects having been excluded because of needing walking aid (2), current smoking (1) and refusal to answer questionnaire (2). There were initially 352 older female historical control subjects. Thirty-two subjects were excluded because of current smoking (31) and missing questionnaire data (1). The comparison of the characteristics of vegetarians and control subjects was made in Table 4c. The vegetarians were significantly older, more likely to be old age home resident and less likely to have ever smoked. Although the vegetarians were more likely to report regular exercise, most of them exercised for less than 20 minutes per day. There was no equivalent data in the control data set. Among vegetarians, regular exercise correlated with old age home residence (R 0.23, p<0.05).

The anthropometric measurements and the calculated fat% of the vegetarians and control subjects were compared in Table 4c. When compared with omnivores, the vegetarians had slightly lower fat percentages but not in BMI. The distribution of subjects with WHO recommended BMI criteria of underweight (<18.5), overweight (= or > 25) and obesity (= or > 30) (WHO, 1998) in the vegetarians and control subjects was compared in Figure 4a. There was no significant group difference in the distribution (Kruskal Wallis test).
<table>
<thead>
<tr>
<th></th>
<th>Vegetarian</th>
<th>Omnivore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no.</td>
<td>108</td>
<td>320</td>
</tr>
<tr>
<td>OAH</td>
<td>66</td>
<td>61%</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>7</td>
<td>7%</td>
</tr>
<tr>
<td>Regular exercise</td>
<td>74 ~</td>
<td>69%</td>
</tr>
</tbody>
</table>

Mean  SD      Mean  SD
Age (yrs)  77.8  7.1     76.6  3  *        
BMI (kg/m²) 23.0  3.8     23.5  3.9
TSF (mm)   14.4  6       17.3  6.8
BSF (mm)   6.5   3.1     7.3   3.4
MAC (cm)   25.7  3.6     26.4  3.5
Fat%       33.1  6.9     34.9  7.0  *
AMC (cm)   21.2  2.4     21    2.4
CAMA (cm²) 29.7  7.9     28.9  7.5

# p<0.005, Chisquare test; * p<0.05, Student t test

1. apparently well community dwelling women (Lau et al., 1996)

~ 48 of these (65%) exercised < 20 minutes per day

Abbreviations: OAH – old age home residence; BMI – body mass index; TSF, BSF – triceps, biceps skinfold thickness; MAC – mid arm circumference; Fat% - fat percentages calculated by equation; AMC – arm muscle circumference; CAMA – corrected arm muscle area.
The average serum albumin concentration of the older vegetarian women was significantly lower than the concentration reported in 38 older community dwelling Chinese women aged 75 or above (37.4 (sd 2.6) versus 42.0 ±3.0 g/L, p<0.001, Student t test) (Woo et al., 1988a). Moreover, 18 out of 103 (17.5%) vegetarian subjects had serum albumin concentrations below the laboratory normal range (<36 g/L). On ANCOVA, adjusting for age, old age home residence, regular exercise, ever smoking, vegetarianism was associated with lower fat percentages (p<0.05). Vegetarianism was however not correlated with BMI, AMC and CAMA. Among vegetarians, serum albumin had no significant correlation with any parameter examined. There was no significant difference between
vegans and lactovegetarians in any of the nutritional parameters, after adjustment of the confounders on ANCOVA.
4.4 Discussion

4.4.1 Dietary Intake

The most significant finding of this study was that the diet of older Chinese vegetarian women had low calorie and protein intakes. This is in contrast with the normal energy and protein intake in older vegetarians reported in Western studies (Brants et al., 1990, Nieman et al., 1989). The majority of the caloric intake of the vegetarian diet came from carbohydrate and the fat content was low. This should be associated with a favourable lipid profile. While low intakes of calories and protein may be detrimental to older people with disease (Sullivan et al., 1995), their long-term effects on health in normal people are uncertain. Low calorie intake has been shown to be associated with longevity in animal studies (Nicolas et al., 1999), but there is little human data on this issue. Moreover, protein energy malnutrition may lower blood pressure (Somova & Moodley, 2000). On the other hand, low protein intake has been associated with increased bone loss in older people and animal models (Hannan et al., 2000; Bourrin et al., 2000).

Protein and energy under nutrition among old age home residents has been well documented (Pinchcofsky-Devin & Kaminski, 1987) and is multifactorial e.g. depression, lack of food choice, chronic disease etc. It has been associated with adverse outcomes (Covinsky et al., 1999). There was however no significant difference in dietary intakes between institutionalized and community dwelling vegetarians, except that the institutionalized vegetarians had higher proportion with subnormal caloric intake. There might have been selection bias, as the frailer vegetarian old age home residents were not included in this study.
The calcium content of vegetarian diet in old age homes was higher than that of omnivore diet, probably because old age home residents were encouraged to drink milk-based supplements. But because their overall dietary intakes were lower, vegetarians and non-vegetarians were equally likely to have subnormal intake of calcium.

The calcium intake has been known to be low among southern Chinese as dairy products are unpopular and lactase deficiency is common. This is one of the major differences between the vegetarian diets in the West and that in Hong Kong. Soya bean products which contain calcium are popular among older Chinese vegetarians. They probably accounted for most of their calcium intake. Green leaf vegetables, on the other hand, were seldom eaten because of poor dental health. Oral calcium supplement may be indicated in those who have poor oral intake or have proven osteoporosis (Lau et al., 1992). Indeed one of the vegetarian old age homes which participated in this study prescribed oral calcium supplements to every residents everyday. The influence of calcium intake on bone mass and blood pressures will be discussed in chapter 5 and 7 respectively.

The vegetarian diets both in the community and in old age homes were deficient in thiamine, riboflavin and niacin. This contrasted with overseas studies showing adequate intakes of B vitamins, except vitamin B12 in vegetarians (Millet et al., 1989). Deficiency in B vitamins causes subtle symptoms e.g. malaise, mental dullness and loss of appetite (Goodwin et al., 1983). As it is difficult to diagnose these deficiency states, it is advisable for older vegetarians to take oral vitamin B complex supplements. Vitamin B12 is also a major worry for
vegetarians, as vegetarian foods are devoid of vitamin B12 except in minute amounts in seaweed and fermented foods. The prevalence of vitamin B12 deficiency will be examined in Chapter 9.

Vegetarian diet was comparable with omnivore diet in iron content. But because of high intakes of phytates in vegetarian diets, vegetarians are still at risk of iron deficiency. Moreover, because of lower dietary intake, more institutionalized than community dwelling vegetarians had subnormal iron intake. The prevalence of iron deficiency and its effect on haemoglobin concentrations in vegetarians will be examined in Chapter 10.

When compared with omnivores, vegetarians tended to have higher intake of potassium. These may tend to lower blood pressure in vegetarians (MacGregor, 1983). Sodium intake cannot be accurately assessed by dietary recall. It can however be estimated by spot urinary sodium adjusted by urinary creatinine. This data and its relationship with blood pressures will be presented in chapter 7.

It is not surprising that vegetarians had higher fibre and vitamin C intakes than omnivores but it is interesting to note that their fibre intake was much lower than that of adult vegetarians reported in Western countries. This was most likely to be caused by the high prevalence of chewing problems. The relationship between dental status and dietary intake in vegetarian old age home residents will be examined in the next chapter 12.

The method of 24-hour recalls may have under-estimated intake, but the same method was used in omnivore subjects, and subjects with subnormal mental test score were excluded. Nevertheless further dietary assessment by 24-hour food
weighing records was undertaken in a vegetarian old age home and will be reported in the next chapter 12.

### 4.4.2 Nutritional Status

Despite the lower caloric and fat intakes, the average BMI of older vegetarians were comparable to that of omnivores. According to BMI, the prevalences of under nutrition and obesity were similar between vegetarians and omnivores. Although the vegetarians were comparable in lean mass as estimated by upper limb anthropometry, they had significantly lower serum albumin concentrations, when compared with omnivores.

There were differences in age and lifestyles between the vegetarian and omnivorous women. The former was older, more likely to be old age home residents, and less likely to have ever-smoked. Smoking has been known to have major influences on body fat (Donkin et al., 1998). As only one of the vegetarians was a current smoker, and nearly 18% of the control subjects smoked, more reliable comparison could be made on exclusion of the current smokers. More vegetarians reported regular exercise than the non-vegetarians. But it was doubtful that they were any more physically active than the latter. Firstly, most of them did the exercises less than 20 minutes per day. Secondly, the vegetarians who reported regular exercise were those in of old age homes where meals were provided. On the other hand, the omnivores had long hours spent in outdoor walking.

The average BMI, AMC and CAMA of both vegetarians and omnivores were all higher than those of community dwelling older women studied about 10
years earlier (Woo et al., 1988b). This suggested an improvement in nutritional status of older people in Hong Kong during that time period. Their average BMI and TSF were comparable to those of urban older people in Mainland China, but their average MAC was significantly higher (Side et al., 1991). This suggested that their protein nutritional status was better than that of Mainland Chinese.

According to WHO guidelines on BMI, the prevalence of obesity (BMI > 30 kg/m²) was low in both vegetarians and omnivores. This was comparable to that reported for middle-aged female vegetarians in the UK (Key & Gwyneth, 1996). However, there are ethnic differences in the relationship between BMI and body fat. For the same BMI, Asians tend to have higher fat percentages (Gallagher et al., 2000). A cross-sectional study of middle-aged adults in Hong Kong showed that the prevalence of diabetes mellitus and hypertension rose after BMI reached 23 kg/m² (Ko et al., 1997). Forty-nine vegetarians (45.4%) and 175 omnivores (54.7%) had BMI greater than this threshold. Therefore nearly half of older vegetarians could be expected to have cardiovascular risk factors e.g. hypertension, diabetes mellitus.

BMI is not reliable in predicting body fat (Woo et al., 1997a). But when it was combined with triceps and biceps skinfold thicknesses, reasonable good estimation of fat percentages could be made in older Chinese people (Kwok et al., 2001a).

Older vegetarians had low protein intake (Table 4a). But they were comparable to omnivores in lean mass according to upper limb anthropometry. However, the correlation between CAMA and lean mass determined by Dual
energy X ray absorptiometry in older Chinese people was modest (correlation coefficient 0.57 in women, Kwok et al., 1997). On the other hand, the vegetarians had lower albumin concentration than omnivores, which may be an indication of protein under nutrition. As many as 17.5% had serum albumin concentrations lower than the reference range (36 g/L). This contrasts with the reported prevalence of subnormal serum albumin (<35 g/L) of 2.2% among older Europeans examined by the SENECA investigators (Lesourd et al., 1996). It is interesting that a 10 days vegetarian protein diet was associated with a lower rate of albumin synthesis than a 10 days animal protein diet of equivalent nitrogen and energy contents (Caso et al., 2000). This suggested that vegetarians should have greater intake of protein to maintain optimal protein nutritional status. A good range of vegetarian foods is also required to provide sufficient essential amino acids. Based on the low dietary protein intake and the lower serum albumin concentrations of our older vegetarians, they were likely to have protein under nutrition.

The inter-observer variation of BSF and TSF measurements between the two research assistants was relatively high (inter-observer coefficients of variation for BSF, TSF were 20% and 15% respectively in 6 subjects), when compared with well-trained technicians (Sullivan et al., 1989). But these two research assistants were also responsible for all the skinfold thickness measurements in the control study, and skinfold thicknesses were combined with BMI in the estimation of fat percentages. This should therefore not have significantly influenced the group comparisons made in this study.
4.5 Conclusion

This chapter showed that in contrast to the Western vegetarians, the older Chinese vegetarians had lower intakes of calories, protein, and B vitamins when compared with older omnivores. Although the fibre intake of vegetarians was higher than omnivores, their fibre intake was much lower than that in Western vegetarians. There was no significant difference in dietary intakes between community dwelling and institutionalized vegetarians, except that the latter were more at risk of subnormal caloric intake.

The older Chinese vegetarian women were slightly leaner than omnivores, nearly half of the vegetarians had body mass index greater than the recommended threshold of 23 kg/m\(^2\) for Chinese. Few vegetarians were underweight. Although the vegetarians were comparable to omnivores in lean mass as estimated by anthropometry, their lower serum albumin concentrations provided evidence of protein undernutrition.

With the low intakes of calcium and protein, and relatively lower body fat\%, vegetarians should be at risk of osteoporosis. This will be explored in the next chapter.
4.6 Key Points

1. The older Chinese vegetarians had lower intake of calories and protein, but higher intake of fibre than omnivores.

2. The vegetarian old age home residents had greater intake of calcium and potassium than community dwelling vegetarians and omnivores, probably because of greater consumption of milk based supplements.

3. Vegetarians in old age homes, when compared with omnivorous old age home residents, were at increased risk of inadequate intakes in calories, protein and B vitamins.

4. Older vegetarians were leaner than omnivores, but nearly half of them were overweight.

5. Older vegetarians had lower serum albumin concentrations than omnivores.
Chapter 5  Bone Density of Older Chinese Vegetarian Women

5.1  Introduction

In Caucasian studies, bone density of vegetarians was either better or similar to that of omnivores (Marsh et al., 1983; Tesar, 1992). But as Chapter 4 demonstrated, the diet of the older Chinese vegetarians was significantly different from Western vegetarian diets which are much more reliant on dairy products. The dietary intakes of older Chinese vegetarians were significantly lower in calories, protein and calcium when compared with those of their Western counterparts. Even when compared with omnivores of similar age in Hong Kong, their intakes of calories and protein were still significantly lower. Moreover, as shown in Chapter 4 (Table 4c), the older Chinese vegetarians had significantly lower fat percentages than age sex matched omnivorous subjects in Hong Kong. These factors were expected to put them at greater risk of osteoporosis. Indeed a recent cross sectional study of Buddhist nuns in Taiwan showed that long-term vegetarianism was associated with lower bone density (Chiu et al., 1997).

The bone density of older Chinese vegetarian women was therefore compared with omnivorous historical control subjects.
5.2 Subjects and Methods

The vegetarian subjects in the main study were selected for comparison with a historical control sample which consisted of female participants of a one-year follow-up study after an initial community survey of vertebral fracture (Lau et al., 1996). They were all community dwelling, aged 71-80 years, and were functionally independent. As age is an important determinant of bone density, only vegetarian subjects aged 67 to 82 were selected for comparison. Both groups of subjects had bone mineral density (BMD) of spine and hip measured by Dual energy X ray absorptiometry (DXA, Hologic QDR-2000 bone densitometer, Software: Enhanced Array Whole Body V5.67A). Osteoporosis was defined according to the WHO diagnostic criteria, i.e. having a body mineral density less than 2.5 standard deviations (SD) below the young adult mean (WHO, 1994). Osteopenia was defined as 1.5 to 2.4 SD below the young adult mean. In a recent study of 4274 Hong Kong subjects aged 9 to 94 years old, the peak bone density was observed at the 20-30 years age group. The mean bone densities at lumbar spine and total hip in female Chinese were 0.99 ± SD 0.10 g/cm² and 0.89± 0.11 g/cm² respectively (Lynn H et al., unpublished data).

Anthropometric measurements included standing height without shoes (Ht), weight with light hospital gown (Wt), triceps and biceps skinfold thicknesses (TSF, BSF), and mid-arm circumference. Body mass index (BMI) was calculated. Body fat percentages were estimated by BMI and skinfold thicknesses according to the following equation for female (Section 3.2.1).
Armspan (AS) was measured with the subject standing erect with back against the wall and arms outstretched at right angles to the body with palms facing forward. With the help of an assistant and a flexible calibrated steel tape measure, the measurement was taken from fingertips to fingertips, passing in front of the clavicles (Kwok & Whitelaw, 1991). The reading was taken to the nearest 0.1 cm and the average of two readings was taken. The difference between AS and Ht was taken as a marker of height loss, because armspan approximates to height at maturity (Kwok & Whitelaw, 1991).

Exercise habit, smoking, alcohol consumption, intake of dairy products, history of backpain in the previous 12 months were enquired in both groups. In the vegetarians only, 24-hour dietary recall data (in those with mental test scores 7 or more). The use of calcium supplements was enquired. Fasting urine samples were analyzed for sodium and creatinine in the Chemical Pathology Department of Prince of Wales Hospital.
5.3 Results

There were 70 vegetarian female subjects who fulfilled the selection criteria. The initial sample size of the vertebral fracture survey was 481. Four hundred of these returned for the one-year follow-up. Seven subjects were excluded because there were technical problems with bone density measurements of hips. Five heavy smokers (15 or more cigarette per day) were excluded. The rationale for this is that smoking was unusual among older female vegetarians and the few vegetarian subjects who had smoked were unlikely to smoke more than this amount. These left a total of 388 omnivorous control subjects.

The average duration of vegetarianism was 38.7 (sd 18.4) years. The anthropometric characteristics of vegetarian and control subjects were compared in Table 5a. The lifestyle characteristics of vegetarian and control subjects were shown in Table 5b. Twelve vegetarian old age home subjects were taking calcium supplement 600 mg daily. The vegetarians were more likely to be old age home residents, perform regular exercise, drink milk and less likely to have ever smoked.

The bone densities of vegetarian and control subjects were shown in Table 5c. There was no significant difference between the two groups. As shown in Table 5d, the majority of subjects in both groups had osteoporosis at lumbar spine and hip as defined by t score < or = -2.5. There was no significant difference in the percentages on Chisquare test.

The correlation coefficients between bone densities and potential variables were shown in Table 5e. The variables correlated most strongly with bone
densities were fat percentages and age. Among vegetarians, the duration of vegetarian diet had no significant correlation with bone densities.

After adjustment for fat percentages, age, old age home residence, dairy foods consumption and ever smoking, vegetarianism was found not to have significant correlation with any of bone densities on univariate analysis.

Twenty-four hour dietary recall was performed in 67 of the vegetarian subjects. Two subjects were rejected because of subnormal mental test scores. Intakes of energy (kcal), protein (g), calcium intakes (g) (oral calcium supplements included), urinary sodium/creatinine ratios, and intake of dairy products were correlated with bone densities at lumbar spine and hips. The correlation coefficients are listed in Table 5f. The only significant correlation was between urinary sodium/creatinine and lumbar spine bone mineral density (correlation coefficient 0.31, p 0.01).
<table>
<thead>
<tr>
<th>Table 5a Anthropometric Characteristics of Vegetarian and Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetarian (N= 70)</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Age  yrs</td>
</tr>
<tr>
<td>Height m</td>
</tr>
<tr>
<td>Armspan m</td>
</tr>
<tr>
<td>Armspan:Height m/m</td>
</tr>
<tr>
<td>Body mass index kg/m²</td>
</tr>
<tr>
<td>Tricep Skinfold mm</td>
</tr>
<tr>
<td>Bicep Skinfold mm</td>
</tr>
<tr>
<td>Mid Arm Circumference cm</td>
</tr>
<tr>
<td>Fat percentage * %</td>
</tr>
</tbody>
</table>

¹ Historical data (Lau et al., 1996)

* p=0.05, Student t test
Table 5b  Lifestyle Characteristics of Vegetarian and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Vegetarian (N=70)</th>
<th>Control (N=388)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OAH</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>Ever smoked</td>
<td>6</td>
<td>91</td>
</tr>
<tr>
<td>Alcohol intake</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>Dairy foods</td>
<td>33</td>
<td>51</td>
</tr>
<tr>
<td>Regular exercise</td>
<td>44</td>
<td>140</td>
</tr>
<tr>
<td>Back pain</td>
<td>22</td>
<td>152</td>
</tr>
</tbody>
</table>

1 Values expressed as number of subjects and percentages

*p<0.05, ** p<0.005, *** p<0.0001

OAH – old age home

Table 5c Bone Densities at Different Sites of Vegetarian and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Vegetarian N=70</th>
<th>Control N=388</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Density (g/cm²)</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
</tr>
<tr>
<td>Lumbar Spine</td>
<td>0.70  0.13</td>
<td>0.71  0.13</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>0.50  0.09</td>
<td>0.53  0.09</td>
</tr>
<tr>
<td>Intertrochanteric</td>
<td>0.70  0.14</td>
<td>0.72  0.14</td>
</tr>
<tr>
<td>Total Hip</td>
<td>0.59  0.11</td>
<td>0.60  0.11</td>
</tr>
</tbody>
</table>
Table 5d Comparison of Percentages of Vegetarian and Control Subjects with Osteoporosis

<table>
<thead>
<tr>
<th></th>
<th>Vegetarian</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lumbar Spine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>10 (14%)</td>
<td>55 (14%)</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>10 (14%)</td>
<td>72 (19%)</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>50 (71%)</td>
<td>261 (67%)</td>
</tr>
<tr>
<td><strong>Total Hip</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>7 (10%)</td>
<td>46 (12%)</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>18 (26%)</td>
<td>122 (31%)</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>45 (64%)</td>
<td>220 (57%)</td>
</tr>
</tbody>
</table>

Osteopenia – t score 1.5 to 2.4; Osteoporosis – t score 2.5 or more

There was no statistical difference between vegetarians and control subjects (Chi Square test).
Table 5e  Correlation between Bone Densities and Potential Predictors in 458 Study and Control Subjects

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Bone Density (g/cm²)</th>
<th>Spine</th>
<th>Femoral Neck</th>
<th>Intertrochanteric</th>
<th>Total Hip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>R</td>
<td>-0.06</td>
<td>-0.20</td>
<td>-0.16</td>
<td>-0.18</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.21</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Fat%</td>
<td>R</td>
<td>0.37</td>
<td>0.46</td>
<td>0.51</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Old age home</td>
<td>R</td>
<td>-0.05</td>
<td>-0.10</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.30</td>
<td>0.02</td>
<td>0.40</td>
<td>0.34</td>
</tr>
<tr>
<td>Dairy food</td>
<td>R</td>
<td>0.03</td>
<td>0.03</td>
<td>0.13</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.47</td>
<td>0.50</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Smoking</td>
<td>R</td>
<td>0.03</td>
<td>-0.09</td>
<td>-0.05</td>
<td>-0.06</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.58</td>
<td>0.06</td>
<td>0.30</td>
<td>0.19</td>
</tr>
<tr>
<td>Alcohol</td>
<td>R</td>
<td>0.02</td>
<td>0.04</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.70</td>
<td>0.36</td>
<td>0.62</td>
<td>0.53</td>
</tr>
<tr>
<td>Exercise</td>
<td>R</td>
<td>0.02</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.72</td>
<td>0.54</td>
<td>0.37</td>
<td>0.41</td>
</tr>
</tbody>
</table>

R = Pearson correlation coefficient

For all categorical data, 0=No, 1=Yes
### Table 5f Correlation Coefficients between Bone Densities and Dietary Factors among 70 Vegetarian Older Female Subjects

<table>
<thead>
<tr>
<th>Bone Site</th>
<th>Urinary Na/Cr</th>
<th>Dairy food</th>
<th>Calcium</th>
<th>Protein</th>
<th>Calories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/mmol</td>
<td>yes-1; no-0</td>
<td>g/day</td>
<td>g/day</td>
<td>Kcal/day</td>
</tr>
<tr>
<td>Lumbar Spine</td>
<td>0.31</td>
<td>-0.01</td>
<td>0.11</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>P value</td>
<td>0.01</td>
<td>0.92</td>
<td>0.40</td>
<td>0.30</td>
<td>0.54</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>0.19</td>
<td>0.04</td>
<td>0.12</td>
<td>0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>P value</td>
<td>0.11</td>
<td>0.74</td>
<td>0.35</td>
<td>0.26</td>
<td>0.22</td>
</tr>
<tr>
<td>Intertrochanteric</td>
<td>0.15</td>
<td>0.11</td>
<td>0.19</td>
<td>0.06</td>
<td>0.20</td>
</tr>
<tr>
<td>P value</td>
<td>0.23</td>
<td>0.38</td>
<td>0.14</td>
<td>0.65</td>
<td>0.10</td>
</tr>
<tr>
<td>Total hip</td>
<td>0.17</td>
<td>0.09</td>
<td>0.18</td>
<td>0.11</td>
<td>0.21</td>
</tr>
<tr>
<td>P value</td>
<td>0.17</td>
<td>0.41</td>
<td>0.16</td>
<td>0.39</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Abbreviation: R = Pearson correlation coefficients; Urinary Na/Cr = urinary sodium and creatinine ratio.
5.4 Discussion

The vegetarians and omnivores were all women and well matched in age. Over two thirds of the older vegetarian women had osteoporosis. But a similar proportion of the omnivores also had osteoporosis. The average bone mineral densities and the proportion of subjects with osteoporosis at the lumbar spine and the hips did not differ significantly between the two groups. The prevalence of back pain and the estimated height loss by AS:Ht of vegetarians were also comparable to omnivores.

When compared with omnivores, the vegetarians had some advantages over bone health. They were less likely to have ever smoked and more likely to consume dairy products. But they were more likely to be in old age homes and had lower fat percentages. After adjustment for these factors, vegetarianism did not correlate with bone mineral densities at lumbar spine and hips. This suggested that vegetarian diet per se did not have a significant effect on bone health.

It was interesting that fat percentage had the highest correlation with bone density. There is a sex difference in the relative importance of fat mass and lean mass in bone health. Studies have consistently shown that lean mass had a stronger association with bone mineral content or density in older men (Baumgartner et al., 1996, Coin et al., 2000). But in older women, some studies showed a stronger association with bone mineral content or density for fat mass than for lean mass (Reid et al., 1994; Baumgartner et al., 1996; Coin et al., 2000). Lean mass is important to bone health because it represents the major part of body weight and thus the mechanical stress to the skeleton. It is also associated with
physical activities. In postmenopausal women, however, adipose tissue may have an important endocrine function in preserving bone mass. Firstly, it converts androgens to oestrogen. Secondly, it secretes leptin which may enhance osteoblast differentiation (Thomas et al., 1999).

Western studies have demonstrated that a high dietary calcium intake had a positive effect on bone mass (Cumming, 1990; Prince et al., 1991). But dairy products, milk predominantly, are seldom consumed among Southern Chinese, whether they are vegetarians or not. The higher percentage of milk consumption in vegetarians was because over half of the subjects resided in old age homes where milk was strongly encouraged. Whether these subjects took milk regularly before admission to old age homes was uncertain. As shown in chapter 4, high percentages of vegetarians and omnivores alike had daily calcium intake of less than the recommended 600 mg. Although the average daily intake of calcium was higher in vegetarians when compared with omnivores, the levels of intakes were not high enough to protect the bones. These factors explained why there was only a weak correlation between dairy food intake and hip BMD in the total sample (including omnivores), and there was no significant correlation between calcium intake or dairy product consumption and bone mineral densities among the vegetarians.

Physical exercise has been shown to be important to bone health. Although over 60% of vegetarians said that they were doing regular exercise, on further questioning about the duration, 23 subjects (32.9%) admitted that they only did it
less than 20 minutes per day. Therefore, the apparent better exercise habit of the vegetarian subjects might not have contributed significantly to their bone health.

There were a couple of dietary differences which were not adjusted for in this study, because they were not measured in control subjects. Among vegetarians, urinary sodium/creatinine ratio was the only dietary factor which correlated with bone mineral density. For some time, high salt intake has been known to increase calcium excretion (Massey et al., 1968). On average, urinary calcium excretion increased by around 0.6 mmol per 100 mmol increase in dietary sodium (Zarkadas et al., 1989). Serum osteocalcin increased by 8% and urinary hydroxy proline by 37% during a high salt diet (McParland et al., 1989). It will be shown in chapter 7 that our vegetarians had significantly higher urinary sodium/creatinine ratios than omnivores. On this basis, vegetarians could be expected to have lower bone densities than omnivores.

Secondly, protein intake may be important to bone health. In chapter 4, the protein intakes of our vegetarians were significantly lower than historical controls of similar age. Some earlier cross sectional studies suggested that high protein intake might be detrimental (Geinez et al., 1993). However low serum albumin was associated with decreased bone densities in geriatric patients (Coin et al., 2000). An animal model showed increase bone loss in dietary protein deficiency (Bourrin et al., 2000). In a Framingham cohort study of 391 older women and 224 older men, low dietary protein intake was associated with greater bone loss over four years, even after adjustment of confounders (Hannan et al., 2000). A randomized trial of 82 hip fracture patients showed that six months of protein
repletion when compared with isocaloric protein free supplement was associated with increased serum concentrations of insulin-like growth factor-I, attenuation of proximal femur bone loss, and shorter stay in rehabilitation hospitals (Schurch et al., 1998).
5.5 Conclusion

The majority of older vegetarian women had osteoporosis. But there was no significant difference in average bone densities at lumbar spine or hips between vegetarians and omnivores, before and after adjustment for body fat, smoking, exercise habit, and dairy food intakes. However, the increased sodium intake and low protein intake of vegetarian subjects were left unadjusted for because of lack of equivalent data in control subjects. As their correlations with bone densities were modest or insignificant among the vegetarians, they would be unlikely to have altered the conclusion that vegetarianism per se would not alter bone densities.

In chapter 4, lower fat intake and relative leanness of vegetarians were demonstrated. Whether these will confer benefits to their cardiovascular health will be the subject of the next chapter.
5.6 Key Points

1. The majority of older vegetarian women had osteoporosis.

2. There was no significant difference between vegetarians and omnivores in bone mineral density at hip and lumbar spine, before and after adjustment for body fat and lifestyles.

3. There was no significant difference between lactovegetarians and vegans in bone mineral densities.

4. Fat percentages and age were the most significant predictors of bone mineral densities in both vegetarians and omnivores.

5. High sodium intake of vegetarians was a significant contributor to their bone loss.
Chapter 6  Ischaemic Heart Disease in Older Chinese Women

6.1 Introduction

Vegetarians have been shown to have lower mortality from ischaemic heart disease (IHD) in epidemiological studies (Key et al., 1999; Fonnebo, 1992). The benefit is attributed to more favourable lipid profile (Sacks et al., 1985). However, the reduction in cardiovascular mortality associated with vegetarian diet in women and in older people had been less significant or not demonstrable (Fonnebo, 1992; Phillips, 1978). The difference may be even harder to demonstrate in Hong Kong where the lipid profiles of older Chinese women are more favourable than that of their western counterparts. Moreover, as demonstrated in Chapter 4, the Chinese vegetarian diet is significantly different from that in Western countries in a number of ways, and the older Chinese vegetarians in Hong Kong have lower socio-economic profile than those in the West.

It would therefore be interesting to compare the prevalence of ischaemic heart disease of older vegetarian women with that of community dwelling older non-vegetarian women in Hong Kong, after adjustment for confounders.
6.2 Subjects and Methods

Chinese vegetarian women 70 years old or over in the main study were selected for this study. The following measurements were used for analysis: Cardiovascular Questionnaire, resting electrocardiograph (ECG), health questionnaire, fasting serum cholesterol and triglyceride concentrations, and body mass index. Because of potential unreliable responses to questionnaires, vegetarian subjects with subnormal mental test scores were excluded.

The omnivorous women consisted of 90 community dwelling Chinese 70 years old or over. They were part of a large-scale community survey of older Hong Kong Chinese people recruited by stratified random sampling (Ho et al., 1994). These control subjects were a subset of people who volunteered to attend Prince of Wales Hospital for electrocardiogram (Woo et al., 1993). They had the same measurements as the vegetarian subjects. Their dietary habit was assessed by food frequency questionnaire. Those found not to consume any meat regularly were excluded. None of the historical control subjects were thought to have dementia.

Using the cardiovascular questionnaire, ECG findings and past history, subjects were classified to have probable (positive symptom, documented history of disease and current medication, or ECG showing probable disease), possible (no history or symptom, and ECG showing possible disease) or no (who did not fall into the above two categories) ischaemic heart disease.
6.3 Results

One hundred vegetarian women were 70 years old or over. One subject refused to cooperate with questionnaire and nine subjects with subnormal mental scores were excluded. A total of 90 vegetarians were therefore included in this study. Fifty-three of them (58.9%) were vegans, and the rest were lacto vegetarians. The average duration of vegetarianism was 41.1 (S.D. 17.2) years. The characteristics of vegetarians and non-vegetarians were compared in Table 6a. The dietary practice of the non-vegetarians as assessed by food frequency questionnaire was shown in Table 6b.
Table 6a Characteristics of Vegetarian and Omnivorous Older Chinese Women

<table>
<thead>
<tr>
<th></th>
<th>Vegetarian</th>
<th>Omnivore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)#</td>
<td>79.0 (5.6)</td>
<td>79.0 (6.5)</td>
</tr>
<tr>
<td>History of IHD</td>
<td>14 (15.6%)</td>
<td>19 (21.1%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>24 (26.7%)</td>
<td>30 (33.3%)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>10 (11.1%)</td>
<td>11 (12.2%)</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>83 (92.2%)</td>
<td>75 (83.3%)</td>
</tr>
<tr>
<td>Exercise &lt;20 min./ day</td>
<td>67 (74.4%)*</td>
<td>38 (42.2%)*</td>
</tr>
<tr>
<td>Old age home resident</td>
<td>55 (61.1%)*</td>
<td>0 (0%)*</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)#</td>
<td>23.0 (3.8)</td>
<td>22.1 (3.8)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L) #</td>
<td>4.8 (1.1)**</td>
<td>5.4 (0.9)**</td>
</tr>
<tr>
<td>Triglyceride (g/L) #</td>
<td>1.6 (1.4)</td>
<td>1.7 (1.1)</td>
</tr>
<tr>
<td>Total</td>
<td>90 (1.4)</td>
<td>90</td>
</tr>
</tbody>
</table>

# mean (sd), 3 missing values for serum cholesterol and triglyceride concentrations

* Chisquare test, p<0.001, ** Student t test, p<0.001

Abbreviations: IHD - ischaemic heart disease
Table 6b Food Frequency of Omnivorous Chinese Women

<table>
<thead>
<tr>
<th>Item</th>
<th>Times/week*</th>
<th>Item</th>
<th>Times/week*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red meat</td>
<td>7 (3,10)</td>
<td>Fruit</td>
<td>7 (3.5, 7)</td>
</tr>
<tr>
<td>White meat</td>
<td>2 (1,3)</td>
<td>Green leafy vegetables</td>
<td>8.5 (7,14)</td>
</tr>
<tr>
<td>Fish</td>
<td>7 (4,10.5)</td>
<td>Non-green leafy vegetables</td>
<td>5 (2,7)</td>
</tr>
<tr>
<td>Egg</td>
<td>2 (1,2)</td>
<td>Milk</td>
<td>0 (0,5)</td>
</tr>
<tr>
<td>Liver</td>
<td>0 (0, 0)</td>
<td>Soya bean products</td>
<td>1 (0,2)</td>
</tr>
</tbody>
</table>

* Median (25th, 75th percentile)

The percentages of subjects with cardiac symptoms and ischaemic heart disease in both groups were compared in Table 6c. Three vegetarian subjects refused ECG. Two of them reported history of ischaemic heart disease with medication and were therefore classified to have probable ischaemic heart disease. The remaining one had no cardiac history or symptom but her ischaemic heart status could not be classified. Another three vegetarians' serum lipid concentrations were missing. They were therefore not included in logistic regression analysis.

The age adjusted odds ratios of all the potential predictors of ischaemic heart disease on logistic regression analysis were shown in Table 6d. Age had a significant association with IHD. Age of 80yrs or more when compared with 70-79 yrs had odds ratios of 0.5 (0.2-1.0) and 0.5 (0.3-1.0) for probable and possible/probable ischaemic heart disease respectively. There were insufficient numbers of subjects with cardiac symptoms or probable ischaemic changes on ECG for logistic regression.
### Table 6c. Cardiac Symptoms and Disease in Vegetarian and Omnivorous Older Chinese Women

<table>
<thead>
<tr>
<th></th>
<th>Vegetarian (N=90)</th>
<th>Omnivore (N=90)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>History of IHD</strong></td>
<td>14</td>
<td>19</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Chest pain</strong></td>
<td>6</td>
<td>19</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Cardiac Symptoms #</strong></td>
<td>3</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td><strong>ECG</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probable IHD</td>
<td>2</td>
<td>10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Possible IHD</td>
<td>24</td>
<td>16</td>
<td>17.7</td>
</tr>
<tr>
<td>Normal</td>
<td>61</td>
<td>64</td>
<td>71.1</td>
</tr>
</tbody>
</table>

**Symptoms and ECG**

<table>
<thead>
<tr>
<th></th>
<th>Vegetarian (N=90)</th>
<th>Omnivore (N=90)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probable IHD</td>
<td>9</td>
<td>28</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Possible IHD</td>
<td>19</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Normal</td>
<td>61</td>
<td>53</td>
<td>58.9</td>
</tr>
</tbody>
</table>

# angina or infarct

* 3 vegetarians refused ECG

** One vegetarian not classifiable

Abbreviations: IHD- ischaemic heart disease; ECG – electrocardiogram
Table 6d. Odds Ratios of Predictors of Ischaemic Heart Disease (IHD) #

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Possible or Probable IHD</th>
<th>Probable IHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetarian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>86</td>
<td>0.7 (0.4-1.3)</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>54</td>
<td>2.4 (1.2-4.6)*</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>155</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21</td>
<td>1.2 (0.5-3.2)</td>
</tr>
<tr>
<td>Physical exercise per day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 20 min</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>&lt; 20 min</td>
<td>102</td>
<td>0.7 (0.3-1.2)</td>
</tr>
<tr>
<td>Smoker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non</td>
<td>147</td>
<td></td>
</tr>
<tr>
<td>Ex + Current</td>
<td>29</td>
<td>1.1 (0.5-2.5)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 5.2</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>&gt; 5.2</td>
<td>76</td>
<td>1.0 (0.5-1.9)</td>
</tr>
<tr>
<td>Triglyceride (g/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 2.0</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>&gt; 2.0</td>
<td>44</td>
<td>1.3 (0.6-2.6)</td>
</tr>
<tr>
<td>Old age home resident</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>52</td>
<td>0.9 (0.4-1.8)</td>
</tr>
</tbody>
</table>

# logistic regression adjusted by age groups (70-79 years and 80+ years)
* p values < 0.05
Abbreviation; OR- age adjusted odd ratio
On stepwise logistic regression, the most significant predictor of probable ischaemic heart disease was vegetarianism (OR 0.2, 95%CI 0.1-0.6, p<0.005). It correctly predicted 79.5% of subjects. None of the other variables: age group, old age home residence, physical exercise and serum triglyceride concentration improved the accuracy of prediction.

For possible or probable disease, the only significant predictor was history of hypertension (OR 2.6, 95%CI 1.3-5.0, p<0.005). It correctly predicted 79.5% of subjects. The addition of age group increased the correction prediction marginally to 65.3%.
6.4 Discussion

This study showed that older vegetarian women had a lower risk of ischaemic heart disease when compared with their omnivorous counterpart. The protective effect was independent of other risk factors of ischaemic heart disease.

All the vegetarian subjects have adopted the vegetarian diet for more than ten years, most starting at young age. Dietary compliance was expected to be extremely good because of communal living in a religious environment. They were different from the vegetarian subjects in Western studies in that they were in advanced age, had a background of working class, and not especially health conscious. Their dietary intakes were described in Chapter 4. The main finding was that the intakes of energy, fat, protein calorie, thiamine, riboflavin and niacin were significantly lower in vegetarians when compared with omnivorous women of similar age.

The control subjects were part of a community survey conducted in Hong Kong in 1991 (Woo et al., 1993). They were representative of older people in Hong Kong in that they were recruited by random sampling in the community, except that the frail elderly and old age home residents were not included. The food frequency profile of the non-vegetarians suggested an adequate intake of vegetables, fruit and fish which were protective factors against ischaemic heart disease. Their prevalence of ischaemic heart disease was however similar to those reported in older Caucasians studies (Woo et al., 1993; Ahto et al., 1998).

The vegetarian and non-vegetarian subjects were comparable in age and body mass index. They were also comparable in the percentages of self-reported
diabetes mellitus and hypertension, which were comparable with local norm (Ho & Woo, 1994). However it is likely that diabetes mellitus was under-diagnosed in both groups as the prevalence, defined by fasting serum glucose concentration, among local Chinese people above 75 years old was reported to be 17.1% (Woo et al., 1987). Although more than half of the vegetarians were recruited from old age homes and none of non-vegetarians was old age home resident, all the vegetarian old age home subjects were self-ambulatory. Furthermore, vegetarian old age home residents did not differ from community dwelling vegetarians in percentages with disease defined either by ECG alone or in combination with questionnaire (data not shown).

As in other studies (West & Hayes, 1968; Sacks et al., 1975), vegetarians had significantly lower serum cholesterol concentrations. This could be attributed to their low fat intake (Lichtenstein et al., 1999). But their high dietary intake of soya bean products could also have a cholesterol lowering effect (Sirtori et al., 1977). It was somewhat surprising that vegetarians were less physically active than omnivores. The difference could not be attributed to institutionalisation, as a greater proportion of old age home residents exercised daily than community dwelling subjects (30.9 % Vs 17.1%, p=0.22, Chi-square test). This was consistent with our assertion that this group of vegetarians was not particularly health conscious.

The WHO cardiovascular questionnaire has been found to possess 79% sensitivity and 98% specificity in a survey of older people (Dewhurst et al., 1991). The sensitivity is expected to increase when the questionnaire is combined with
ECG appearance and medical history (Woo et al., 1993). However, only a minority of subjects reported cardiac chest pain, as silent coronary ischaemia is common in old age (Miller et al., 1990), and the sedentary lifestyle may protect older people from the symptom of angina. Although 15 to 20% of the subjects were diagnosed to have ischaemic heart disease in the past, few of them had significant ECG changes, especially so in vegetarians.

Vegetarianism was an independent protective factor of probable ischaemic heart disease defined by questionnaire, history and ECG. Apart from lower serum cholesterol concentrations, there might therefore be additional protective factors associated with vegetarianism. It has been suggested that an active religious lifestyle might protect against IHD, but it has not yet been proven (Jarvis & Northcott, 1987).

It is more likely that there are additional dietary factors that protect against ischaemic heart disease (Dwyer, 1988; Fraser 1994). The most likely candidate is isoflavones. There is increasing evidence that soy protein, abundant in isoflavones, is a protective factor against cardiovascular disease. Anti-oxidants e.g. beta carotene and vitamin C may render LDL particles less atherogenic (Sato et al., 1990; Esterbauer et al., 1989); ε 3 and ε 6 families of polyunsaturated fats may reduce platelet aggregation (Fraser et al., 1994); dietary arginine abundant in certain vegetarian food (e.g. nuts) may have a positive influence on endothelial functions (Cooke et al., 1992).

There has been recent interest in the association between hyper-homocysteinemia and ischaemic heart disease (Nygad et al., 1977). This may be
caused by genetic predisposition (Jacques et al., 1996), but it can be caused by folate deficiency or more indirectly by cobalamin deficiency (Savage et al., 1994). Vegetarians have high folate intakes but are at great risk of vitamin B12 deficiency. Some Western studies have demonstrated hyperhomocysteinaemia in vegetarians (Mezzano et al., 1999). Whether the particularly high folate intakes of the Chinese vegetarian diet may neutralize the adverse effect of vitamin B12 deficiency is a subject of Chapter 13.

The association of serum triglyceride concentration but not cholesterol concentration with probable ischaemic heart disease on logistic regression analysis is interesting, even though it was just excluded from the stepwise logistic regression model. The association between total cholesterol and ischaemic heart disease (IHD) in older people has been inconsistent (Harris et al., 1988; Krumholz et al., 1994; Simons et al., 2001). The problem seems to lie in the associations among mortality, poor health status and low serum cholesterol concentration in older people. In a five-year cohort study of 4066 older Americans, the unadjusted fatal IHD events were associated with the lowest serum total cholesterol (<= 4.15 mmol/L). But when risk factors of IHD and markers of poor health i.e. serum iron and albumin concentrations were adjusted for and deaths within one year were excluded, elevated total cholesterol levels predicted death from IHD (P for trend =0.005) (Corti et al., 1997). Triglyceride concentration is increasingly recognised to be an independent predictor of ischaemic heart disease (Jeppesen et al., 1998; Mann et al., 1999). However the apparent association observed in this study could be mediated through diabetes mellitus that has not been fully adjusted for because
of problem of under-diagnosis.

Over one fifth of vegetarians had possible ischaemic heart disease, primarily because of non-specific T wave changes. The significance of these changes in older people is controversial (Simonson, 1972; Caird et al., 1974; Yasumura & Shibata, 1989). Hypertension is likely to have an important role, as it was the only independent predictor of possible/probable IHD on multivariate logistic regression. The self reported prevalence of hypertension in vegetarians was comparable to that in omnivores. But undiagnosed hypertension is common among Hong Kong people (Janus, 1997). Whether our older vegetarians were more hypertensive than non-vegetarians is the subject of the next chapter. In addition, the prevalence of diabetes mellitus in vegetarians and omnivores will be compared.
6.5 **Key Points**

1. Vegetarians had lower prevalence of probable ischaemic heart disease defined by history and significant ischaemic ECG changes.

2. Vegetarians had significantly lower serum cholesterol concentrations, but this did not appear to account for the lower prevalence of ischaemic heart disease.

3. The serum triglyceride concentrations of vegetarians were comparable to that of omnivores.

4. Over one fifth of vegetarians had possible ischaemic heart disease.

5. Hypertension was the most significant predictor of possible ischaemic heart disease in both vegetarian and omnivorous older Chinese women.
Chapter 7 Hypertension and Diabetes Mellitus in Older Chinese Vegetarian Women

7.1 Introduction

In the last chapter, hypertension was found to be an important predictor of possible ischaemic heart disease, while serum cholesterol and history of diabetes mellitus did not appear to correlate with ischaemic heart disease in older Chinese women.

Vegetarian diets in Western countries have been shown to be associated with lower blood pressure. In addition, short term randomized trials of vegetarian diets have also shown a reduction in blood pressure. However in chapter 4, the Chinese vegetarian diet was found to be low in protein and calcium, but rich in potassium. The former two factors elevate blood pressure while the latter lowers it (Hajjar et al., 2001). But sodium intake, which is thought to be the most important dietary determinant of blood pressure, has not been investigated in older Chinese vegetarians.

In the last chapter, vegetarians and omnivores did not differ in the history of diabetes mellitus. This was unsatisfactory as under diagnosis was common. As demonstrated before, older vegetarians were leaner and consumed less calories and fat than omnivores. They might therefore have a lower prevalence of diabetes mellitus.

This study therefore determined the prevalence of hypertension and diabetes mellitus in older Chinese vegetarian women and compared with local
norms. The dietary determinants of blood pressures among vegetarians were also examined.
7.2 Subjects and Methods

Vegetarian subjects in the main study as described in chapter 3 were used in this study. The measurements used were as follows: supine blood pressures, body mass index, fasting serum glucose, lipids, and albumin, fasting urinary sodium, potassium and creatinine, health questionnaire, dietary assessment by twenty-four hour recall method.

Those who had history of diabetes mellitus or fasting glucose more than 7.8 mmol/L were classified as diabetic (WHO, 1980). Those with history of hypertension and supine blood pressure greater than 140/90 were classified as hypertensive.
7.3 Results

One hundred and eleven vegetarian subjects of the main study were included in this study. Urinary cations data was available in all except one subject. Dietary intake data was available in 95 subjects (9 subjects excluded because of subnormal Clifton mental test scores, 7 missing data). Eighteen subjects in a vegetarian old age home were routinely given calcium and multivitamin supplements, amounting to an additional intake of 600 mg of calcium per day. The contribution calcium supplement was included into the total calcium dietary intake in data analysis.

7.3.1 Prevalence of Hypertension and Diabetes Mellitus of Older Vegetarians

Twenty-nine subjects (26.1%) had history of hypertension, but only 22 (75.9%) were on medication. Another 42 (37.8%) subjects were found to be hypertensive. The overall prevalence of hypertension was therefore 64.0%. If the cut-off value of hypertension was increased to 160/90 or 160/95, the prevalence rate decreased to 43.2%.

The blood pressure and urinary cations of vegetarian subjects who were not on antihypertensive medication were compared with those of a group of apparently healthy and community dwelling older Chinese people studied in the late 1980’s (Woo et al., 1988c) in Table 7a. The vegetarians were older, and had increased urinary excretion of sodium and potassium. There was no significant difference in systolic and diastolic blood pressures.
Eleven subjects (10%) gave a history of diabetes mellitus. Another 4 subjects had raised fasting glucose concentrations. Eight subjects had missing blood glucose data. The overall prevalence of diabetes mellitus in this vegetarian population was therefore 15/103 (14.6%).
Table 7a  Comparison of Blood Pressures, Dietary Intakes and Urinary Cations of Older Female Vegetarians and Omnivores

<table>
<thead>
<tr>
<th></th>
<th>Vegetarian</th>
<th></th>
<th>Omnivore</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>Age (years)</td>
<td>78.4</td>
<td>7.1</td>
<td>88</td>
<td>70.7*</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>22.9</td>
<td>3.8</td>
<td>88</td>
<td>22.6</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>146.2</td>
<td>25.6</td>
<td>88</td>
<td>143</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>74.9</td>
<td>12.2</td>
<td>88</td>
<td>79</td>
</tr>
<tr>
<td>Urinary ratio (mmol/mmol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium/Creatinine</td>
<td>29.8</td>
<td>17.3</td>
<td>86</td>
<td>17.5*</td>
</tr>
<tr>
<td>Potassium/Creatinine</td>
<td>7.1</td>
<td>3.2</td>
<td>86</td>
<td>4.3*</td>
</tr>
<tr>
<td>Sodium/Potassium</td>
<td>4.6</td>
<td>2.7</td>
<td>86</td>
<td>4.2</td>
</tr>
</tbody>
</table>

1 - normal and active community dwelling female subjects with mean age of 70.7 ± 4.7 years (Woo et al. 1988c); neither vegetarians nor omnivores were on anti-hypertensive medications.

* p<0.001, Student t test

Abbreviations: BP – blood pressure
The demographic, clinical and dietary characteristics of hypertensive and normotensive vegetarian subjects were compared in Table 7b. Normotensive subjects had significantly lower urinary sodium/potassium ratio, higher dietary calcium intake and were more likely to take calcium supplements. None of the demographic and clinical characteristics showed significant difference between hypertensives and normotensives, except that the former had been vegetarian for longer.

On correlation analysis in the subjects who were not taking antihypertensive or diuretic drugs, the significant variables which correlated with systolic blood pressures were calcium intake ($R = -0.41$), urinary sodium/creatinine ($R = 0.39$), urinary sodium/potassium ratio ($R = 0.30$) and age ($R = 0.22$) for systolic blood pressures. The only significant variable for diastolic blood pressures was urinary sodium/creatinine ($R = 0.29$). As urinary sodium/creatinine and urinary sodium/potassium ratios were highly correlated and the former had better correlation, only the former was entered into the linear regression model for systolic blood pressure.

On linear regression for systolic blood pressure, urinary sodium/creatinine alone had explained variance ($R^2$) of 0.16. When dietary calcium intake was added into the model, the $R^2$ increased to 0.26. The addition of age resulted in a marginal increase of explained variance ($R^2 = 0.28$). It was
therefore not included in the final model. On linear regression for diastolic blood pressure, urinary sodium accounted for 0.09 of the variance.
Table 7b Characteristics of Hypertensive and Normotensive Older Female Vegetarians

<table>
<thead>
<tr>
<th></th>
<th>Hypertensive (N=71)</th>
<th>Normotensive (N=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Vegan</td>
<td>46</td>
<td>65</td>
</tr>
<tr>
<td>Aged home resident</td>
<td>42</td>
<td>59</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Calcium supplement</td>
<td>7</td>
<td>10!</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>Mean</td>
<td>78.7</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>77.1-80.3</td>
</tr>
<tr>
<td>Vegetarian years</td>
<td>42</td>
<td>38-46*</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>165</td>
<td>160-170**</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>81</td>
<td>79-83**</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>22.9</td>
<td>22.1-23.8</td>
</tr>
<tr>
<td>Mental score (max.12)</td>
<td>9.3</td>
<td>8.8-9.9</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.9</td>
<td>4.6-5.2</td>
</tr>
<tr>
<td>Triglyceride (g/L)</td>
<td>1.7</td>
<td>1.3-2.1</td>
</tr>
<tr>
<td>Urine Na/creatinine</td>
<td>32.6</td>
<td>27.8-36.8</td>
</tr>
<tr>
<td>Urine Na/K (mmol/mmol)</td>
<td>4.7</td>
<td>4.0-5.3*</td>
</tr>
<tr>
<td>Urine K/creatinine (mmol/mmol)</td>
<td>7.7</td>
<td>6.8-8.6</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>37.5</td>
<td>36.9-38.2</td>
</tr>
<tr>
<td>Energy intake (kcal/day)</td>
<td>1169</td>
<td>1084-1254</td>
</tr>
<tr>
<td>Calcium intake (g/day)</td>
<td>323</td>
<td>322-480#</td>
</tr>
<tr>
<td>Fibre intake (g/day)</td>
<td>7.3</td>
<td>7.4-10.7</td>
</tr>
<tr>
<td>Potassium intake (mg/day)</td>
<td>1039</td>
<td>974-1276</td>
</tr>
<tr>
<td>Protein calorie%</td>
<td>11.2</td>
<td>10.5-11.9</td>
</tr>
</tbody>
</table>

*, ** - P<0.05, <0.001, Student t test
# p<0.05, Mann Whitney; ! p<0.05, Chisquare test
A - missing 10 in dietary data, 6 in diabetes mellitus, 3 in blood test, 1 in alcohol and 1 in mental score
B- missing 6 in dietary data, 3 in diabetes mellitus, 2 in blood test, 1 in urinary data
Abbreviations: 95% CI – confidence interval of mean; Na – sodium, K - potassium
In order to further examine the importance of dietary sodium, potassium and calcium intakes, we compared the blood pressure and prevalence of hypertension among subjects with high or low calcium intake and urinary sodium/potassium ratio. The median urinary sodium potassium ratio and daily calcium intake of all the available subjects were 3.9 (mmol/mmol) and 346 g respectively. Among the 88 subjects who were not on antihypertensive or diuretics, 66 had dietary data. These subjects were divided into four groups, depending on whether their urinary sodium/potassium ratio and calcium intake were greater or smaller than the respective median values. As can be seen in Table 7c, there was a significant group difference in age in that the group with high urinary sodium/potassium ratio and low calcium intake was significantly older than the other groups. There was a significant group difference in systolic blood pressure, after adjustment for age. The main difference in systolic blood pressure lay between the high urinary sodium/potassium ratio-low calcium intake and low urinary sodium/potassium ratio-high calcium intake groups (159 ± 26 mmHg versus 130 ± 15 mmHg). The blood pressure of two other dietary groups was intermediate between these. In contrast, there was no significant group difference in diastolic blood pressure. There was also a significant group difference in the prevalence of hypertension (p=0.01, Chi-squared test). Again, the main difference was between the high urinary sodium/potassium ratio-low calcium intake and low urinary sodium/potassium ratio-high calcium intake groups (78% versus 25%).
Table 7c Comparison of Blood Pressures and Prevalence of Hypertension in Subjects with Different Urinary Sodium/Potassium Ratios and Calcium Intakes

<table>
<thead>
<tr>
<th></th>
<th>High Na/K</th>
<th>High Na/K</th>
<th>Low Na/K</th>
<th>Low Na/K</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Ca intake</td>
<td>High Ca intake</td>
<td>Low Ca intake</td>
<td>High Ca intake</td>
<td></td>
</tr>
<tr>
<td>No. of subjects</td>
<td>23</td>
<td>14</td>
<td>12</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>80.5±5.5</td>
<td>76.9±6.7</td>
<td>75.8±8.6</td>
<td>74±7.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Urine Na/K</td>
<td>6.7±1.8</td>
<td>6.6±3.0</td>
<td>2.2±0.7</td>
<td>2.4±0.9</td>
<td>0.00</td>
</tr>
<tr>
<td>Ca intake (g/day)</td>
<td>254±62</td>
<td>553±285</td>
<td>199±85</td>
<td>736±376</td>
<td>0.00</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>159±26</td>
<td>145±21</td>
<td>143±28</td>
<td>130±15</td>
<td>0.01</td>
</tr>
<tr>
<td>(mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>78±12</td>
<td>78±13</td>
<td>73±15</td>
<td>73±10.8</td>
<td>0.54</td>
</tr>
<tr>
<td>(mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>18(78%)</td>
<td>8(57%)</td>
<td>6(50%)</td>
<td>4(25%)</td>
<td>0.01</td>
</tr>
<tr>
<td>(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Comparison of blood pressures by ANCOVA, using age as covariate; comparison of prevalence of hypertension by Chi-square test; comparison of age by ANOVA

Abbreviations: Na/K urinary sodium/potassium ratio (mmol/mmol)

Definitions: Cut-off values for urinary sodium/potassium ratio and total daily calcium intake were 3.9 (mmol/mmol) and 346 g respectively
7.4 Discussion

7.4.1 Comparison of Older Vegetarians with Normal Older People in Prevalence of Hypertension

The blood pressures of the older vegetarians were comparable to those of historical control subjects, even though they were older and had increased urinary sodium intake as suggested by their greater urinary sodium excretion. The greater potassium intake as suggested by the urinary potassium excretion data of this study and the potassium intake data in chapter 4, might have exerted a blood pressure lowering effect.

Consistent with the above findings, the prevalence of hypertension in older vegetarians was found to be similar to that of a local survey. The survey examined a random local territory-wise sample of 2881 subjects of all ages from 25 years (Janus et al., 1997), the prevalence of definite hypertension (systolic BP \(\geq 160\) and/or diastolic BP \(\geq 95\) mmHg or on treatment for hypertension) and borderline hypertension (systolic BP 140-159 and/or diastolic BP 90-95 mmHg) was 35.7% and 18.8% respectively in 65-74 years old female (total number of 154). The corresponding prevalence in 65-74 yrs old male subjects (totaling 162) was 26.5% and 25.3% respectively.

Direct comparison with these local studies is hampered by the difference in the method of blood pressure measurement. In these studies, blood pressures were taken in sitting position, whereas they were taken in the supine position for the sake of convenience in this study. One study showed that if the arms were left dependent at the side of the body, there was no significant difference between
systolic blood pressures taken supine or seated, though diastolic pressures taken seated were higher than those taken supine by an average of 5 mmHg (Netea et al., 1998). But if the arms are elevated to the level of the right atrium, systolic pressures taken in sitting position are lower than those taken supine by an average of 8 mmHg (Terent & Breig-Asberg., 1994; Netea et al, 2002). In the Woo’s study (Woo et al., 1988c), the arms were not elevated to the level of the heart when sitting blood pressures were taken. The positioning of the arm was not mentioned in the Janus study (Janus et al., 1997).

Blood pressure readings are variable, particularly in the elderly (Parati et al., 1993). To make the diagnosis of hypertension, the averages of two consecutive blood pressure readings in three separate occasions are generally recommended. Even then, there is discrepancy between clinic and home blood pressures. The true pressure may be taken as the average of pressure over a prolonged period of time and appears to be a more important predictor of target organ damage than clinic pressure (O’Brien, 1994). 24-hour ambulatory pressure monitoring may be a better means to assess blood pressures of individuals. It removes observer error, reduces white coat effect and gives a profile of diurnal variation of blood pressures. The mean 24-hour ambulatory blood pressure readings are also more reproducible than clinic blood pressures in the elderly (Fotherby and Potter, 1993).
7.4.2 Comparison of Older Vegetarians with Normal Older People in Prevalence of Diabetes Mellitus

On the basis of history of diabetes mellitus or fasting glucose of greater or equal to 7.8 mmol/L, 14.6% of older vegetarians were found to be diabetic. This was comparable to local norms. In a local community survey of 1467 ambulatory older people (17% male) attending social centers in 1994, history of diabetes mellitus was present in 10.7% and 11.3% of 60-80 year olds and >80 yrs old respectively. Another 4.9% and 6.0% of 60-80 yrs old and >80 yrs old respectively had fasting glucose greater than 7.8 mmol/L. The overall prevalence of diabetes mellitus in 60-80 yrs old and >80 yrs old was therefore 15.7% and 17.3% respectively (Kung et al., 1996). In an earlier study (Woo et al., 1987), another local study of 427 community dwelling older people (41.5% male) with an average age of 70.6 years, the prevalence of diabetes mellitus, defined by either random glucose greater than 12.5 mmol/L or abnormal glucose tolerance tests for those with random glucose between 7.8 and 12.5 mmol/L or elevated concentrations of fructosamine or glycosylated haemoglobin, was found to be 9.8%. The prevalence rose from 8% in the 60-64 yrs old to 17% in the 75+ yrs olds.

Oral glucose tolerance test is the gold standard test for diabetes mellitus (DM). But for practical reasons and for the comparison of DM prevalence with previous local studies, fasting glucose was used as a criterion of DM. Cockram et al. (1991) showed that the sensitivity of the cutoff for fasting glucose of 7.8 mmol/L in detecting DM as defined by oral glucose tolerance test was only 40%.
The sensitivity was increased to 57% if the cutoff was lowered to 7.0 mmol/L. The true prevalence of DM in our older vegetarians was therefore very likely to be greater than 14.6%.

7.4.3 Dietary Determinants of Blood Pressures

Apart from medication, there has been a lot of interest in lowering blood pressure by dietary means. It was worrying that longer duration of vegetarianism was associated with hypertension. This implies that a certain aspects of the Chinese vegetarian diet may have predisposed the vegetarians to hypertension. The most consistent and powerful dietary determinant of blood pressure is sodium intake (MacGregor, 1985; Law, 1997; Hajjar, 2001). The association was more apparent in inter-countries comparisons than in local studies, because the local range of intake may be too narrow (MacGregor, 1985). This might explain why two local studies of adults and older people failed to demonstrate an association between blood pressure and urinary sodium:creatinine ratio (Woo et al., 1988c; Woo et al., 1992).

In contrast, a clear association between urinary sodium excretion and blood pressure among the relatively small number of vegetarian subjects was shown in this study. The very high level of urinary sodium excretion and the greater variability might explain the difference. The reason for the high salt intake in the vegetarians when compared with omnivores was probably due to the lack of tastes in the local Chinese vegetarian diet and the liberal use of soya sauce.
Salt intake is difficult to assess reliably. This is particularly the case in Chinese foods, as salt and different kinds of soya sauce are invariably added in the cooking process. It is therefore recommended to use 24-hour urinary sodium excretion adjusted by creatinine as a marker of sodium intake. But in older people, reliable 24 urine collections are difficult to achieve.

The association between low dietary calcium intake and hypertension has been controversial and inconsistent in Western studies (MacGregor, 1985). While epidemiological data consistently showed an inverse relationship between dietary calcium and blood pressure, clinical trials of calcium supplementation have not been as consistent in outcome, probably because of high baseline calcium intake (McCarron et al., 1994). Recent meta analysis did confirm the association between calcium intake and blood pressure (Cappuccio et al., 1995). Calcium supplementation could also reduce blood pressure significantly but no more than 2 mmHg on average (Allender et al., 1996).

It was somewhat surprising that this small sized study did indeed show the association between low calcium intake and elevated blood pressure. This contrasts with another local study of older Chinese people which showed a slight positive correlation between calcium intake and systolic blood pressure (Woo et al., 1988c). The female subjects of this sample were compared with the vegetarians in Table 7a. The vegetarians were older, and had greater urinary excretion of sodium and potassium. Whether any of these factors were relevant to the apparent hypotensive effect of higher dietary calcium required further investigations.
The exact mechanism whereby calcium influences blood pressure is as yet uncertain. A plausible mechanism is that hypertension is associated with a defect in calcium handling characterized by a renal calcium leak, increased circulating parathyroid hormone, and increased intracellular calcium levels, which may be a consequence of abnormal calmodulin activity and may lead to increase in vascular tone (McCarron et al., 1994; Postnov et al., 1984). Increasing dietary calcium appears to improve calmodulin activity in the spontaneously hypertensive rat (McCarron et al., 1994).

As shown in Chapter 4, the calcium intakes of our vegetarian subjects were in fact higher than the omnivores. In Asian countries, calcium intakes are frequently low at all ages (Iso et al., 1991). The main reason is the unpopularity of dairy products in this part of the world. It is not easy to increase calcium intake significantly in our older vegetarians as they had poor dental health (see Chapter 12). Realistically, the simplest solution was for them to drink milk. Lactase intolerance in Chinese has also been well documented. But its clinical significance has been over-stated. Moreover low lactose milk powder preparations are now commercially available and have been shown to be well tolerated by Chinese old age home residents (Kwok et al., 2001a).

Observational studies have demonstrated an inverse relationship between potassium intake and blood pressure (Intersalt Cooperative Research Group, 1988). Oral potassium supplementation lowers blood pressure (Whelton et al., 1997), and this effect is more pronounced in subjects with a high sodium intake. There was no association between potassium intake either measured by dietary
recall or by urinalysis, and hypertension or blood pressure in this study. The urinary data suggested that the vegetarians had generally higher potassium intake with less variation than omnivores. This might explain the lack of correlation observed. One local study did observe the negative association between urinary potassium/creatinine and blood pressure (Woo et al., 1992), but another one on older people by the same research group did not (Woo et al., 1988c). Despite the lack of correlation observed in this study, the high potassium intake was likely to have counterbalanced to a certain degree the detrimental effect of high sodium intake on the blood pressure in this group of vegetarians.

No association between hypertension and protein intake, or between hypertension and serum albumin was found in this study. The evidence for a relationship between protein intake and blood pressure is at present confused. High protein intake estimated by 24-hour urinary nitrogen and urea was shown to be independently associated with high blood pressure in an epidemiological study of over 10,000 men and women (Stamler et al., 1996a). But protein energy malnutrition was found to lower blood pressure in hypertensive rats by improving insulin sensitivity (Somova et al., 2000). Interestingly a recent epidemiological study showed that animal protein intake as estimated by 3-Methylhistidinidine inversely correlated with systolic and diastolic blood pressures in Chinese (Liu et al., 2000). Soy protein which contains phyto-oestrogen, on the other hand, was found to lower diastolic blood pressure by an average of 5 mm Hg in a randomized placebo controlled trial of perimenopausal women (Washburn et al., 1999).
Dietary components should not be examined in isolation, as they frequently interact with each other (Stamler et al., 1996b). In this study, a clear dietary pattern of high sodium, low calcium intake was associated with higher systolic blood pressure. From the point of view of the older vegetarian subjects, if they can reduce their salt intake to that of the general older population, and embrace dairy products, their blood pressure may be reduced significantly.
7.5 Conclusion

Based on this study and the study in last chapter, one can conclude that older vegetarians were equally exposed to the detrimental effects of hypertension, diabetes mellitus and hypertriglyceridaemia as normal older people in Hong Kong. The only advantage was a decrease in serum cholesterol concentrations because of the combination of low fat and high soya intakes.

In the next chapter, a method of detection of metabolic vitamin B12 deficiency which is common in vegetarians will be described.
7.6 Key Points

1. The prevalence of hypertension and diabetes mellitus in older vegetarians was comparable to omnivores.

2. Low calcium and high sodium intakes were associated with higher systolic blood pressure in the older vegetarians.
Chapter 8  Relationship between Urinary and Serum Methylmalonic acid Concentrations in Older Chinese Vegetarians

8.1  Introduction

The reliability of serum vitamin B12 concentration in detection of vitamin B12 deficiency has been questioned. Apart from earlier problems with reliability of laboratory methods, studies have shown that a quarter of subjects with serum vitamin B12 concentrations as low as <100 pmol/L could have no clinical or biochemical evidence of deficiency (Moelby et al., 1990), and that 10-40% of older people within serum vitamin B12 concentrations between 150 and 300 pmol/L had metabolic deficiency, as shown by elevated serum methylmalonic acid (MMA) concentrations (Lindenbaum et al., 1994).

Serum MMA has been suggested to be a more reliable measurement of metabolic vitamin B12 deficiency than serum vitamin B12 concentrations (Savage et al., 1994). Compared to serum MMA, urinary MMA is biochemically more stable and is more reliably measured because of its greater concentrations. It is generally agreed that the cut-off value for urinary MMA/creatinine ratio is 4.8 \( \mu \text{mol/mmol creatinine} \) (Matchar et al., 1987). However this cut-off value was derived from cases of clinical vitamin B12 deficiency, mostly in the form of pernicious anaemia.

In order to prevent the occurrence of clinical manifestations of vitamin B12 deficiency, it is necessary to screen for preceding metabolic vitamin B12 deficiency (Herbert, 1994), which is a common in old age (Lindenbaum et al., 1994; Pennypacker et al., 1992). The importance of treating vitamin B12
deficiency early was borne out by the observation that neurological symptoms might develop in the absence of anaemia (Lindenbaum et al., 1988; Healton et al., 1991), and that only recent onset of dementia could benefit from vitamin B12 supplementation (Martin et al., 1992). In addition, hyperhomocysteinaemia associated with raised MMA is now a recognized independent cardiovascular risk factor (Clarke, 2001).

Therefore the cut-off value of urinary MMA/creatinine ratio should be determined by its sensitivity and specificity in detecting raised serum MMA which is considered to be a more reliable indicator of metabolic vitamin B12 deficiency. As there is evidence that urinary MMA is increased by food intake (Rasmussen, 1989), fasting urinary MMA was used.
8.2 Subjects and Methods

All the 117 female vegetarian subjects who attended the research clinic in Prince of Wales Hospital were included in this study. In order to increase the sample size, the additional 13 female vegetarian subjects recruited from Chi Lin old age home residents were included in this study. The following data was used for analysis: fasting serum vitamin B12, folate, MMA and renal function, and fasting urinary MMA/creatinine ratio. The methods of MMA assays were described in Chapter 3. Serum MMA $\geq 0.4 \, \mu\text{mol/L}$ was defined to be abnormal (Allen et al., 1990; Savage et al, 1994). Those subjects with serum creatinine $>150 \, \mu\text{mol/L}$ were excluded.
8.3 Results

Complete data was available in 118 out of 130 vegetarian subjects (6 missing serum MMA, 4 missing renal function test, 1 missing urine MMA because of incontinence); one other subject with missing serum vitamin B12 concentration was also included. Five were excluded because of serum creatinine concentrations being above 150 mmol/L. The total number of subjects for analysis was 114. The average age of the subjects was 77.4 (sd 7.6, range 55-94) yrs. All except 4 subjects had been vegetarian for more than ten years. The concentrations of serum vitamin B12 and MMA, and urinary MMA/creatinine were shown in Table 8a. The correlation coefficient between serum and urinary MMA/creatinine was 0.94. The scatter plot of urinary MMA/creatinine against serum MMA was shown in Figure 8a. When urinary MMA/creatinine was regressed against serum MMA, the explained variance was 0.88. There was no significant increase in explained variance when age was added into the regression model (0.89).
Table 8a  Ranges of Vitamin B12 and Methylmalonic Acid Concentrations in Older Vegetarians

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12*  pmol/L</td>
<td>145</td>
<td>37</td>
<td>1771</td>
<td>&gt;150</td>
</tr>
<tr>
<td>Folate* nmol/L</td>
<td>50.1</td>
<td>14.5</td>
<td>54</td>
<td>&gt;10</td>
</tr>
<tr>
<td>SMMA μmol/L</td>
<td>0.63</td>
<td>0.06</td>
<td>11.7</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>UMMA μmol/mmol creatinine</td>
<td>2.5</td>
<td>0.5</td>
<td>85.9</td>
<td>&lt;5.0</td>
</tr>
</tbody>
</table>

Abbreviations: SMMA – serum methylmalonic acid; UMM – urinary MMA

* lowest recordable vitamin B12 concentration was 37 pmol/L, the highest recordable folate concentration was 54 nmol/L.
Figure 8a Scatter Plot of Urinary Methylmalonic Acid:Creatinine Ratio against Serum Methylmalonic Acid Concentrations

Abbreviations: SMMA – serum methylmalonic acid; UMMA – urinary methylmalonic acid; CR – creatinine
80 out of 114 subjects (70%) had raised serum MMA (≥ 0.4 μmol/L).

When urinary MMA/creatinine ratio was used to predict raised serum MMA, the ROC area of 0.88. The ROC curve was shown in Figure 8b.

**Figure 8b Receiver Operating Characteristic (ROC) curves for the Use of Fasting Urinary Methylmalonic Acid (MMA) to Predict Serum MMA > 0.4 μmol/L.**

The thick arrow indicates the cutoff value considered optimal for the purpose of screening; the thin arrow indicates the previously recommended cutoff value of urinary MMA of 4.8 μmol/mmol creatinine.
The most optimal cut-off values appeared to be $2 \mu \text{mol/mmol creatinine}$, as shown in Figure 8b. It had a sensitivity of 78.8%, positive predictive value of 92.6%, specificity of 85.3% and 64 out of 68 subjects (94.1%) with urinary MMA/creatinine > $2 \mu \text{mol/mmol creatinine}$ had serum vitamin B12 concentration < 300 pmol/L, and 32 (47.1%) had vitamin B12 concentrations < 150 pmol/L.
8.4 Discussion

This study showed that the recommended cut-off value of normal urinary MMA/creatinine was too insensitive to an elevated serum MMA concentration. The lower cut-off value of 2 μmol/mmol creatinine improved sensitivity while giving a good positive predictive value.

Increased urinary excretion of MMA in vitamin B12 deficiency was first described by Cox and White in 1962. Gas chromatography mass spectroscopy was first used to assay urinary MMA in the early 1980’s (Norman et al., 1979). Norman first suggested the upper normal range limit of 5 μg/mg creatinine, equivalent to 4.8 μmol/mmol creatinine (Norman, 1984). In a prospective study of 96 patients with suspected vitamin B12 deficiency, this cut-off value of spot urinary MMA was found to have sensitivity and specificity close to 100% in detecting the 7 cases of reversible classic vitamin B12 related haematological anomalies (Matchar et al., 1987). In a later study, raised spot urinary MMA was found to have high predictive value in detecting subnormal serum vitamin B12 or raised serum MMA in older people (Norman & Morrison, 1993).

Capillary gas chromatography-mass spectrometry was later developed to detect the much lower concentrations of MMA in human serum (Marcell et al., 1985). With this technological advance, it was found that urinary MMA increased significantly after foods, while serum MMA remained relatively stable (Rasmussen, 1989). Rasmussen et al. further demonstrated that the renal clearance of MMA was only about half of that of creatinine, suggesting renal reabsorption of MMA. The correlations between serum MMA and urinary MMA
adjusted by creatinine were fairly good in normal subjects and very good in subjects with raised serum MMA ($R = 0.57, 0.98$ respectively). The normal range of urinary MMA was $0.24 - 1.92 \mu\text{mol/mmol creatinine}$ (Rasmussen et al., 1989).

The normal range of serum MMA measured by capillary gas chromatography – mass spectrometry as tert.-butyldimethylsilyl derivative in older people was reported to be $0.02 - 0.40 \mu\text{mol/L}$ (Starczek et al., 1993). Using this upper limit, 98.4% of 434 episodes of definite vitamin B12 deficiency defined by clinical features and response to treatment had raised serum MMA (Savage et al., 1994). Because its concentrations are more stable and not affected by food, serum MMA is a more reliable indicator of metabolic vitamin B12 deficiency.

In this study, the overall reproducibility of MMA assays was not as good as that reported in the literature. Slight difference in equipment used might account for some of the differences. As expected, urinary MMA showed better reproducibility than that of serum MMA, because the concentration of MMA in serum is much lower than that in urine. Renal impairment can lead to a moderate rise in serum MMA. Such patients were therefore excluded from this study. Urinary MMA/ mmol of creatinine has theoretically adjusted for renal impairment. But its reliability in established renal failure requires further study.

The correlation between urinary and serum MMA was greater than reported. This may be attributed to the fact that all urine samples were collected after an overnight fast. Food has been shown to increase urinary MMA. More variation in urinary MMA can therefore be expected if urine samples are taken at random. This may also explain the much lower cut-off value elicited in this study.
The recommended cut-off value of serum MMA was also based on clinical cases of vitamin B12 deficiency. It was interesting that a study showed that subjects with serum MMA between 0.3 and 0.4 µmol/L also showed a significant decrease upon vitamin B12 supplementation. It is therefore possible that the lower cut-off value of normality may be as low as 0.3 µmol/L.

This study had several limitations. Firstly, the subjects were strict vegetarians with a high prevalence of vitamin B₁₂ deficiency. The positive predictive value of urinary MMA for elevated serum MMA and subnormal vitamin B₁₂ concentrations is likely to lower when the underlying prevalence of deficiency is much lower. Secondly, the protein intakes of these vegetarians were significantly lower than normal older people; the average proportion of calories due to protein was 12% in vegetarians versus 16% in omnivores (Table 4a). The influence of habitually low protein intake on the serum MMA concentrations, and on the urinary MMA excretion has not investigated, except that restricted protein intake has been effectively used to prevent neurological damage in young children with congenital methylmalonic academia (van der Meer et al., 1994). Lastly, subjects with renal impairment were excluded from this study. The validity of urinary MMA:creatinine ratio in detecting metabolic vitamin B₁₂ deficiency in renal impaired subjects therefore requires further study. In view of these limitations, further validation by screening a large group of older people in different settings is warranted.

It was concluded that urinary MMA taken after an overnight fast had a strong correlation with serum MMA. It was highly sensitive and specific for the
detection of raised serum MMA. The optimal cut-off value was 2 μmol/mmol creatinine. With this simple screening tool, the prevalence of vitamin B12 deficiency of vegetarians was compared with that of omnivores in the next chapter.
8.5 Key Points

1. Urinary MMA has high correlation with serum MMA in older people without significant renal impairment.

2. Urinary MMA > 2 μmol/mmol creatinine is highly sensitive and specific for elevated serum MMA concentrations.
Chapter 9  Vitamin B12 Status of Older Chinese Vegetarians

9.1 Introduction

Vegetarians are at risk of vitamin B12 deficiency. Dietary deficiency is by far the commonest cause (Hokin & Butler, 1999). The main sources of vitamin B12 in lactovegetarians are from dairy products and eggs. Vegans have to rely on fortified foods – meat analogues, soya milk, cereals, and fortified yeast products. Fortified foods are commonly available in the United States. But vegans in countries where food fortification is more strictly controlled or unavailable are at greater risk of vitamin B12 deficiency. Mushrooms and bacterial contaminated or fermented foods could provide small amount of vitamin B12 (Herbert, 1988). Seaweeds contain cobalamin analogues but they may not be biologically active (Dagnelie, 1997).

Vitamin B12 taken during the years prior to vegetarianism could be preserved for years by enterohepatic re-circulatory mechanism (Herbert, 1994). That is why the recommended daily intake of vitamin B12 is only 1.9 mcg (Joint FAO/WHO, 1988). A study in New Zealand showed that 100% of vegans and 42% of lactovegetarians had less than 2 mcg daily (Alexander et al., 1994).

The older Chinese vegetarians seldom consume dairy products and eggs because of religious and cultural reasons. Vitamin B12 fortified foods are not commonly available in Hong Kong. It is therefore likely that the older Chinese vegetarians have a very high prevalence of vitamin B12 deficiency.

This study therefore ascertained the prevalence of vitamin B12 deficiency in older Chinese vegetarians and compared it with omnivores. To prevent vitamin
B12 deficiency, potential risk factors among vegetarians, e.g. age, duration of vegetarianism, disabilities etc. were examined by logistic regression.
9.2 Subjects and Methods

Apart from the 117 vegetarian subjects who attended the research clinic in Prince of Wales Hospital, 40 additional vegetarian subjects were recruited from four vegetarian old age homes (see section 3.4).

In one of these homes (Cheuklam) which was essentially sheltered housing for older people within a Taoist temple, 26 omnivores were recruited. These omnivorous subjects shared the same lifestyle of the vegetarian subjects. Another 107 residents in an omnivorous old age hostel were recruited for comparison. But these subjects only had fasting urinary MMA/creatinine ratio estimation. All subjects were above 55 years old.

Blood samples were analyzed for serum vitamin B12, and methylmalonic acid (MMA) concentrations. Fasting urine samples were analyzed for MMA: creatinine ratio. Definite vitamin B12 deficiency was defined as serum MMA \( \geq 0.4 \ \mu\text{mol/L} \) and serum vitamin B12 \(<150 \ \text{pmol/L}; \) possible deficiency when either criterion was met; no deficiency when no criterion was met. As suggested in the last chapter, urinary MMA \( > 2 \ \mu\text{mol/mmol creatinine} \) was regarded as indicative of possible vitamin B12 deficiency.

The duration of vegetarianism, intake of dairy products, eggs and oral vitamin supplements were recorded.
9.3 Results

One hundred and forty five vegetarians, and 26 omnivores had complete data on blood and urine tests. The average ages of vegetarians and omnivores were 79.3 (sd 7.5) and 79.8 (7.5) yrs respectively. The majority of vegetarian and omnivorous subjects were female (95% and 74% respectively). 32 of vegetarian subjects were on vitamin supplements, but none of the supplements contained any vitamin B12.

The prevalence of subnormal vitamin B12 (<150 pmol/L) and raised serum MMA (≥ 0.4 μmol/L) was 66.1% and 74.2% in vegans, 37.3% and 55.4% in lactovegetarians, 11.5% and 32.6% in omnivores. The overall prevalence of definite vitamin B12 deficiency in vegetarians was 40.7%. The comparison of vitamin B12 status defined by serum vitamin B12 and MMA among vegans, lactovegetarians and omnivores was shown in Table 9a. Vegans had the higher risk of vitamin B12 deficiency than lactovegetarians (p<0.005, Chisquare test).
Table 9a  Comparison of Vitamin B12 Status between Vegetarians and Omnivores

<table>
<thead>
<tr>
<th>Vitamin B12 Deficiency*</th>
<th>Total</th>
<th>No</th>
<th>Possible</th>
<th>Definite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegan</td>
<td>62</td>
<td>10(16%)</td>
<td>17(27%)</td>
<td>35(57%)</td>
</tr>
<tr>
<td>Lactovegetarian</td>
<td>83</td>
<td>30(36%)</td>
<td>29(36%)</td>
<td>24(29%)</td>
</tr>
<tr>
<td>Omnivore</td>
<td>26</td>
<td>16(62%)</td>
<td>8(31%)</td>
<td>2(8%)</td>
</tr>
</tbody>
</table>

*Definite deficiency: Serum Methylmalonic acid (SMMA) $\geq 0.4$ μmol/L and vitamin B12 $<150$ pmol/L; Possible deficiency: only one of above criteria met; No deficiency: none of criteria met. There were significant differences in vitamin B12 status among the three dietary groups (Chisquare test, $p<0.000$)

Ten out of 26 (38.5%) omnivore subjects had raised urinary MMA/creatinine ratio. Similarly, 37 out of 107 (34.6%) omnivore old age home residents had raised urinary MMA/creatinine ratio. The difference was not significant.

The odds ratios of potential predictors of definite vitamin B12 deficiency, determined by logistic regression, were shown in Table 9b. The significant predictors were intakes of milk products or eggs.
Table 9b Odds Ratios of Predictors of Definite Vitamin B12 Deficiency among Older Vegetarians

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>OR</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55-80</td>
<td>78</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>&gt;80</td>
<td>67</td>
<td>0.76</td>
<td>0.33-1.76</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>138</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>7</td>
<td>1.51</td>
<td>0.14-16.74</td>
</tr>
<tr>
<td><strong>Milk intake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>61</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>84</td>
<td>0.34*</td>
<td>0.14-0.78</td>
</tr>
<tr>
<td><strong>Egg intake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>114</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>31</td>
<td>0.29*</td>
<td>0.09-0.90</td>
</tr>
<tr>
<td><strong>Vegetarian</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;= 10 yrs</td>
<td>23</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>&gt;10 yrs</td>
<td>119</td>
<td>0.49</td>
<td>0.20-1.20</td>
</tr>
<tr>
<td><strong>Old age home resident</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>51</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>94</td>
<td>1.1</td>
<td>0.41-2.98</td>
</tr>
</tbody>
</table>

1. Definite deficiency: Serum Methylmalonic acid $\geq 0.4 \mu$mol/L and vitamin B$_{12} <150$ pmol/L
2. included dairy products and oral nutritional supplements

Abbreviations: OR – odds ratio, CI – confidence interval


9.4 Discussion

This study confirmed the high prevalence of vitamin B12 deficiency amongst older Chinese vegetarians. The largest prevalence study of vegetarians was Hokin & Butler (1999) who found that 53% of Seventh-day Adventist primarily lactovegetarian church ministers had serum vitamin B12 less than 171 pmol/L. Up to 50% of people on macrobiotic diet had serum vitamin B12 less than 150 pmol/L (Miller et al., 1991). But one small study of Seventh-day Adventist New Zealanders showed no significant difference in serum vitamin B12 concentrations from omnivores (Harman & Parnell, 1998).

The intakes of milk and eggs were significant protective factors against deficiency. This highlighted the importance of these foods in maintaining vitamin B12 status of older Chinese vegetarians. A recent study showed that the older people could absorb vitamin B12 from milk efficiently (Russell et al., 2001). Unfortunately, these foods were forbidden by Taoism and discouraged by Buddhism. But even without these prohibitions, milk is not a popular food among the older people in the Southern part of China. The reason why a reasonable number of lactovegetarians were recruited was because old age home residents in general were very much encouraged to drink milk powder or oral nutritional supplements on a daily basis. The relevance of lactase deficiency in Chinese people has been over-stated. Moreover, low lactose milk powder preparations are available for those who have lactase deficiency and it was found to be well tolerated by local old age home residents (Kwok et al., 2001b). Apart from rejection of eggs for religious reasons, older people in Hong Kong also had the
perception that its cholesterol content was bad for health. In a community survey of over 2,000 older people in Hong Kong, a quarter avoided eggs (Ho & Woo, 1994).

It is noteworthy that the prevalence of vitamin B12 deficiency in omnivores was significant. Even with the generally used cut-off value of 5 μmol/mmol creatinine (which was shown to be very insensitive in detecting raised serum MMA in chapter 8), 9.8% of the omnivorous subjects were deficient. This was higher than that in an American study in which 3-5% of a total of 809 elderly people at four different sites had spot urinary MMA greater than 5 μmol/mmol creatinine (Norman & Morrison, 1993). But it was consistent with other studies which showed prevalence of 11 to 15% for older subjects with elevated serum MMA and borderline low cobalamin concentrations (Lindebaum et al., 1994; Pennypacker et al., 1992). The prevalence of vitamin B12 deficiency in the older Chinese people in Hong Kong warrants further investigation.

At both extremes of serum vitamin B12 concentrations, there were unexpected SMMA concentrations. For example, there were five vegetarian subjects with vitamin B12 concentrations close to or greater than 1000 pmol/L, yet one of them had raised serum MMA. It was possible that some of them had received vitamin B12 injections in a recent doctor’s consultation. Another possibility was the ingestion of seaweeds. The cobalamin analogues in seaweed are mostly bio-inactive (Dagnelie, 1997).

There was one female vegan who had definite vitamin B12 deficiency as her vitamin B12 concentration was only 41.5 pmol/L and her macrocytosis and
pancytopenia responded to vitamin B12 supplementation subsequently. However, both her serum and urine MMA were well within the normal ranges. The failure of MMA to rise in response to vitamin B12 deficiency has been thought to be rare and attributed to changes in gut flora (Lindenbaum et al., 1990).

It was notable that oral vitamin supplements (vitamin B complex and multivitamin) were commonly prescribed in vegetarian old age homes. Yet none of the preparations contained any vitamin B12. In view of the high prevalence of vitamin B12 deficiency, and their reluctance to consume dairy products or eggs, older Chinese vegetarians should be advised to take oral vitamin B12 supplements on a daily basis. Enriched foods are equally effective in supplementing vitamin B12. In a randomized controlled trial, enriched foods containing as little as 2.5 μg vitamin B12 and 0.25 mg folic acid daily were effective in decreasing serum MMA by 30% in frail older people over a four month period (de Jong et al., 2001).

It was concluded that vitamin B12 deficiency was very common among older Chinese vegetarians. Vegans were particularly at risk. All older Chinese vegetarians should be advised to take oral vitamin B12 supplements or fortified foods. In the next chapter, the independent effect of vitamin B12 deficiency on haematological status in older vegetarians was investigated.
9.5 Key Points

1. Vitamin B12 deficiency is very common among older Chinese vegetarians.

2. Intakes of dairy products and eggs protect against vitamin B12 deficiency.

3. All older Chinese vegetarians should be advised to take vitamin B12 supplements or consume fortified foods.
Chapter 10 The Influence of Vitamin B12 Deficiency on Haematological Status of Older Chinese Vegetarian Women

10.1 Introduction

In the last chapter, the prevalence of vitamin B12 deficiency was found to be very high in older Chinese vegetarians. It is well known that vitamin B12 deficiency could cause macrocytic anaemia. Although Western vegetarians are also at risk of vitamin B12 deficiency, comparative studies of vegetarians and omnivores have so far shown no or minor differences in haemoglobin concentrations and mean corpuscular volumes (Tungtrongchitr et al., 1993; Sanders et al., 1978; Inamdar-Deshmurkh et al., 1976), except when there was a high prevalence of iron deficiency (Pongstaporn & Bunyaratavej, 1999). Macrocyanosis was remarkably rare in these studies. It was possible that the high folate intake and iron deficiency of vegetarians masked the classic haematological manifestations of vitamin B12 deficiency. It was also possible that the degree of vitamin B12 deficiency, presumably dietary in origin, in adult vegetarians was not severe enough to cause macrocytosis or anaemia (Herbert, 1994). There is evidence that the older people may be more susceptible to macrocytosis with vitamin B12 deficiency. In a study of fifty-six geriatric outpatients with low serum B12 concentrations showed that thirteen (23%) had macrocytosis (Stott et al., 1997).

These studies defined vitamin B12 deficiency by serum vitamin B12 concentrations. As discussed in chapter 8, this definition was unsatisfactory. With the addition of serum methylmalonic acid concentration, the vitamin B12 status
can be better defined. It was therefore possible to examine the independent effect of varying degrees of vitamin B12 deficiency on the haematological status of older Chinese people. The confounding effects of iron deficiency and renal impairment were avoided by exclusion.
10.2 Subjects and Methods

All the 117 female vegetarian subjects who attended the research clinic in Prince of Wales Hospital were included in this study. In order to increase the sample size, the additional 13 female vegetarian subjects recruited from Chi Lin old age home residents were included in this study.

The following data was used in analysis: complete blood count, serum iron, total serum iron binding capacity, serum iron saturation, renal function test, serum vitamin B12 and folate, and serum methylmalonic acid concentrations (MMA).

Anaemia was defined to be haemoglobin concentration less than 12 g/dl. Normal range of mean corpuscular volume was 80-97 fl. Lower limits of normal white cell count and platelet count were $4.0 \times 10^9$ /L and $140 \times 10^9$ /L respectively.

Iron deficiency was defined to be iron saturation <10%. Iron saturation between 10-15% was considered to signify possible iron deficiency. Lower limit of normal serum folate concentration was 2.1 nmol/L.

Lower limit of serum vitamin B12 concentration was 150 pmol/L, and the upper normal limit of serum MMA was 0.4 $\mu$mol/L (Allen et al., 1990). Subjects with low vitamin B12 concentrations and raised serum MMA were considered to be definitely vitamin B12 deficient. Those with low vitamin B12 or raised MMA were considered to be possible cases of vitamin B12 deficiency.

For the analysis of the influence of vitamin B12 status and haematological indices, subjects with possible or definite iron deficiency, those with serum creatinine $>150$ mmol/L and those with low serum folate concentrations were
excluded. The rationale for excluding patients with renal failure was that it would affect the haemoglobin concentrations and serum MMA concentrations.
10.3 Results

One hundred and nineteen female vegetarian subjects had complete data on serum vitamin B12 and MMA, complete blood count and renal function test. Twenty-six subjects in a vegetarian old age home took multivitamin and mineral supplement which contained 5 mg of iron, but no vitamin B12, daily. There were nine missing values for iron status because of insufficient samples. There were 4 subjects with definite iron deficiency and 7 subjects with possible deficiency. The prevalence of subnormal iron status was therefore 11/110 (10%). If one excluded those subjects on iron supplement, the prevalence of iron deficiency was 11/89 (12.4%). Out of the 11 subjects with compromised iron status, 5 had normocytic anaemia, and 2 had microcytic anaemia (both had definite deficiency).

The prevalence of definite vitamin B12 deficiency (low vitamin B12 concentration and raised MMA) was 50/119 (42%). Another 39/119 (32.8%) had possible vitamin B12 deficiency (low vitamin B12 or raised MMA alone). No subject had subnormal serum folate concentrations. There were four subjects with abnormally high serum vitamin B12 concentrations (>1000 pmol/L). Three of them had normal serum MMA. Two of the four subjects had macrocytic anaemia, one of them had normal serum MMA.

Three subjects were further excluded because of raised serum creatinine concentrations, the maximum concentration being 290 $\mu$ mol/L. After further excluding subjects with possible and definite iron deficiency, and those with missing serum iron concentrations, 96 subjects were available for further analysis. The characteristics and haematological profile of subjects with different vitamin
B12 status were compared in Table 10a. The deficient groups were more likely to be vegans and have lower haemoglobin concentrations.
Table 10a Characteristics and Haematological Profile of Older Chinese Vegetarian Women With and Without Vitamin B12 Deficiency

<table>
<thead>
<tr>
<th>Vitamin B12 Deficiency</th>
<th>No (N=23)</th>
<th>Possible (N=28)</th>
<th>Definite (N=45)</th>
<th>P value#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old age home resident</td>
<td>13 57%</td>
<td>18 64%</td>
<td>23 51%</td>
<td>NS</td>
</tr>
<tr>
<td>Milk intake*</td>
<td>19 83%</td>
<td>20 72%</td>
<td>18 40%</td>
<td>0.000</td>
</tr>
<tr>
<td>Egg intake</td>
<td>7 30%</td>
<td>9 32%</td>
<td>6 13%</td>
<td>NS</td>
</tr>
<tr>
<td>Mean SD Mean SD Mean SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>78.1 9.1</td>
<td>80.2 7.2</td>
<td>78 6.8</td>
<td>NS</td>
</tr>
<tr>
<td>Vegetarian years</td>
<td>35.3 15.4</td>
<td>43 22.1</td>
<td>33.4 17.6</td>
<td>NS</td>
</tr>
<tr>
<td>Iron saturation (%)</td>
<td>25.9 8.2</td>
<td>26 7</td>
<td>26 7</td>
<td>NS</td>
</tr>
<tr>
<td>Vitamin B12 (pmol/L)</td>
<td>489 420</td>
<td>273 316</td>
<td>87 29</td>
<td>0.000</td>
</tr>
<tr>
<td>Serum MMA (umol/L)</td>
<td>0.2 0.1</td>
<td>0.8 0.6</td>
<td>1.5 2</td>
<td>0.000</td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
<td>49 9.2</td>
<td>46 9</td>
<td>45.4 10.5</td>
<td>NS</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13 1.3</td>
<td>12.1 1</td>
<td>12.4 1.2</td>
<td>0.030</td>
</tr>
<tr>
<td>Mean Corpuscular Volume (fl)</td>
<td>90.6 9.6</td>
<td>88.8 8.2</td>
<td>90.2 9</td>
<td>NS</td>
</tr>
<tr>
<td>White blood cell (per 10^6)</td>
<td>6.3 1.7</td>
<td>7.1 2.3</td>
<td>6.6 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Platelet (per 10^6)</td>
<td>221 57</td>
<td>226 66</td>
<td>206 62</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: SD – standard deviation; MMA – methylmalonic acid; NS – not significant

* nutritional supplements included

# ANOVA for continuous data, Kruskal Wallis test for nominal data
Serum MMA but not vitamin B12 correlated with haemoglobin concentrations (correlation coefficient \(-0.34, p 0.001\)), MCV (0.31, \(p 0.003\)), and platelet count (-0.20, \(p 0.05\)). Neither serum MMA nor vitamin B12 correlated with white cell count. On univariate analysis, adjusting for age, old age home residence, partial dependency, iron saturation and folate concentration, serum MMA correlated inversely with haemoglobin concentration and platelet count, and positively with MCV (\(p 0.000, 0.03, 0.005\), respectively). The prevalence of anaemia, macrocytosis and thrombocytopenia at different concentrations of serum MMA was shown in Table 10b. There was no significant increase in anaemia until serum MMA was greater than 1.0 \(\mu\)mol/L (\(p 0.03\), Chisquare test). There was no significant association found between vitamin B12 and any haematological indices. Moreover, there was no association between low vitamin B12 concentrations (defined as either <150 pmol/L or <300 pmol/L) and any of the haematological indices (Chisquare test).
Table 10b  Percentages of Haematological Abnormalities at Different Degrees of Vitamin B12 Deficiency

<table>
<thead>
<tr>
<th>Serum Methylmalonic Acid</th>
<th>Normal</th>
<th>Borderline Raised</th>
<th>Raised</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.4 $\mu$ mol/L</td>
<td>6</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>0.4-1.0 $\mu$ mol/L</td>
<td>19%</td>
<td>23%</td>
<td>48%</td>
</tr>
<tr>
<td>&gt;1.0 $\mu$ mol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Anaemia

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Borderline Raised</th>
<th>Raised</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>19%</td>
<td>23%</td>
<td>48%</td>
</tr>
</tbody>
</table>

Macrocytosis

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Borderline Raised</th>
<th>Raised</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>7%</td>
<td>8%</td>
<td>16%</td>
</tr>
</tbody>
</table>

Thrombocytopenia

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Borderline Raised</th>
<th>Raised</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>3%</td>
<td>13%</td>
</tr>
</tbody>
</table>

Total

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Borderline Raised</th>
<th>Raised</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Definitions: Anaemia – haemoglobin <12g/dl; Macrocytosis – mean corpuscular volume >97 fl; Thrombocytopenia – platelet count < 140 x 10^9/L.
10.4 Discussion

This study showed vitamin B12 deficiency was associated with a decrease in haemoglobin concentrations. However, anaemia tended to occur only when metabolic deficiency was moderately severe. Although there was a correlation with an increase in MCV and a decrease in platelet count, macrocytosis and thrombocytopenia were uncommon. Serum vitamin B12 had no significant correlation with haematological indices.

Anaemia was present in nearly one third of the older vegetarian subjects. This prevalence of anaemia was even higher than that reported in postmenopausal South Asians who were infrequent meat eaters in the UK (Fishbacher et al., 2001).

The prevalence of iron deficiency anaemia in this group of older vegetarians, after excluding the ones with iron supplements, was 7/110 (6.4%). This was higher than the 2.5% reported in 427 older Chinese people with low socioeconomic status in Hong Kong (Woo et al., 1989). However it was at the lower end of the range reported in vegetarians in Asians and Caucasians (Pongstaporn & Bunyaratavej, 1999, Reddy & Sanders, 1990). Although our vegetarian subjects had dietary iron intakes comparable to those of omnivores of similar age (Table 4a), they were still at risk of iron deficiency because of inhibitors of absorption of non-hem iron from vegetable sources, e.g. fiber and phytate (Alexander et al., 1994). The majority of iron deficient subjects were anemic. By excluding them from analysis, the independent effect of vitamin B12 could be examined.
As expected, the serum folate concentrations of these vegetarians were high, 45% of the 96 subjects having concentrations higher than the upper limit of normality (54 nmol/L) (data not shown). It has been shown that high folate intake could delay the haematological manifestations of vitamin B12 deficiency but yet ineffective in preventing its neurological complications (Herbert, 1994) or a rise in metabolite (homocysteine) concentration (Mann et al., 1999; Mezzano et al., 1999). Therefore, the association between vitamin B12 deficiency and haematological problems might have been attenuated by generally high folate intakes in this study group, even though adjustment was made for serum folate concentration in univariate analysis.

The inverse correlation between vitamin B12 deficiency and haemoglobin was consistent and significant. The confounding effects of iron deficiency and renal failure were excluded, and other potential confounders were adjusted for. The lack of correlation between vitamin B12 concentrations and haematological indices was consistent with other studies, one of which involved Hong Kong Chinese subjects (Oosterhuis et al., 2000; Chui et al., 2001). This confirmed that serum MMA was a more specific indicator of deficiency state than vitamin B12 concentrations (Savage et al., 1994). Although serum MMA can be artificially raised in significant renal impairment (Savage et al., 1994), such subjects have been excluded in this study.

The correlation between vitamin B12 deficiency and an increase in MCV was not as strong, as the latter was not significantly increased in deficient subjects. MCV should therefore not be relied upon to distinguish vitamin B12 related
anaemia from other causes of anaemia. Vitamin B12 deficiency should therefore be screened for in all cases of anaemia in old age, especially that multiple deficiencies of haematinics are not uncommon (Harant & Goldberger, 1975).

The average difference in haemoglobin concentrations between the deficient and the non-deficient subjects was only up to 0.9 g/dl. This might not be sufficient to render the subjects anemic. The current recommended cutoff value of normal haemoglobin concentrations is lower in women because of the common phenomenon of a sex difference in haemoglobin concentrations. This sex difference was also shown to in our local older population (Woo et al., 1989; Janus et al., 1997). But the validity of this statistical approach in defining anaemia in women has recently been called into question, because many women with iron deficiency were included in establishing reference ranges (Rushton et al., 2001).

Interestingly, data from the Baltimore Women’s health and aging study in the US, involving 598 community dwelling disabled women older than 65 years showed a decreasing trend of three-year all causes mortality risk within the Hb range of <12 g/dl to 14 g/dl of Hb, after adjusting for confounders, e.g. chronic diseases, depression and creatinine etc. It was suggested that the optimal Hb cutoff for anaemia in older women should be above 12g/dl (Chaves et al., 2001). It is therefore likely that the generally low Hb concentrations of our older vegetarians are detrimental to their health.

Vitamin B12 deficiency is a recognized cause of pancytopenia. In one study from north-west India, 80% of patients with nutritional megaloblastic anaemia has thrombocytopenia (Sarode et al., 1989). Our study demonstrated a significant
but weak negative association between metabolic vitamin B12 deficiency and platelet counts. But this was unlikely to have any clinical significance.

There were four subjects with abnormally high serum vitamin B12 concentrations. Some popular vegetarian foods e.g. sea-weeds contain vitamin B12 analogs which may be indistinguishable from bioactive cobalamin by competitive protein binding assay (Dagnelie, 1997). But the assay which we used was more specific with little cross reactivity with vitamin B12 analogs such as cobinamide. We could not exclude the possibility that the subjects had received recent vitamin B12 injections by general practitioners, as this was unfortunately not enquired about. Myelodysplasia was another possibility. Two subjects had macrocytic anaemia with no associated thrombocytopenia or leucopenia. Although the anemic subjects’ MMA concentrations were either undetectable or slightly raised (0.6 µmol/L), partially treated vitamin B12 related megaloblastic anaemia could not be excluded. The exclusion of these four subjects, however, did not significantly alter any of our results.

It was concluded that vitamin B12 deficiency is associated with a significant decrease in haemoglobin concentrations and anaemia in moderate cases. Because macrocytosis was seldom present in vitamin B12 deficiency, mean corpuscular volume is not a reliable indicator of vitamin B12 status. If vegetarians are given vitamin B12 supplements routinely, as suggested in the last chapter, a consequential increase in haemoglobin concentrations may confer significant health benefits. In the next chapter, the potentially casual relationship between
vitamin B12 deficiency and cognitive impairment was examined by a randomised controlled trial.
10.5 Key Points

1. Vitamin B12 deficiency is associated with a significant decrease in haemoglobin concentration.

2. Moderate vitamin B12 deficiency is required to cause anaemia.

3. Anaemia associated with vitamin B12 deficiency is usually not macrocytic.
Chapter 11 Randomized Trial of Vitamin B12 Supplementation on the Cognitive Function of Older People with Subnormal Serum Vitamin B12 Concentrations

11.1 Introduction

Vitamin B\textsubscript{12} is a very important vitamin for well-being of the brain. It holds a key position in the synthesis of DNA and S-adenosylmethionine (SAM) (Regland & Gottfries, 1992). A major concern of vitamin B12 deficiency is its effect on cognitive function in older people.

There is cross sectional and longitudinal data to support the role of vitamin B12 deficiency in the cognitive decline and dementia in old age (Selbub et al., 2000; Wang et al., 2001). The crucial question is whether vitamin B12 deficiency contributes to brain dysfunction or merely co-exists with dementia. The benefit of vitamin B12 supplementation in vitamin B12 deficient people with dementia was based on case studies and not subjected to controlled trials (Chiu, 1996). Open prospective trials showed variable and often incomplete responses. Those with more recent onset of dementia symptoms were more likely to respond (Martin et al., 1992; Cunha et al., 1995). A more recent observational study showed that demented patients with low serum vitamin B12 concentrations did not improve with supplementation when compared with their counterparts with normal vitamin B12 concentrations, but patients with cognitive impairment did (Eastley et al., 2000).

A randomized controlled trial of vitamin B12 supplementation on a group of older people with subnormal serum vitamin B12 concentrations. The hypothesis
was that vitamin B12 deficiency always caused a degree of cognitive impairment in older people, and that the impairment could be reversed by supplementation.
11.2 Subjects and Methods

Several groups of people above 60 years old were screened for vitamin B12 deficiency by serum vitamin B12 concentration. The majority of the subjects were vegetarians living in their own homes or aged homes in Hong Kong. The minority of non-vegetarians were recruited after they were found to have subnormal vitamin B12 concentrations in hospital medical outpatient clinics or wards. Serum vitamin B12 concentration below 120 pmol/L was defined as subnormal. Those subjects who could not cooperate with the neuropsychological tests because of severe confusion or communication problems, and those with anaemia (Haemoglobin < 9.0 g/dl), unstable medical condition and signs of subacute combined degeneration of cord were excluded.

At recruitment, all subjects had the following neuropsychological tests (see section 3.6.1.1 and appendix B):

1. Mini Mental State Examination (MMSE)
2. Digit Span
3. Wechsler Memory Scale (revised) - Logical Memory and Visual Reproduction
4. Wechsler Adult Intelligence Scale (revised) - Similarities, Block Design
5. Adult Luria-Nebraska Neuropsychological Battery - Motor Function Scale
6. General Health Questionnaire

This battery of tests was selected to cover different aspects of cognitive function, and was thought to be feasible in normal older people. The tests were administered by three master level clinical psychology trainees and took about
one hour to complete. The number of years of formal education was enquired and noted.

Fasting blood was taken for serum cobalamin, folate, methyl-malonic acid (MMA), and complete blood count. The laboratory method was described in chapter 3. Serum MMA concentrations greater than 0.40 \( \mu \text{mol/L} \) (Allen et al., 1990; Starczek et al., 1993) were regarded as elevated. History of hypertension, diabetes mellitus, cerebrovascular disease, ischaemic heart disease was recorded and regarded as risk factors of cerebrovascular disease. Subjects with dementia diagnosed by psychogeriatrician, and MMSE score less than 20 were regarded as demented (Chiu et al., 1994).

The subjects were randomised into supplement and control groups by the last of the 6 digit numbers of the Hong Kong Identity Card (an official document of identity for every citizen). If the last digit number was odd number, the subject was assigned to supplement group. Subjects with even last digit numbers were assigned to control group. The treatment regime was as follows: three doses of Cyano-cobalamin 1mg given intramuscularly in the first week, one dose weekly for further three weeks, followed by one monthly dose thereafter. The injections were given by nurses in old age homes or in local health centres. The control group received no intervention and were advised not to take any oral vitamin supplements until the end of trial. Written consent was obtained before randomization.

All subjects were reviewed at three to six months. They had blood tests and neuropsychological battery repeated as at recruitment. The psychologists were
blinded from the treatment option. This study was approved by the Clinical Research Ethics Committee, the Chinese University of Hong Kong. The study was funded by a Direct Grant from the Chinese University of Hong Kong. All subjects were volunteers and unpaid, and there was no conflict of interests between investigators and the subjects.
11.3 Results

Fifty subjects completed the trial. Their characteristics were shown in Table 11a. The supplement group tended to have more demented, uneducated and institutionalized subjects, though none of the parameters reached statistical significance. Two subjects were excluded, one in the supplement group because of adverse reaction to parenteral cyanocobalamin, one in the control group because of vitamin B12 injections given by a private doctor.
Table 11a  Characteristics of Trial Subjects with Subnormal Serum Vitamin B12 Concentrations *

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>77.4(6.4)</td>
<td>76.6(6.8)</td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Vegetarian</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Institutionalized</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Risk of CVA</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Dementia †</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>No education</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Vitamin B12(pmol/L)</td>
<td>77.9(27.8)</td>
<td>87.3(24.0)</td>
</tr>
<tr>
<td>Raised MMA ‡</td>
<td>21 (77.8%)</td>
<td>18 (85.7%)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>12.4(1.1)</td>
<td>12.2(1.1)</td>
</tr>
<tr>
<td>Macrocytosis (MCV &gt;97 fl)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Follow-up time (wk)</td>
<td>16.1(3.2)</td>
<td>17.3(5.5)</td>
</tr>
</tbody>
</table>

* data presented in average ( standard deviation ) when appropriate
† diagnosed by psychogeriatrician or MMSE < 20
‡ serum MMA > or = 0.40 μmol/L; maximum 2.48 μmol/L; two missing samples in control group
At follow-up of supplement group, all except 3 subjects had serum vitamin B12 concentration above 200 pmol/L. The lowest concentration was 169 pmol/L in a subject reviewed at six months. Five subjects had slightly raised MMA concentrations, the highest concentration being 0.85 μmol/L. The compliance of supplement regime was satisfactory, and the minimum amount of cyanocobalamin given was 7 mg before review.

At follow-up of control group, serum vitamin B12 concentrations remained subnormal in 23 out of 24 subjects. MMA concentrations were elevated in 19 out of 23 subjects, 2 of whom had normal MMA concentrations at recruitment. Two subjects refused blood tests at follow-up; one blood sample for MMA was not analyzed. Most subjects of both trial groups had very high serum folate concentrations both at recruitment and at follow-up, the lowest concentration being 13.8 nmol/L (reference range 7-39 nmol/L).

The neuropsychological at baseline and follow-up were shown in Table 11b. The supplement group had significantly lower baseline performance IQ. The control subjects deteriorated significantly in verbal memory and drawing skill but improved in oral motor skill. The supplement group improved significantly in performance IQ, but deteriorated in motor skills: kinesthesia (sense of position and movement), drawing and fine motor. When their changes in scores were compared with those of the control subjects, the supplemented subjects fared worse in motor scores: kinesthesia, fine motor and oral motor.

When the demented subjects were excluded from both trial groups, the supplement group still had significantly lower performance IQ at baseline
(p<0.05). The overall trends of changes at follow-up remained the same, except that the deterioration in kinesthesia based movement and fine motor skill in the supplement group were no longer significant. But the group differences in favour of control group in the changes of kinesthesia based movement and oral motor skill remained significant (p<0.05).

Seven demented subjects received vitamin B12 supplementation. Three of them improve significantly. One subject improved by more than one point in MMSE (from 18 to 21), digit span, verbal memory and performance IQ. One subject improved in MMSE (19 to 24), verbal IQ, performance IQ and oral motor skill. One subject improved in MMSE (5 to 9), verbal memory and oral motor skill. The remaining subjects showed no significant improvement. None of the subjects improved in visual memory and motors skills: drawing, fine motor and spatial movement.
Table 11b  Changes in Neuropsychological Scores of Trial Subjects

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-up</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control N=27 Mean</td>
<td>Supplement N=23 Mean</td>
<td>Control N=27 Mean</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>SD</td>
<td>Supplement N=23 Mean</td>
</tr>
<tr>
<td>MMSE</td>
<td>23.8 4.7</td>
<td>22.2 4.7</td>
<td>24 3.7</td>
</tr>
<tr>
<td>Digit span</td>
<td>11.6 3.5</td>
<td>10.4 3.8</td>
<td>10.6 2.9</td>
</tr>
<tr>
<td>GHQ</td>
<td>10.4 4.3</td>
<td>9.2 3.9</td>
<td>11.1 4.7</td>
</tr>
<tr>
<td>Memory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual</td>
<td>15.3 15.0</td>
<td>12.7 9.0</td>
<td>11.6 12.3</td>
</tr>
<tr>
<td>Verbal</td>
<td>11.4 6.8</td>
<td>7.8 6.1</td>
<td>9.3 † 6.2</td>
</tr>
<tr>
<td>IQ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal</td>
<td>60.1 7.1</td>
<td>58.2 7.1</td>
<td>58.9 7.1</td>
</tr>
<tr>
<td>Performance</td>
<td>84.3 15.3</td>
<td>74.9* 13.1</td>
<td>85.8 16.7</td>
</tr>
<tr>
<td>Motor skill a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kinesthesia</td>
<td>3 3.4</td>
<td>2.6 2.4</td>
<td>2.3 2.6</td>
</tr>
<tr>
<td>Drawing</td>
<td>7.3 3.5</td>
<td>7.2 2.8</td>
<td>9.1 † 3.9</td>
</tr>
<tr>
<td>Fine</td>
<td>7.6 3.2</td>
<td>8.6 3.4</td>
<td>7.1 3.7</td>
</tr>
<tr>
<td>Spatial</td>
<td>3.4 4.3</td>
<td>2.8 3.3</td>
<td>2.5 3.3</td>
</tr>
<tr>
<td>Oral</td>
<td>1.1 1.5</td>
<td>0.6 1.3</td>
<td>0.2 ‡ 0.6</td>
</tr>
</tbody>
</table>

Abbreviations: 95%CI – confidence interval of mean; MMSE – mini-mental state examination; GHQ – General Health Questionnaire; IQ – intelligence quotient

*higher sub-score indicated slower or incorrect movement

* significant difference between supplement and control groups, Student t test, p<0.05

† p<0.05, ‡ p<0.005, significant difference from baseline, Paired t test

‡ significant group difference in the change of scores, Student t test, p<0.05
11.4 Discussion

This was the first randomized trial of vitamin B12 supplementation on the cognitive function of older people. It showed no benefit in cognitive function from vitamin B12 supplementation in older people with vitamin B12 deficiency. The only parameter, which improved in supplement group, was performance IQ. But control group also improved in this respect, probably because of practice effect and the amount of improvement in both groups was not significantly different (p=0.15, ANOVA).

The only other randomized trial of vitamin B12 supplementation in the literature showed that both vitamin B12 and placebo intra-muscular injections improved the subjective ratings of psychiatric and general well being of older community dwelling subjects. It was concluded that the apparent benefit was placebo effect. No neuropsychological testing was performed (Hughes et al., 1970). In this trial, General Health Questionnaire which measured mood and anxiety level did not change significantly in supplemented subjects, but the sample size was inadequate to detect modest differences.

Subnormal serum vitamin B12 concentration is not specific for vitamin B12 deficiency (Stabler et al., 1990). The importance of raised metabolites in defining vitamin B12 deficiency was borne out by a recent study showing that in early and moderately demented people, only those with raised homocysteine concentrations improved with vitamin B12 and folate supplementation (Nilsson et al., 2001). But as the majority of our subjects (72%) had serum vitamin B12 concentration lower than 100 pmol/L, and 81% of subjects had elevated serum
MMA concentrations, one could be confident that the subjects were vitamin B12 deficient. Moreover, the exclusion of those subjects with normal or missing serum MMA did not alter the findings of this study.

Subjects with significant anaemia were excluded partly because of ethical consideration and partly because the correction of anaemia by vitamin B12 supplementation might improve the results of the neuropsychological scores. As the neuropsychiatric complications of vitamin B12 deficiency are independent of the development of anaemia (Lindenbaum et al., 1988), the exclusion of anaemic subjects should not bias the results of this trial.

Most subjects of this study were apparently normal older people recruited after voluntary screening. Their mental state ranged from normal to demented. Subjects at both extremes of cognitive functioning were not excluded because it was hypothesized that vitamin B12 deficiency impaired the cognitive function of all older people and that the impairment was reversible. As it was, the inclusion of demented subjects did not alter the study findings. Apart from the ten subjects who had psychiatric diagnosis of dementia, the remaining subjects did not have a full clinical evaluation. It was therefore possible that some of the subjects with "normal" MMSE had undetected dementing illnesses. It is noteworthy that the local cut-off MMSE score for dementia is lower than that in Western countries (19/20 vs 23/24) (Chiu et al., 1994; Folstein et al., 1985). Low educational level of the older Chinese people in Hong Kong, as in our study subjects, is thought to be responsible (Chiu et al., 1994).
The trial groups were not well matched in that supplement group tended to have more demented, uneducated and institutionalized subjects, as reflected in their generally lower neuropsychological scores. This might explain the relatively greater deterioration of supplement group as compared with control group at follow-up.

Both trial groups appeared to have deteriorated in memory and motor skills, even after the exclusion of demented subjects. This could not be attributed to ageing as normal older people showed no decline in cognitive test performance in a two-year longitudinal study (Flicker et al, 1993). It was therefore probable that some of the subjects in both groups had dementing illness from causes other than vitamin B12 deficiency.

Another possible cause for the observed cognitive decline in some of the subjects was nutritional deficiencies. The majority of the trial subjects were strict life long vegetarians. Their intakes of B vitamins were found to be much below recommended levels in chapter 4. These vitamins had been shown to be associated with cognitive impairment in older people (Goodwin et al., 1983). In retrospect, oral supplements of these vitamins should have been prescribed to all subjects.

The mean memory and motor function scores had wide confidence intervals, and the sample size was far from adequate for these parameters. The apparent differences might therefore be type 1 errors.
11.5 Critique of the Trial

1. Choice of Neuropsychological tests

The Chinese version of Mini mental state examination (Chiu et al., 1994) and General health questionnaire (Chi et al., 1994) were validated in older people in Hong Kong in 1994. But there was lack of local data on older people when the trial was conducted. Furthermore, the other neuropsychological tests have not been validated in older people in Hong Kong, and there was no local data on older people. In retrospect, more preliminary work should have been done on the validation of the instruments, the influence of practice effect and inter and intra observer variation. Judging from the results of this study, one could conclude motor function scores and the verbal and visual memory tests had too much variation among individuals and overtime to be useful in clinical trials.

Although the three research assistants were postgraduate psychology students who were used to conducting neuropsychological tests, no inter-observer variation study was done, and the follow-up assessments were not necessarily done by the same assistant. This might have accounted for some of the variation between individuals and over time.

2. Choice of subjects

It would have been helpful to assess the cognitive status of the subjects clinically before the trial, so that the subjects could be categorized into normal, mild cognitive impairment, early dementia, and established dementia. This would help in the interpretation of the results. After literature review, the early demented people would be the most likely group to respond to vitamin B12 supplementation.
In retrospect, it was not necessary to lower the vitamin B12 concentration to 120 pmol/L for inclusion. A higher cutoff point for inclusion could have made recruitment easier.

3. Sample size

Because of the lack of local data on these neuropsychological tests in older people in Hong Kong at the time of the study, it was not possible to estimate sample size beforehand. Based on the baseline means and standard deviations of the test in the supplemented subjects, this trial had a 80% chance of detecting a 10% increase in verbal IQ, 15% increase in performance IQ, and a 18% increase in MMSE. The sample size was far from adequate to detect changes in other neuropsychological tests which had greater individual variations.

Despite the limited sample size, a significant improvement (about 10%) in performance IQ was detected. But what was unexpected was that a proportion of control subjects also improved at follow-up, probably because of practice effect. If that was also taken into account, the sample size was likely to be not adequate to detect a modest (10-15%) increase in performance IQ.

4. Duration of trial

The follow-up period of this trial varied from 3-6 months, because of the availability of research assistants. It was preferable to have a uniform trial period of six months to allow for neuronal regeneration from vitamin B12 supplementation. However, in the published case series, most subjects whose neuropsychiatric manifestations responded to vitamin B12 supplementation did so within three months (MacDonald Holmes, 1956, Healton et al., 1991, Cunha et al.,
Moreover, because of the potential risk of neuropsychiatric complications from untreated vitamin B12 deficiency, a trial period of six months might have been regarded as too long.

5. Lack of Placebo

This trial was not a placebo-controlled trial. Intramuscular injections were preferred to oral vitamin B₁₂ supplements on the ground of the high prevalence of vitamin B₁₂ malabsorption in older people, and it was deemed unethical to give placebo intramuscular injections. Moreover, the neuropsychological tests were performed in a blinded fashion. The compliance of intramuscular injection was not a problem, as the local Chinese people, especially the older ones, believed that intramuscular injections were more therapeutics than oral medication.

As there is now evidence to support the use of oral vitamin B₁₂ (Seal et al., 2002) in vitamin B₁₂ deficiency in older people and the deficiency in vegetarian is likely to be dietary in origin, an alternative could have been to supplement the trial subjects with oral vitamin B₁₂ tablets. In this case, oral placebo tablets could have been given.
11.6 Conclusion

This trial did not support the hypothesis that vitamin B12 deficiency causes reversible cognitive impairment in older people. But because of its limited sample size for the tests adopted in the trial, this should be regarded as a pilot study. The trend of improvement in performance IQ, and the improvement in some demented subjects after supplementation should encourage further research in the role of vitamin B12 deficiency in cognitive impairment in older people.
11.7 **Key Points**

1. Older subjects with vitamin B12 deficiency showed no significant treatment effect on cognitive function with parenteral vitamin B12 supplementation.

2. Vitamin B12 deficiency does not invariably cause cognitive impairment in older people.
Chapter 12  The Association between Dental Status and Dietary Intakes among Chinese Vegetarian Old Age Home Residents

12.1  Introduction

Many vegetarian foods require a lot of chewing. Good dental health is therefore important for vegetarians. However, the current older population in Hong Kong has low socio-economic status and received minimal dental care in the past. Moreover, dental services have not been a priority for public funding in Hong Kong. Consequently, older people often neglect dental problems and their dental status is generally poor (Schwarz & Lo, 1995). As in other countries, the dental status of older people in institutional care is most unsatisfactory (Yu, 1997).

The association between dental status and dietary intake or nutritional status in normal older people is probably insignificant, because they are likely to overcome their chewing problem by selecting a soft yet adequate diet. But in frail old age home residents, there is an association between dental status, dietary intake and nutritional status (Mojon et al., 1999). In a local study of institutionalised elderly, chewing problem was associated with poor nutritional status (Woo et al., 1994). The impact of poor dental status on dietary intake may be even more important in vegetarian old age home residents because the choice of food is more limited, and many vegetarian foods require much chewing.

This study investigated the dental status of older vegetarian Chinese women in a vegetarian old age home in Hong Kong. The correlation with dental status and dietary intake was examined.
12.2 Subjects and Methods

The old age home which participated in this study provided an exclusively vegan diet. It was subsidized and run by a Taoist group for the benefit of their followers who required residential care in old age. The dependency level varied from independence to infirmary. All the subjects were female. Those on enteral feeding were excluded.

In the year prior to this study, because of the concern about the poor nutritional status of the residents, the Home started to provide a milk-based supplement “Ensure” to the frailer residents. Yet this was met with limited tolerance and acceptance, consequently only the equivalent of half a glass of “Ensure” was consumed daily, providing 84 kcal, 3 g protein, 43 mg calcium and 210 iu vitamin A.

The dietary intakes of the subjects were measured by a 24 hours food record by two postgraduate students in dietetics at weekdays. Food intake at breakfast, lunch and dinner was estimated by firstly weighing and averaging individual items of three standard portions, and then the food left over by individual residents was directly observed and recorded by research assistants. Snacks consumed during the day were also recorded by the researchers. But the bedtime snacks consumed the previous night were recorded and samples of the snacks were presented to research assistants by the nursing home staffs. Most subjects consumed half a glass of oral nutritional supplement (Ensure) daily. The nutritional composition of the consumed foods was calculated by Food tables from multiple sources – UK and two Mainland Chinese institutions (Holland et al.,
In addition, body weight (Wt, kg), height (Ht, m) and/or knee height (m) were measured. Functional status was measured by Barthel index (BI) with maximum score of 20 indicating full independence (Mahoney & Barthel, 1965). Body mass index was calculated using the formulae: Wt/Ht² kg/m². When standing height was unavailable because of immobility, Ht was calculated by a knee-height prediction using a locally derived formula (Li et al., 2000).

The dental status of all the residents of the same vegetarian old age home was examined by a fully trained dental surgeon. All residents underwent a standardized oral examination procedure with the diagnostic criteria based on the WHO standard. Data included the Decayed-Missing-Filled-Teeth value (DMFT), number of functional teeth unit(s) (see 5.2.1), denture status, subjective view on masticatory function and reasons for difficulty in eating were recorded.

The dental status of vegetarian subjects who reported chewing difficulties and those without was compared by appropriate statistical tests.
12.3 Results

The dental surgeon examined a total of 76 out of 86 residents. One subject refused examination, six subjects were in hospital, and three on home leave on the day of survey. None of the examined subjects were on enteral feeding. Food record was available in 68 out of the 76 subjects. The remaining subjects were not in old age home on the day of dietary survey for the following reasons: admitted after dietary survey 5, away 3.

The average DMFT score was 28.55 (SD 5.5). 43 out of 76 (63%) subjects were edentulous (DMFT=32), and 36 (53%) subjects had less than six functional teeth units. A comparison of the dental status of the vegetarian old age home subjects who reported chewing difficulties and those without was shown in Table 12a. The commonest reasons for chewing difficulties as assessed by the dentist were no denture 29 (56%) and unfit denture 18 (35%). Oral mucosal disease or neurological deficits accounted for the remaining 4% and 6% of cases respectively.

The distribution of functional teeth units of the vegetarian subjects was shown in Figure 12a. As having functional teeth units of 5 or less was previously reported to be associated with lower dietary intake (Mojon et al., 1999), the subjects were divided into those with 6 or more functional units and those with 5 or less functional units. The dietary intakes of these two groups of vegetarian subjects were compared in Table 12b. The former group had significantly lower BI than the ones with more functional unit, and they had significantly less fiber intake, even after adjustment by BI.
Table 12a. Comparison of the Dental Status of the Vegetarian Old Age Home Subjects who reported Chewing Difficulties and Those Without *

<table>
<thead>
<tr>
<th></th>
<th>Chewing Difficulty</th>
<th>No Chewing Difficulty</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N 52)</td>
<td>(N 16)</td>
<td></td>
</tr>
<tr>
<td>Age years</td>
<td>87.0±5.5</td>
<td>85.9±7.2</td>
<td>0.56</td>
</tr>
<tr>
<td>Body mass index kg/m²</td>
<td>20.0±3.7</td>
<td>21.7±4.4</td>
<td>0.15</td>
</tr>
<tr>
<td>Barthel Index &lt;20</td>
<td>35 (67%)</td>
<td>6 (37%)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>17 (33%)</td>
<td>10 (63%)</td>
<td></td>
</tr>
<tr>
<td>Food Texture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hard</td>
<td>19 (37%)</td>
<td>14 (88%)</td>
<td>0.00</td>
</tr>
<tr>
<td>Medium</td>
<td>19 (37%)</td>
<td>2 (13%)</td>
<td></td>
</tr>
<tr>
<td>Soft</td>
<td>14 (27%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Functional teeth units &lt;6/16</td>
<td>29 (56%)</td>
<td>2 (13%)</td>
<td>0.00</td>
</tr>
<tr>
<td>DMFT (median, range)</td>
<td>32 (20-32)</td>
<td>32 (10-32)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

* Data presented as mean ± standard deviation or number (percentage) unless stated otherwise; Student t-test for age, body mass index; Chisquare test for Barthel index, functional teeth units; Mann Whitney test for DMFT

DMFT – number of decayed, missing and filled teeth.
Figure 12a  Distribution of Functional Teeth Units among Vegetarian Old Age Home Residents

![Bar chart showing distribution of functional teeth units among vegetarian old age home residents. The chart displays the number of subjects in each category of functional tooth units: 0, 1-5, 6-15, and 16. The number of subjects ranges from 0 to 35.]
Table 12b Comparison of the Characteristics and Dietary Intakes of Vegetarian Old Age Home Residents with Adequate and Inadequate Functional Teeth Units #

<table>
<thead>
<tr>
<th></th>
<th>FTU &lt; or = 5 (N=32)</th>
<th>FTU &gt; 5 (N=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>87.4±5.2</td>
<td>85.9±6.3</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>19.5±4.4</td>
<td>20.90±3.1</td>
</tr>
<tr>
<td>Barthel Index =20</td>
<td>9, 29%</td>
<td>18, 49%</td>
</tr>
<tr>
<td>(max. 20) (N,%) &lt;=20</td>
<td>22, 71%</td>
<td>19, 51%</td>
</tr>
<tr>
<td>Calories (Kcal)</td>
<td>1436±333</td>
<td>1494±340</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>41.7±11.5</td>
<td>43.7±12.2</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>31.7±8.8</td>
<td>34.8±10.4</td>
</tr>
<tr>
<td>Vitamin A (I.U./day)</td>
<td>2110±1218</td>
<td>2727±5362</td>
</tr>
<tr>
<td>Vitamin B1 (mg/day)</td>
<td>0.8±0.2</td>
<td>0.7±0.2</td>
</tr>
<tr>
<td>Vitamin B2 (mg/day)</td>
<td>0.8±0.3</td>
<td>0.7±0.2</td>
</tr>
<tr>
<td>Vitamin B3 (mg/day)</td>
<td>11.1±3.1</td>
<td>10.1±2.9</td>
</tr>
<tr>
<td>Vitamin C (mg/day)</td>
<td>120±41</td>
<td>125±52</td>
</tr>
<tr>
<td>Vitamin D (mg/day)</td>
<td>49.7±31.5</td>
<td>29.3±23</td>
</tr>
<tr>
<td>Calcium (mg/day)</td>
<td>395±130</td>
<td>436±209</td>
</tr>
<tr>
<td>Iron (mg/day)</td>
<td>13.1±3.6</td>
<td>13.9±3.7</td>
</tr>
<tr>
<td>Zinc (mg/day)</td>
<td>8.2±2.9</td>
<td>7.3±2.2</td>
</tr>
<tr>
<td>Fibre (g/day)</td>
<td>6.5±2.3</td>
<td>8.2±2.8 *</td>
</tr>
</tbody>
</table>

# values presented as mean ±standard deviation unless stated otherwise

* p<0.05, by ANCOVA adjusted with Barthel index categories
12.4 Discussion

This study showed that the dental status of vegetarian old age home residents was poor and many reported chewing difficulties. Consequently, many of them had to eat softer foods. The main problem appeared to be lack of dentures and their maintenance care. The fiber intake of the subjects with less functional units was significantly reduced.

The BI and BMI of the vegetarian old age home subjects were comparable to those reported in a local study of omnivorous subjects in three old age homes (Kwok et al., 2001b). In contrast with the study in Chapter 4, the caloric intakes of the vegetarian subjects were in fact greater than those of the omnivorous old age home residents (Kwok et al., 2001b). The same 24-hour food record was used in the latter study. This could be explained by relatively better nursing care provided in this vegetarian home.

This home also participated the main study in 1995, as described in Chapter 3. It is noteworthy that the dietary intakes measured by 24 food record in this study were generally higher than those estimated by 24-hour recall in the previous study. This is to be expected, as dietary intakes are liable to be under-estimated by the latter method. With the present more accurate method of dietary assessment, the caloric intakes of the vegetarians were close to the recommended 1600 kcal per day. But the protein and calcium intakes were still significantly lower than the recommended 55g and 600 mg daily respectively.

The dental status of the vegetarian subjects was poor. But it was comparable to that found in another survey performed in omnivorous old age
homes by the same dentist (Yu, 1997). In that survey, the average DMFT was 27.5. The prevalence of edentulism was 33.7%, and over half of the elderly (57%) had no dentures. The percentages of vegetarian subjects who had dentures and of those reporting chewing difficulties were also comparable to those found in a local community survey involving a random sample of 879 men and 737 women (Woo et al., 1994).

There have not been large-scale comparative studies of dental status between vegetarians and non-vegetarians. There is a small case control study in Finland of a higher rate of dental erosions in lactovegetarians, probably because of consumption of acidic foods (Linkosalo & Markkanen, 1985). The key determinants of dental decay are dental hygiene, intake of fermentable sugars and individual’s susceptibility. Vegetarianism is therefore unlikely to have a significant effect on dental status (Walls, 1999; Dwyer, 1988).

The dental health of those who reported and those who did not report chewing difficulties was equally bad. But those who had difficulties had significantly less functional units, primarily because of problems with dentures. This highlights the importance of the care of dentures in the chewing ability of older people in institutional care. Chewing difficulties are however multifactorial. In a community study, chewing difficulties were associated with poor health and depression (Woo et al., 1994). Its association with disability level in this study is consistent with that.

It is interesting to note that lack of functional teeth units was associated with dependency. In Hong Kong, dental service is primarily not publicly funded.
The disabled older people have great problems in gaining access to dental care because of lack of financial resources and difficulties in arranging transport.

The relationship between poor dental status and insufficient dietary intakes in older people is often weak (Ranta et al., 1988; Norlen et al., 1993; SENECA investigators, 1996). In a nursing home study, the association between lack of functional teeth units and poor nutritional status was only demonstrable in the dependent residents (Mojon et al., 1999). Compromised dental status probably has to be combined with severe disability before nutritional status is affected. Moreover with good dietary advice and careful food preparation, the nutritional values of soft diets can be as good as normal foods.

However devising a nutritious vegetarian diet for old age home residents with very few functional teeth or dentures is no easy task. The exclusive vegan diet restricted the diet to grains, vegetables, fruits, legumes, nuts, seeds and Soya products, eliminating any milk, egg or meat products. Majority of the older residents came from a strong Chinese cultural background that would classify some vegetables like Chinese Cabbage, Mung Bean Sprouts, and Winter Melon commonly served at the table as “cold” food, which the elderly tend to avoid. For those on pureed or blenderized diet, the choices are even more restricted and the diet tend to be quite monotonous with little variation due to manpower constraint and lack of skills in menu planning.

It was therefore not surprising that the fibre intake was lower in those with compromised dental status. One should also point out that despite the fact that the food was all vegetarians; the fiber intakes of all except one subject were lower
than the recommended 16 g daily (World Health Organization, 1990). It has to be pointed out that the total fiber intakes were under-estimated because the food composition tables which we used were only capable of quantifying crude fibre (Woo et al., 1997b). Nevertheless, it is therefore possible that despite the good intention to provide sufficient fiber in the menu, the actual fiber intakes of the residents were insufficient. The Chinese vegetarian diet is primarily based on grains and Soya bean products, e.g. To Fu. Encouraging the intake of beans, high fiber snacks like fruits and nuts, skillfully served in mechanical soft diet and using more fiber rich nutritional supplement may be helpful in this setting.
12.5 Conclusion

It was concluded that having less than six functional units is associated with chewing difficulties and soft diets in vegetarian old age home residents. Physically frailty has an additive effect on chewing difficulties. Compromised dental status was independently associated with reduced fiber intake in older institutionalized vegetarians. Dietetic supports for vegetarian old age home are warranted.
12.6 Key Points

1. Nearly two thirds of institutionalised older vegetarians had no teeth.

2. Poor dental status was associated with chewing difficulties and soft diets.

3. The lack of denture and ill-fitting denture accounted for 90% of the chewing problems.

4. Poor dental status did not however compromise nutritional intake except that of fibre.
Chapter 13 Plasma Homocysteine Concentrations of Chinese Vegetarians

13.1 Introduction

Hyperhomocysteinemia is increasingly recognized to be an independent cardiovascular risk factor. It is well known that vegetarians are at risk of vitamin B12 deficiency which may cause hyperhomocysteinaemia. There are recent studies showing that plasma homocysteine concentrations are elevated in vegetarians, and the concentrations correlated with serum vitamin B12 concentrations. (Mann et al., 1999; Mezzano et al., 1999).

The Chinese vegetarian diet is different from those in Western countries. Some Chinese vegetables are particularly rich in folate content. As folic acid has a more direct effect on the metabolism of homocysteine, and folate supplementation has been shown to lower homocysteine in normal people, it is possible that the high folate intake may compensate the vitamin B12 deficiency, resulting in less elevation in plasma homocysteine concentration.

Because plasma homocysteine concentrations are subject to variation in old age because of renal impairment, and there is no available normative data in older people in Hong Kong, a new group of younger Chinese vegetarians from the same religious group as those in the main study was recruited and compared with those of local omnivorous subjects in their plasma homocysteine concentrations.
13.2 Subjects and Methods

Subjects were recruited among Taoism followers in Hong Kong. The inclusion criteria are as follows: 1. aged 18 to 70 years; 2. community dwelling; 3. vegetarian for five years or more; 4. no known major disease, e.g. vascular disease, renal disease, diabetes mellitus, hypertension, hyperlipidaemia; 5. not on regular medication, including vitamin supplements containing vitamin B.

All the subjects attended the research clinic in Prince of Wales Hospital after an overnight fast. Fifteen ml of blood (11 ml in EDTA, 4 ml serum gel) was taken. The blood samples were kept in an ice-box and delivered to a laboratory in the hospital within one hour. The plasma was isolated and frozen at -40 °C. The following blood analysis were performed:

1. Homocysteine (see Section 9.2.1)
2. Folate and vitamin B12 (Solid-phase no-boil Dualcount Radioimmunoassay),
3. Renal function test
4. Glucose by Haemostix read by Glucometer 4 (Bayer)

Food frequency questionnaire was administered to estimate intake of energy, folate and methionine. Body weight was measured by hospital beam balance (Delco), and standing height with shoes off was measured. Sitting blood pressure was measured by electronic sphygmomanometer (Omron HEM-720C). Standard 12 lead electrocardiogram was performed. Those with blood pressure greater than 140/90, Haemostix greater than 7 mmol/L, ischaemic ECG changes and abnormal renal function tests were excluded.
The control subjects were part of a bigger comparative study of homocysteine concentrations of Hong Kong and Mainland Chinese. Thirty-two female in Hong Kong matched within one year of the vegetarian subjects were arbitrarily selected to be control subjects. The same inclusion and exclusion criteria were applied, and the same research assistant in the same laboratory undertook all the biochemical assays. Blood pressure, body weight and height were also measured. Fasting glucose was measured by laboratory assay in control subjects, but by Haemostix only in vegetarian subjects.
13.3 Results

Thirty-eight vegetarians volunteered. Three were excluded because of history of ischaemic heart disease, vitamin supplementation and failure of venesection respectively. Because only three vegetarian men participated the study, and there is sex difference in homocysteine concentrations, only female subjects were included in this study.

The mean age of the 32 remaining subjects was 45.6 (sd 7.9, range 26-61) years old. The average length of vegetarianism was 22.1 (sd 12.6, 5-55) years. Eight of them (25%) became vegetarians before 18 years old. Twenty-eight subjects (88%) consumed dairy products. According to food frequency questionnaire, the mean daily energy intake was 1568 (sd 428) kcal. The mean folate intake from high folate containing foods per 1000 kcal was 54.8 (sd 27.2) g/1000 kcal. As none of the subjects consumed meat, their intakes of methionine were negligible.

The characteristics of vegetarian and control subjects were compared in Table 13a. Six (19%) vegetarians and 2 control subjects had sitting blood pressure >140/90. The difference was not significant by Chisquare test. None of the subjects had abnormal renal function test. The plasma concentrations of vitamin B12, folate, and homocysteine of the vegetarian subjects were also compared with control subjects in Table 13a.
Table 13a. Comparison of the Characteristics and Plasma Homocysteine Concentrations of Vegetarians and Omnivores

<table>
<thead>
<tr>
<th></th>
<th>Vegetarian (N=32)</th>
<th>Omnivore (N=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>45.6</td>
<td>7.7</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>22.7</td>
<td>2.6</td>
</tr>
<tr>
<td>Vitamin B12 (pmol/L)#</td>
<td>199</td>
<td>134</td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
<td>67.9</td>
<td>23.5</td>
</tr>
<tr>
<td>Homocysteine (μmol/L)</td>
<td>9.4</td>
<td>2.5</td>
</tr>
</tbody>
</table>

** p < 0.001, Student t test

# 3 missing data

Abbreviations: 95% CI – confidence interval of mean

Six vegetarian subjects (19%) and none of control subjects had raised plasma homocysteine concentration (>12 μmol/L). The difference was highly significant (p= 0.02, Chisquare test). Fifteen subjects (47%) had subnormal vitamin B12 concentration (< 150 pmol/L), which was the lower limit of the normal reference range. Another 12 subjects (38%) had low normal vitamin B12 (150-300 pmol/L). On the other hand, only 5 subjects (17%) of control subjects had marginal vitamin B12 concentration (150-300 pmol/L), and none had subnormal concentration(< 150 pmol/L).

The Spearman coefficients of rank correlation between the three categories
of vitamin B\textsubscript{12} concentrations and homocysteine concentrations was 0.61 (p<0.0001). Age and plasma folate concentration were positively correlated with homocysteine concentrations (Spearman coefficient 0.32, p <0.01; 0.50, p<0.0001 respectively). High blood pressure or fasting glucose had no significant correlation with homocysteine concentration.
13.4 Discussion

This study showed that plasma homocysteine concentrations of vegetarian women were significantly higher than omnivorous female subjects of similar age. Compromised vitamin B12 status was primarily responsible. In this respect, higher than normal folate intake was unable to compensate the negative effect of vitamin B12 deficiency.

The vegetarian subjects were carefully selected for absence of diseases which have been associated with hyperhomocysteinaemia. The control subjects were part of a large-scale local study of hyperhomocysteinaemia in Hong Kong, in which similar selection criteria were applied.

The prevalence of vitamin B12 deficiency as defined by subnormal vitamin B12 concentrations in this group of younger vegetarians was surprisingly high, especially that the majority were lactovegetarians. The most likely explanation was that although dairy products were not entirely excluded, they were not consumed sufficiently to prevent vitamin B12 deficiency. Their long duration of vegetarianism and early age of starting vegetarian diet were also important factors (Herbert, 1994).

The plasma folate concentrations of our vegetarians were higher than control subjects as suggested earlier. Moreover their intake of methionine was negligible. These factors should tend to depress plasma homocysteine concentrations. Despite these, their homocysteine concentrations were still significantly higher than those of the control subjects, which is consistent with the previous reports of raised homocysteine concentrations in vegetarians (Mann et al., 1999). This suggested
that high folic intake is unable to prevent the hyperhomocysteinaemia associated with vitamin B12 deficiency. The positive correlation between plasma folate concentration and homocysteine concentration was most likely to be caused by the confounding effect of vegetarian subjects who are more likely to have high folate and low vitamin B12 concentrations at the same time.

In the general population, however, folate intake or status is a more important determinant of plasma homocysteine concentrations than that of vitamin B12 (Shimakawa et al., 1997; Lussier-Gacan et al., 1996; Jacobsen et al., 1994). Moreover, folate supplement is more effective than vitamin B12 supplement in lowering homocysteine concentrations (Homocysteine lowering trialists’ collaboration, 1998). These could be explained by the much lower prevalence of vitamin B12 deficiency in the general population. However the influence of vitamin B12 status can be expected to be more significant in older people and in vegetarians. It is noteworthy that the oral folate supplement reaches its ceiling effect at the relatively low dose of 0.5 mg daily, and that its effect is proportional to the baseline concentrations of homocysteine. These suggest that folate supplement is effective in lowering homocysteine concentration by correcting folate deficiency and may be less effective in people who have satisfactory folate status.

Hyperhomocysteinaemia has been associated with vascular diseases and thromboembolism (Verhoef et al.; 1994, Arnesen et al., 1995) in the Western populations. It has also been shown to impair endothelial function in local Chinese people. On the other hand, the vegetarian diets are associated with
protective factors against vascular diseases e.g. favorable lipid profile, greater intake of anti-oxidants, phytooestrogen etc., which may over-ride the deleterious effects of hyper-homocysteinaemia. This could explain why many epidemiological studies in the West still showed lower cardiovascular mortality risk in vegetarians.

However, the balance may be less favorable in Chinese vegetarians as the prevalence of vitamin B12 deficiency is even higher than the western counterparts. It is interesting that in chapter 6, the prevalence of possible ischaemic heart disease of our older Chinese women was comparable to that of the omnivorous women of similar age. In addition, in a meta-analysis of three major vegetarian cohort studies, vegans did not show significant reduction in cardiovascular death.

Since this study, 30 of our young study subjects further volunteered to have endothelial function tests. When compared with age sex matched local control subjects, it was found that the vegetarians had significantly greater carotid intima-media thickness (IMT) (p<0.05), and marginally lower flow mediated dilatation (p=0.15). On multiple regression analysis, homocysteine concentration was an independent predictor of carotid IMT (Woo et al., 2001). This was consistent with a recent reported randomized placebo controlled trial showing that vitamin B12 and folate supplementation lowered plasma homocysteine concentrations and significantly improved brachial artery flow-mediated dilatation (endothelium dependent) in patients with ischaemic heart disease (Chambers et al., 2000).

The prevention of vitamin B12 deficiency in vegetarians may therefore be important to their cardiovascular health. One strategy, which has been
successfully adopted overseas, is to increase intake of dairy products and fortified breakfast cereals. As these two foods are not our habitual foods, they need to be actively promoted in this at risk group.
13.5 **Key Points**

1. Vitamin B12 deficiency is common among Chinese vegetarians.

2. Their plasma homocysteine concentrations are significantly higher than those of omnivores.

3. High folate intakes cannot prevent hyperhomocysteinaemia associated with vitamin B12 deficiency.
Chapter 14  Conclusion

14.1 Adequacy of the Diet of Older Chinese Vegetarians

In contrast with Western vegetarian studies, major areas of inadequacy of the diet consumed by the older Chinese vegetarians were identified. High percentages of older Chinese vegetarians had below recommended intakes in calories, protein, calcium and B vitamins (Table 4b). As in Caucasian studies, however, their fat intakes were low and they obtained most calories from carbohydrates. The fibre intakes of Chinese vegetarians were higher than local omnivores, but were much lower than those of Western vegetarians.

The older Chinese vegetarians had lower intakes of calories, fat, protein and B vitamins than local omnivores. The vegetarian old age home residents had higher intakes of calcium and potassium than community dwelling vegetarians and omnivores, probably because greater consumption of milk based supplements (Table 4a). But they were at greater risk of subnormal intakes in calories, protein, and B vitamins than omnivorous old age home residents.

Poor dental status and chewing problems were common among vegetarian old age home residents (Section 12.3). Denture related problems were strongly associated with chewing problems. The lack of functional teeth unit was associated with decreased fibre intake but other dietary intakes were maintained by soft diets.
14.2 Nutritional Status of Older Chinese Vegetarians

The older vegetarians had significantly lower percentage of body fat than omnivores, but they were not thin (Table 4c). Nearly a half of them were overweight. Their anthropometric indices of lean mass, i.e. arm muscle circumference and corrected arm muscle area were comparable to those of omnivores, but their serum albumin concentrations were significantly lower (Section 4.3.2). There was therefore evidence of protein under nutrition in older vegetarians.
14.3 Cardiovascular Problems of Older Chinese Vegetarians

Although older vegetarians were leaner, hypertension (supine blood pressure > 140/90 mmHg or history) and diabetes mellitus (fasting glucose > 7.8 mmol/L or history) were as prevalent among them as in omnivores (Section 7.3.1). Their fasting serum cholesterol concentrations were significantly lower than those of omnivores, because of low fat intake and the cholesterol lowering effect of soya foods (Table 6a). But there was no significant difference in fasting triglyceride concentrations between vegetarians and omnivores.

On the other hand, in a separate study of younger Chinese vegetarians, plasma homocysteine concentrations were found to be significantly higher than those of age matched omnivores, because of high prevalence of vitamin B12 deficiency (Table 13a). The very high folate intakes as suggested by the serum folate concentrations did not appear to obviate the negative effect of vitamin B12 deficiency on homocysteine concentrations. Hyperhomocysteinaemia, an emerging cardiovascular risk factor, is therefore a major concern among Chinese vegetarians.

Overall, the prevalence of definite ischaemic heart disease based on history, cardiovascular questionnaire and resting electrocardiogram (ECG), was lower in vegetarians than in omnivores (Table 6c). But over one fifth of vegetarians had possible ischaemic heart disease which was primarily based on ECG changes and was associated with hypertension.
14.4 High Salt Intake and its Consequences in Older Chinese Vegetarians

The older Chinese vegetarians had higher salt intakes than omnivores, as suggested by their significantly greater urinary sodium/creatinine ratios (Table 7a). This could be attributed to the increased use of soya sauce or salt to improve the tastes of vegetarian foods. At the same time, associations between urinary sodium/creatinine ratio and systolic and diastolic blood pressures were found in older vegetarians. A very interesting finding was that lower calcium intake had an additive effect on raising systolic blood pressure (Table 7c). This association was not consistently observed in Western studies.

If these vegetarians could reduce their sodium intake to the equivalent level of normal older people in Hong Kong, and achieve calcium intake as little as 350 mg daily, their blood pressures could be expected to be lowered significantly.

Apart from causing hypertension, high salt intake of these vegetarians had a negative effect on their bones, by increasing urinary calcium excretion (Section 5.3). Together with relative leanness and lower protein intakes, it was surprising that the overall bone mineral densities at lumbar spine and hips of vegetarians were comparable to those of omnivores. Phyto-oestrogenic isoflavones in soy protein might have exerted their bone preserving effects.
14.5 Vitamin B12 Deficiency

A major finding was that vitamin B₁² deficiency was very prevalent among Chinese vegetarians (Table 9a), even in younger subjects (Table 13a). Vegans were much more at risk than lactovegetarians. The very high prevalence strongly argues for fortified foods or oral vitamin B₁² supplements for Chinese vegetarians.

To screen for vitamin B₁² deficiency, serum vitamin B₁² concentrations were not sensitive or specific enough, fasting urinary MMA could be recommended. It correlated very well with serum MMA, and the cut-off value of 2 µmol/mmol creatinine had high sensitivity and specificity in detecting elevated serum MMA (Table 8b). Urinary MMA is superior to serum MMA as it does not involve venesection and its assay is more reproducible (Section 8.4).

14.5.1 Vitamin B₁² Deficiency and Anaemia

Moderate metabolic vitamin B₁² deficiency was associated with anaemia, but contrary to common belief, macrocytosis was uncommon (Table 10b). Vitamin B₁² deficiency should therefore be looked for in all cases of anemia in old age. Even in non-anaemic vegetarians, vitamin B₁² supplementation is likely to bring about a significant increase in haemoglobin concentration.
14.5.2 Vitamin B12 Deficiency and Cognitive Impairment

The effect of vitamin B\textsubscript{12} deficiency on cognitive function of older people is a major concern, as the changes can occur imperceptibly and may not be reversible in later stages. In order to examine if older subjects with vitamin B12 deficiency had reversible cognitive impairment, a randomized controlled trial of parenteral vitamin B12 supplementation in fifty older people with subnormal vitamin B12 concentrations was performed. Over an average of four-month period, the supplemented group showed a significant improvement in the block design test as an indicator of performance IQ. But because the non-supplemented group also improved somewhat probably because of practice effect, there was no significant group difference in the changes in Performance IQ. On the other hand, the supplemented group appeared to have fared worse in motor functions, but this could have been a type 1 error or the effect of subclinical dementia (Table 11b).

This pilot study did not support the hypothesis that vitamin B12 deficiency caused reversible cognitive impairment in older people. But the trend of improvement in performance IQ, and the improvement in some demented subjects after supplementation should encourage further research in the role of vitamin B12 deficiency in cognitive impairment in older people.
14.6 Strengths and Limitations

This series of studies provided comprehensive health status and nutritional data of older Chinese vegetarians, which have been lacking in the medical literature. The majority of vegetarians had been vegetarians for years, and could be expected to have excellent dietary compliance. The results confirmed that the Chinese vegetarian diets were significantly different from those commonly adopted in Western countries. Consequently the health impact of the Chinese vegetarian diet was significantly different from that of those reported in Western countries, though the concern over hyperhomocysteinaemia should be relevant to vegetarians elsewhere. Finally, the randomized controlled trial of the effect of vitamin B12 supplementation on the cognitive function of older people was first of its kind.

There were, however, a number of weaknesses:

1. More than half of the subjects in the main survey were old age home residents, and almost all the community dwelling vegetarians were Taoists. This limited the representativeness of the sample as older Chinese vegetarians. Excluding those who were not self-ambulatory minimized the potential differences between old age home residents and community dwelling older people. The differences between the Taoist vegans and the lactovegetarian Buddhists were remarkably small, except in vitamin B12 status, probably because of the relatively low intake of dairy products among lactovegetarians.

2. All the vegetarian subjects were women. The impact of the vegetarian diet on older men may be different, especially in cardiovascular and bone health.
3. The historical subjects were all independent community dwelling older people in Hong Kong. The sample size of community dwelling vegetarian subjects was not adequate to detect small differences between vegetarians and omnivores. This was partially compensated by the additional of old age home subjects and statistical adjustment for the influence of confounders.

4. There were significant lifestyle differences between vegetarians and historical omnivorous subjects. Although adjustment for confounders has been made, the influence of the religious lifestyle of the vegetarians could not be entirely excluded. The ideal control subjects would be omnivores with similar lifestyle and religious practices as in the vegetarian subjects, as was the case of the Seventh Adventist Church followers. Unfortunately, the sources of vegetarian subjects I managed to find were very strict on dietary practices, and the number of omnivores in these settings was too small to form a control group.

5. The use of historical controls limited the choice of data collected. In addition, some important confounders were not accounted for, e.g. diabetes mellitus for comparison of blood pressures, because of differences in protocols and questionnaires. In retrospect, an age sex matched control study would be better. But this would have involved much more resources which were not available at the time.

6. The limitations of the randomized trial of vitamin B12 supplementation on the cognitive function of older people with deficiency have been discussed (Section 11.5).

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14.7 Implications of Research Findings

These studies have demonstrated dietary inadequacies and high salt intake of the older Chinese vegetarians, resulting in hypoalbuminaemia, hypertension, vitamin B12 deficiency and its associated hyperhomocysteinaemia and anaemia. A more balanced vegetarian diet with dietary advice and better dental care can bring about major health benefits to these vegetarians, in that blood pressure can be lowered, protein undernutrition corrected, and bone mass increased. A well-balanced vegetarian diet is more difficult to maintain in old age and in poverty. More nutritional studies for these at-risk groups should be conducted elsewhere.

Vitamin B12 deficiency remains a significant health threat to vegetarians, especially to vegans. Chinese vegetarians should be encouraged to consume more milk and commercially available vitamin fortified vegetarian foods, e.g. breakfast cereals. As soya milk and soya foods are popular among Chinese vegetarians, manufacturers of these products, especially those targeted for vegetarians should consider fortifying their products with vitamin B12.

Alternatively, oral vitamin B12 supplements are indicated. There is evidence that even small doses (2-37.5 mcg daily) of oral vitamin B12 are adequate in preventing metabolic vitamin B12 deficiency in older people (Garcia et al., 2002) in the majority of normal older people. In two of the vegetarian old age homes involved in the main study, they have subsequently adopted the policy of three monthly intramuscular vitamin B12 injections for all residents, which were well accepted by their residents. Intramuscular injections were preferred to oral supplements because of the worry of vitamin B12 malabsorption in old age,
and the prevalence of polypharmacy among old age home residents in general. Both types of vitamin B12 supplementation have low financial costs.

Hypertension, diabetes mellitus and hypertriglyceridaemia are as prevalent in older vegetarians as in omnivores. Case finding and treatment for these cardiovascular risk factors should therefore be just as vigorous as in omnivores.
14.8 Future Research

1. The negative association between calcium intake and systolic blood pressure needs confirmation by a larger scale cross sectional study with more precise measurement of calcium intake, e.g. seven-day food records and documentation of other confounding factors, e.g. 24-hour urinary sodium excretion. If confirmed, a randomized placebo controlled trial of calcium supplements on the blood pressures of older Chinese people who have very low calcium intake by Western standard is warranted.

2. The high prevalence of hyperhomocysteinaemia in vegetarians is worrying. Its importance as a cardiovascular risk factor can be confirmed by comparing the carotid intima-media thickness and flow mediated dilatation of vegetarians at different quartiles of serum homocysteine concentrations, after adjustment for age, sex, hypertension, diabetes mellitus, smoking and fasting lipids concentration.

3. The homocysteine lowering effect of folate has been demonstrated. But the effect of vitamin B12 supplementation on homocysteine concentrations and endothelial functions in at risk groups, e.g. vegetarians, older people warrants a randomized controlled trial.

4. The casual relationship between vitamin B12 deficiency and cognitive decline in old age has eluded many investigators because of the definition of deficiency, and the ethical concern of leaving vitamin B12 deficiency untreated. The most likely group of older subjects who would show cognitive improvement upon supplementation would be those with early dementia. A randomised placebo controlled trial of oral vitamin B12 supplements in this selected group of patients.
for one year or more may be the ideal research design to address this question. The ethical concern can be addressed by excluding those with definite deficiency i.e. low serum vitamin B12 and elevated metabolites concentrations, and only including those with low normal serum vitamin B12 concentrations with or without elevated metabolites concentrations. There is now normative data of Mattis Dementia Rating scale (Chan et al., 2001) for older people in Hong Kong. This could be used as a sensitive measurement of cognitive function in early dementia subjects.
Appendix A Questionnaires

A) Mental state (Clifton assessment procedure for the elderly)

1. What is your name? □ 1=correct MENTAL 1 □ 2=incorrect
2. How old are you? □ 1=correct MENTAL 2 □ 2=incorrect
3. When is your birthday? □ 1=correct MENTAL 3 □ 2=incorrect
4. What year is it now? □ 1=correct MENTAL 4 □ 2=incorrect
5. What month is it now? □ 1=correct MENTAL 5 □ 2=incorrect
6. What day is it today? □ 1=correct MENTAL 6 □ 2=incorrect
7. Which block is this building? □ 1=correct MENTAL 7
   Which floor are we on? □ 2=incorrect
   Which street are we on?
8. What is the name of this estate? □ 1=correct MENTAL 8
   Which district does it belong to? □ 2=incorrect
9. Which city are we in? □ 1=correct MENTAL 9 □ 2=incorrect
10. What is the name of governor of the Specific
    Administrative Region? □ 1=correct MENTAL 10 □ 2=incorrect
11. What is the name of the Chinese
    Premier/Leader? □ 1=correct MENTAL 11 □ 2=incorrect
12. What is the color of national flag of
    China/Taiwan? □ 1=correct MENTAL 12 □ 2=incorrect

Total: ________ MSCORE
B) General health state

1. What do you think of your present health status? □ 1 = Excellent □ 2 = Good □ 3 = Fair □ 4 = Poor □ 5 = Very poor

2. How do you compare your health status with people of your age, better or worse? □ 1 = Better □ 2 = Same □ 3 = Worse □ 8 = Don’t know

3. How do you compare your health status with that of a year ago? □ 1 = Better □ 2 = Same □ 3 = Worse □ 8 = Don’t know

4a) How many times did you go to see the doctor in the past one year? (90 = none, go to 5)

b) What kind of doctor did you go to? For how many times?
I) Private doctor □ (90 = none)
II) Government general clinic □ (90 = none)
III) Traditional Chinese Medicine □ (90 = none)

5a) Have you been admitted to the hospital in the past one year? □ (90 = no)

b) If yes, how long did you stay in the hospital? □ days

6) Are you taking any drugs prescribed by doctors? □ 1 = no □ 2 = yes
7) Are you taking any drugs not prescribed by doctors? (including Vitamins) □ 1 = no □ 2 = yes NPDRUG

8) Altogether how many kinds of drugs are you taking now? □ (90=none, go to 10) DRUGNO

9a) (for clients taking some kind of drugs) Which drugs are you taking now?

(Column A are answers from client and column B are checks by interviewer)

<table>
<thead>
<tr>
<th>(A)</th>
<th>(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>I) Diuretics</strong></td>
<td></td>
</tr>
<tr>
<td>□ 1=no</td>
<td>□ 1=no</td>
</tr>
<tr>
<td>□ 2=yes</td>
<td>□ 2=yes</td>
</tr>
<tr>
<td>□ 8=not sure</td>
<td>□ 8=not sure</td>
</tr>
</tbody>
</table>

| **II) Antihypertensive drugs (apart from diuretics)** |      |
| □ 1=no | □ 1=no |
| □ 2=yes | □ 2=yes |
| □ 8=not sure | □ 8=not sure |

| **III) Drugs for cardiovascular disease (apart from antihypertensive drugs and diuretics)** |      |
| □ 2=yes | □ 2=yes |
| □ 8=not sure | □ 8=not sure |

| **IV) Bronchodilators** |      |
| □ 1=no | □ 1=no |
| □ 2=yes | □ 2=yes |
| □ 8=not sure | □ 8=not sure |

| **V) Drugs for respiratory disease (apart from bronchodilator)** |      |
| □ 1=no | □ 1=no |
| □ 2=yes | □ 2=yes |
| □ 8=not sure | □ 8=not sure |

| **VI) Antacid or H2 receptor antagonist (drugs for dyspepsia)** |      |
| □ 1=no | □ 1=no |
| □ 2=yes | □ 2=yes |
| □ 8=not sure | □ 8=not sure |

259
<table>
<thead>
<tr>
<th>No.</th>
<th>Category</th>
<th>1=no</th>
<th>2=yes</th>
<th>8=not sure</th>
<th>Drug Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>VII</td>
<td>Laxative</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG7A-B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG7A-B</td>
</tr>
<tr>
<td>VIII</td>
<td>Drugs for neurological diseases (e.g.</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG8A-B</td>
</tr>
<tr>
<td></td>
<td>drugs for Parkinsonism)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG8A-B</td>
</tr>
<tr>
<td>IX</td>
<td>Antipsychotic drugs/Sedatives/Hypnotics</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG9A-B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG9A-B</td>
</tr>
<tr>
<td>X</td>
<td>Drugs for DM</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG10A-B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG10A-B</td>
</tr>
<tr>
<td>XI</td>
<td>NSAIDS or analgesics</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG11A-B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG11A-B</td>
</tr>
<tr>
<td>XII</td>
<td>Steroids or other kinds of hormone</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG12A-B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG12A-B</td>
</tr>
<tr>
<td>XIII</td>
<td>Vitamins or minerals (e.g. iron or calcium)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG13A-B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG13A-B</td>
</tr>
<tr>
<td>XIV</td>
<td>Antibiotics</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG14A-B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG14A-B</td>
</tr>
<tr>
<td>XV</td>
<td>Antihistamines</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG15A-B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>DRUG15A-B</td>
</tr>
<tr>
<td>XVI) Medications for skin</td>
<td>□ 1= no  □ 1= no</td>
<td>DRUG16-B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------</td>
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</tr>
<tr>
<td></td>
<td>□ 2= yes  □ 2= yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>□ 8= not sure  □ 8= unsure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>XVII) Traditional Chinese Medicine:</th>
<th>□ 1= no  □ 1= no</th>
<th>DRUG17A-B</th>
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<tr>
<td></td>
<td>□ 2= yes  □ 2= yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>□ 8= not sure  □ 8= unsure</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>XVIII) Others:</th>
<th>□ 1= no  □ 1= no</th>
<th>DRUG18A-B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>□ 2= yes  □ 2= yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>□ 8= not sure  □ 8= unsure</td>
<td></td>
</tr>
</tbody>
</table>

b) To be filled by the interviewer:

I) How many of the drugs taken by the elderly are unmarked?  

II) How many of the drugs taken by the elderly are marked?  

Please write down the drugs:

1. ________________________________  
2. ________________________________  
3. ________________________________  
4. ________________________________  
5. ________________________________  
6. ________________________________  
7. ________________________________  
8. ________________________________  
9. ________________________________  
10. ________________________________
### C) Past medical history

1. Have you been told by doctors that you have the following diseases?

<table>
<thead>
<tr>
<th>Disease</th>
<th>PMH</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Cerebrovascular disease (stroke)</td>
<td>PMH1</td>
</tr>
<tr>
<td>□ 1 = no</td>
<td></td>
</tr>
<tr>
<td>□ 2 = yes</td>
<td></td>
</tr>
<tr>
<td>□ 8 = do not know</td>
<td></td>
</tr>
<tr>
<td>b) Parkinson’s disease (Parkinsonism)</td>
<td>PMH2</td>
</tr>
<tr>
<td>□ 1 = no</td>
<td></td>
</tr>
<tr>
<td>□ 2 = yes</td>
<td></td>
</tr>
<tr>
<td>□ 8 = do not know</td>
<td></td>
</tr>
<tr>
<td>c) Heart disease (including coronary heart disease, heart failure, arrhythmia)</td>
<td>PMH3</td>
</tr>
<tr>
<td>□ 1 = no</td>
<td></td>
</tr>
<tr>
<td>□ 2 = yes</td>
<td></td>
</tr>
<tr>
<td>□ 8 = do not know</td>
<td></td>
</tr>
<tr>
<td>d) Hypertension</td>
<td>PMH4</td>
</tr>
<tr>
<td>□ 1 = no</td>
<td></td>
</tr>
<tr>
<td>□ 2 = yes</td>
<td></td>
</tr>
<tr>
<td>□ 8 = do not know</td>
<td></td>
</tr>
<tr>
<td>e) Chronic bronchitis or emphysema</td>
<td>PMH5</td>
</tr>
<tr>
<td>□ 1 = no</td>
<td></td>
</tr>
<tr>
<td>□ 2 = yes</td>
<td></td>
</tr>
<tr>
<td>□ 8 = do not know</td>
<td></td>
</tr>
<tr>
<td>f) Asthma</td>
<td>PMH6</td>
</tr>
<tr>
<td>□ 1 = no</td>
<td></td>
</tr>
<tr>
<td>□ 2 = yes</td>
<td></td>
</tr>
<tr>
<td>□ 8 = do not know</td>
<td></td>
</tr>
<tr>
<td>g) Pulmonary tuberculosis</td>
<td>PMH7</td>
</tr>
<tr>
<td>□ 1 = no</td>
<td></td>
</tr>
<tr>
<td>□ 2 = yes</td>
<td></td>
</tr>
<tr>
<td>□ 8 = do not know</td>
<td></td>
</tr>
<tr>
<td>h) Peptic ulcer</td>
<td>PMH8</td>
</tr>
<tr>
<td>□ 1 = no</td>
<td></td>
</tr>
<tr>
<td>□ 2 = yes</td>
<td></td>
</tr>
<tr>
<td>□ 8 = do not know</td>
<td></td>
</tr>
</tbody>
</table>
i) DM

☐ 1 = no PMH9
☐ 2 = yes
☐ 8 = do not know

j) Arthritis

☐ 1 = no PMH10
☐ 2 = yes
☐ 8 = do not know

k) Old fractures

☐ 1 = no PMH11
☐ 2 = yes
☐ 8 = do not know

l) Dementia

☐ 1 = no PMH12
☐ 2 = yes
☐ 8 = do not know

m) Psychosis (apart from dementia)

☐ 1 = no PMH13
☐ 2 = yes
☐ 8 = do not know

n) Cancer

☐ 1 = no PMH14
☐ 2 = yes
☐ 8 = do not know

o) Others :

☐ 1 = no PMH15
☐ 2 = yes
☐ 8 = do not know
D) Cardiovascular Questionnaire

a). Have you ever had any chest pain or chest discomfort?
   □ 1= yes
   □ 2= no \(\Rightarrow\) finished

b). Do you get this chest pain or chest discomfort when you walk quickly or walk uphill?
   □ 1= yes
   □ 2= no \(\Rightarrow\) go to Q. h)

c). Do you get this chest pain or chest discomfort when you walk on level ground at an ordinary speed?
   □ 1= yes
   □ 2= no

d). When you get this chest pain or chest discomfort, what do you do?
   □ 1= stop to rest
   □ 2= slow down your walking
   □ 3= continue walking at the same speed

e). Does the chest pain or chest discomfort go away when you stop?
   □ 1= yes
   □ 2= no

f). How long does it take before the pain goes away?
   □ 1= 10 min or less
   □ 2= > 10 min

g). Please tell me the position where you get this chest pain or chest discomfort
   □ 1= mid chest
   □ 2= left anterior chest and left arm
   □ 3= other: ________

h). Have you ever had a severe pain at the front of your chest lasting for half an hour or more?
   □ 1= yes
   □ 2= no
E) Health Behavior

I) Activities

1. Do you exercise?
   □ 1 = no (go to II)  
   □ 2 = yes  

2. What kind of exercise?
   a) Morning exercise
      □ 1 = often (> twice weekly)  
      □ 2 = occasionally (< once weekly)  
      □ 3 = no  
   b) Fast walk
      □ 1 = often (> twice weekly)  
      □ 2 = occasionally (< once weekly)  
      □ 3 = no  
   c) Tai Chi
      □ 1 = often (> twice weekly)  
      □ 2 = occasionally (< once weekly)  
      □ 3 = no  
   d) Qi Gong
      □ 1 = often (> twice weekly)  
      □ 2 = occasionally (< once weekly)  
      □ 3 = no  
   e) Lok-Tong Kuen (a form of martial art)
      □ 1 = often (> twice weekly)  
      □ 2 = occasionally (< once weekly)  
      □ 3 = no  
   f) Others: ______________________
      □ 1 = often (> twice weekly)  
      □ 2 = occasionally (< once weekly)  
      □ 3 = no  

3. On average, for how long do you exercise daily?
   □ 1 = > 4 hours  
   □ 2 = 3-4 hours  
   □ 3 = 1-2 hours  
   □ 4 = 20-59 minutes  
   □ 5 = < 20 minutes
4. Usually how do you exercise?

- 1=slowly
- 2=just as usual
- 3=quickly
- 4=very quickly

5. Comparing with one year before, do you exercise more or less now?

- 1=same
- 2=more
- 3=less
- 4=far less
II) Smoking and drinking

1a) Do you drink?                      □ 1= no (go to 2)    DRINK1
                     □ 2= yes

b) Do you drink very often?  □ 1=less than once    DRINK2
                           weekly (uncertain)
                           □ 2= 1-2 times per week
                           □ 3= 3-4 times per week
                           □ 4=daily/ ≥ 5 per week

2a) Did you ever smoke daily for a whole year?
               □ 1= no (go to the end)    SMOKE1
               □ 2= yes

b) How long have you been smoking?  ______ years    SMOKE2

3) Which kind do you smoke?            □ 1=cigarette     SMOKE3
                                   □ 2=tobacco
                                   □ 3=pipe
                                   □ 4=cigar

4a) Are you still smoking?             □ 1= no     SMOKE4
                                    □ 2= yes (go to c)

b) How long have you stopped smoking?  ______ yrs (90: <1 year)    SMOKE5

c) How many cigarettes do you smoke daily?  _____    SMOKE6
Appendix B  Neuropsychological Tests

1. Mini-Mental State Examination (10 minutes)

11 items – screening of severe dementia, organ brain damage. It has been translated and validated in older people in Hong Kong. The cutoff values for the detection clinical dementia was 20 (Chiu et al., 1994).

2. Wechsler Memory Scale (15 minutes)

1. Visual memory – It consisted of four items, of which the first three contained a single figure, and on the fourth card had two figures. The cards were shown to the subject for five seconds, and the subject was asked to draw the figure by memory. Thirty minutes later, subjects were asked to reproduce the four figures again. Scoring guidelines were available in the manual. The maximum combined score was 82. Uneducated people tended to do badly in this test.

2. Verbal memory - A contemporary story was told by the examiner. The subject then recalled the story. The story contained 23 memory units. The subject gained one point for each idea recalled. Thirty minutes later, the subject was asked to retell the story. The maximum combined score was therefore 46. This test was subject to the interpretation of the examiner, and the education level of the subject.

3. Digit span (immediate numerical recall)- It consisted of two different tasks – 1. immediate recall for the repetition of increasing numbers of digits, 2. repeat backwards an increasing series of numbers. The scoring is
simple: the item is passed if repeated or reversed exactly correct. Two trials are given for each digit length and both count toward the raw score. Digits forward begins with a three-digit number and proceed until both trials of a series are failed. Digits backwards begins with two three-digit practice items and proceed with a two-digit series and continues until both trials of a series are failed. Digits forward had maximum span of 8 digits, and digit backward had maximum span of 7 digits. The maximum total score was 24. Lezak suggested that span of six forward was normal, five marginal, and three defective (Lezak 1983, p. 268)

3. Wechsler Adult Intelligence Test – Revised (15 minutes)

1. Similarities – It was designed to assess abstract thinking. It asked the client to identify how 14 successive pairs of nouns were alike. Testing began with the first item for all clients and proceeded throughout the series. The clients' responses were scored 2, 1, 0 points for satisfactory, partially satisfactory and unsatisfactory answers. Subjective scoring by examiners was required, though general criteria and examples were available in the manual. The maximum score was 28.

2. Block design – It was a measure of general intelligence, and required a significant degree of visual-spatial ability. It was considered to culturally free and relatively less affected by educational levels.

The client was presented with nine cards depicting geometric designs in white and red, and with a number of identical plastic cubes each having
two red, two white, and two red and white sides split on the diagonal. The task was to reproduce the design on the card with the cubes. The client’s performance was timed; after the second item, additional points were awarded for rapid, errorless performance.

The first design was copied by the client from a model constructed by the examiner. In the second design, the examiner demonstrated how to construct model by copying a stimulus figure on the card. A second demonstration and trial was allowed for initial failure, and half credit awarded if successful. The first five designs were constructed with four blocks and one minute was allowed. From design 6 onward, the designs were constructed with nine blocks, and two minutes were allowed. In the final design, the figure was presented after being rotated by 45 degrees by the examiner.

Scores from Similarities were used to estimate the Verbal IQ, while scores of Block design were used to estimate Performance IQ, by referring to published Tables for conversion (Brooker & Cyr, 1986).

4. Luria Nebraska Neuropsychological Battery

The examination techniques were first developed by AR Luria as qualitative tests of the brain damaged adults. They were collected and organized by AL Christensen. Golden and his colleagues (Golden, 1981) later selected items from Christensen’s manual to form a battery so that Luria’s test procedures can be administered and evaluated in a standardized manner.
There are 11 clinical scales: Motor functions, Rhythm, Tactile functions, Visual functions, Receptive speech, Expressive speech, Writing, Reading, Arithmetic, Memory, and Intellectual processes, involving 269 items. Performance on each item is evaluated on a three-point scale, from 0 for no impairment to 2 for severely impaired.

The Motor function was the only scale used in the studies in Chapter 11. It consists of 51 items. In 15 items, there is a time limit of 10-20 seconds. In 6 items, quality of drawing was scored. The total score is the simple summation of the scores in all items. The scale can be divided into 5 subscores: Kinesthesia, Drawing, Fine motor, Spatial movement, and Oral movement. This clinical scale takes about 20 minutes to administer.

5. General Health Questionnaire

This is a generic questionnaire consisting of 30 questions to assess the general mood and anxiety of adults. To make it easier for older people to answer, the original four graded answers were reduced to yes and no answers. It has been translated into Chinese and validated in Hong Kong (Chi & Boey, 1994, Chi, 1995).
**Mini Mental State Examination**

**Orientation**

<table>
<thead>
<tr>
<th>Question</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the Year? (1)</td>
<td></td>
</tr>
<tr>
<td>Season? (1)</td>
<td></td>
</tr>
<tr>
<td>Month? (1)</td>
<td></td>
</tr>
<tr>
<td>Day? (1)</td>
<td></td>
</tr>
<tr>
<td>Day of week? (1)</td>
<td></td>
</tr>
</tbody>
</table>

**Where are we now?**

<table>
<thead>
<tr>
<th>Location</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>City (Hong Kong) (1)</td>
<td></td>
</tr>
<tr>
<td>Region (HK/KLN/NT) (1)</td>
<td></td>
</tr>
<tr>
<td>District (1)</td>
<td></td>
</tr>
<tr>
<td>Name of Clinic (1)</td>
<td></td>
</tr>
<tr>
<td>Floor (1)</td>
<td></td>
</tr>
</tbody>
</table>

**Registration**

3. Ask patient to remember the names of 3 objects (e.g. apple, key, bicycle) then tell them slowly once. Then ask patient to repeat them and score 1 mark for each correct answer: (3) 

Repeat the 3 names until patient able to tell all 3 correctly or up to 5 trials. Warn patient that he will be asked on them later.

**Attention and calculation**

4. Perform the serial 7's up to 5 times. (Prompt only once) Score 1 mark for each correct answer. (5) 

5. Ask the patient for the names of the 3 objects in Q. 3 (3) 

6. Point to 2 objects (e.g. watch, pencil) and ask patient to give their names. (2) 

7. Ask the patient to repeat the sentence after saying it once. (1) 

8. Ask the patient to listen to your commands and follow accordingly. Hand a piece of paper to patient.
   - 「請用左手接住這張紙」 (1) 
   - ("Please use your left hand to hold this sheet of paper") 
   - 「把紙對摺一次」 (1) 
   - ("Fold the sheet of paper into half") 
   - 「把紙放在地上」 (1) 
   - ("Place it onto the floor")

9. Ask patient to read out aloud and follow as written below. (1) 

10. Ask patient to write a sentence of own choice. 「請随便寫出一句說話」 ("Please say out one complete sentence of your own choice.") (Score 1 mark if the sentence makes sense to you. Wrong words are ignored). (1)
11. Ask patient to copy the figure below to the adjacent space. (5 sides preserved with intersecting sides forming a quadrangle) (1)

![Figure](image)

**DIGIT SPAN**  Discontinue after failure on both trials of any item.  
Administer both trials of each item, even if the first trial is passed.

<table>
<thead>
<tr>
<th>Item</th>
<th>Trial I Pass-Fail</th>
<th>Trial II Pass-Fail</th>
<th>Score 2, 1, or 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>6-2-9</td>
<td>3-7-5</td>
<td>Max. = 12</td>
</tr>
<tr>
<td>2.</td>
<td>5-4-1-7</td>
<td>8-3-9-6</td>
<td>Total Forward</td>
</tr>
<tr>
<td>3.</td>
<td>3-6-9-2-5</td>
<td>6-9-4-7-1</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>9-1-9-4-2-7</td>
<td>6-3-5-4-8-2</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>1-2-8-5-3-4-6</td>
<td>2-8-1-4-9-7-5</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>3-8-2-9-5-1-7-4</td>
<td>5-9-1-8-2-6-4-7</td>
<td></td>
</tr>
</tbody>
</table>

**DIGITS FORWARD**  Administer Digits Backward even if examinee scores 0 on Digits Forward.

<table>
<thead>
<tr>
<th>Item</th>
<th>Trial I Pass-Fail</th>
<th>Trial II Pass-Fail</th>
<th>Score 2, 1, or 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5-1</td>
<td>3-8</td>
<td>Max. = 12</td>
</tr>
<tr>
<td>2.</td>
<td>4-9-3</td>
<td>5-2-6</td>
<td>Total Backward</td>
</tr>
<tr>
<td>3.</td>
<td>3-8-1-4</td>
<td>1-7-9-5</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>6-2-9-7-2</td>
<td>4-8-5-2-7</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>7-1-5-2-8-6</td>
<td>8-3-1-9-6-4</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>4-7-3-9-1-2-8</td>
<td>8-1-2-9-3-6-5</td>
<td></td>
</tr>
</tbody>
</table>

**LOGICAL MEMORY (IMMEDIATE)**

(In the village next door, / there lived a middle-aged woman / named Chan, / who depended on / growing vegetables / for a living. / One day, / she went to the police station / to report a crime: / she claimed that on the day before, / she had been robbed of / 50 dollars / by a bad guy / on the street. /)

The woman had four / kids / at home. / She now did not have money to pay rent / or to buy food. / The policemen / all pitied her, / so they chipped in some money / and gave it to her./)
LOGICAL MEMORY (DELAYED)

Subject asked to repeat the same story
### Motor Functions Scale

<table>
<thead>
<tr>
<th>Item No.</th>
<th>Description</th>
<th>Quantitative Score</th>
<th>Qualitative Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>Thumb-finger sequential touch, RIGHT hand. # of sequences in 10 seconds: [    ]</td>
<td># sequences</td>
<td>0 1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 = &gt; 6</td>
<td>1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 = 5-6</td>
<td>0 1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = 0-4</td>
<td>0 1 2</td>
</tr>
<tr>
<td>(2)</td>
<td>Thumb-finger sequential touch, LEFT hand. # of sequences in 10 seconds: [     ]</td>
<td># sequences</td>
<td>0 1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 = &gt; 8</td>
<td>1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 = 5-8</td>
<td>0 1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = 0-4</td>
<td>0 1 2</td>
</tr>
<tr>
<td>(3)</td>
<td>Alternating clench / extension, RIGHT hand. # of sequences in 10 seconds: [  ]</td>
<td># sequences</td>
<td>0 1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 = &gt; 12</td>
<td>1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 = 7-12</td>
<td>0 1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = 0-6</td>
<td>0 1 2</td>
</tr>
<tr>
<td>(4)</td>
<td>Alternating clench / extension, LEFT hand. # of sequences in 10 seconds: [    ]</td>
<td># sequences</td>
<td>0 1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 = &gt; 15</td>
<td>1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 = 8-15</td>
<td>0 1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = 0-7</td>
<td>0 1 2</td>
</tr>
</tbody>
</table>

For the next several items, I am going to blindfold you and put your hand in a certain position. After I am finished, I want you to put your hand in the same position.

(5) Right thumb against fifth finger for 2 seconds, then separate. 0 = correct 2 = incorrect 0 2

(6) Left thumb against fifth finger for 2 seconds, then separate. 0 = correct 2 = incorrect 0 2

Now I am going to put your hand in a certain position against, but this time I will ask you to repeat the position with your other hand.

(7) Left thumb against middle finger for 2 seconds. 0 = correct 2 = incorrect 0 2

(8) Right thumb against middle finger for 2 seconds. 0 = correct 2 = incorrect 0 2

[Remove blindfold.] I am going to show you some hand movements. Please copy them exactly and make sure that you use the same hand as I do and that your whole arm and hand match mine. [Verbally correct if wrong hand used initially.]

(9) DEMONSTRATE: Right hand with bent fingers under chin. 0 = correct 2 = incorrect 0 2

(10) DEMONSTRATE: Left hand with bent fingers under chin. 0 = correct 2 = incorrect 0 2

(11) DEMONSTRATE: Tips of vertical right-hand fingers, palm left, touch chin. 0 = correct 2 = incorrect 0 2

(12) DEMONSTRATE: Tips of vertical left-hand fingers, palm right, touch chin. 0 = correct 2 = incorrect 0 2

(13) DEMONSTRATE: Tips of horizontal right-hand fingers (palm down) placed against palm of vertical left hand. 0 = correct 2 = incorrect 0 2

(14) DEMONSTRATE: Tips of horizontal left-hand fingers (palm down) placed against palm of vertical right hand. 0 = correct 2 = incorrect 0 2

275
<table>
<thead>
<tr>
<th>Item No.</th>
<th>Description</th>
<th>Quantitative Score</th>
<th>Qualitative Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>(15)</td>
<td>DEMONSTRATE: Raise right hand, palm out, to height of your head.</td>
<td>0 = correct 2 = incorrect</td>
<td>0 2</td>
</tr>
<tr>
<td>(16)</td>
<td>DEMONSTRATE: Raise left hand, palm out, to height of your head.</td>
<td>0 = correct 2 = incorrect</td>
<td>0 2</td>
</tr>
<tr>
<td>(17)</td>
<td>DEMONSTRATE: Right hand points to left eye.</td>
<td>0 = correct 2 = incorrect</td>
<td>0 2</td>
</tr>
<tr>
<td>(18)</td>
<td>DEMONSTRATE: Left hand points to right eye.</td>
<td>0 = correct 2 = incorrect</td>
<td>0 2</td>
</tr>
<tr>
<td>(19)</td>
<td>Point to your left eye with your right hand. [Do not demonstrate.]</td>
<td>0 = correct 2 = incorrect</td>
<td>0 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Item No.</th>
<th>Description</th>
<th>Quantitative Score</th>
<th>Qualitative Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>(20)</td>
<td>Touch your right ear with your left hand. [Do not demonstrate.]</td>
<td>0 = correct 2 = incorrect</td>
<td>0 2</td>
</tr>
<tr>
<td>(21)</td>
<td>I want you to copy what I do and change the positions of your two hands like this. First, you are to clench the fingers of your right hand and at the same time extend the fingers of your left hand. Then I want you to reverse the positions of your two hands. That is, I want you to clench the fingers of your left hand and extend the fingers of your right hand at the same time, changing smoothly from one hand to the other. [Demonstrate with palms facing down and allow S to practice.] Do it as quickly as possible.</td>
<td># sequences 0 = &gt; 8 1 = 6-8 2 = 0-5</td>
<td>0 1 2</td>
</tr>
<tr>
<td>(22)</td>
<td>Tap your right hand twice and your left hand once, changing from one hand to the other without interruption. [Demonstrate and allow S to practice.] Do this as fast as you can until I tell you to stop.</td>
<td># sequences 0 = &gt; 8 1 = 7-8 2 = 0-6</td>
<td>0 1 2</td>
</tr>
<tr>
<td>(23)</td>
<td>Now, tap your left hand twice and your right hand once, changing from one hand to the other without interruption. [Demonstrate and allow S to practice.] Do this as fast as you can until I tell you to stop.</td>
<td># sequences 0 = &gt; 10 1 = 5-10 2 = 0-4</td>
<td>0 1 2</td>
</tr>
<tr>
<td>(24)</td>
<td>Card D1 [Present Patient Response Booklet.] I want you to draw this pattern [present Card D1] as quickly as you can without lifting your pencil from the paper. Make sure that you draw the figure exactly as it is drawn on the card, that this is the same size and has the same number of forms as in the figure. [Allow 20 seconds. Permit second trial if S lifts pencil on first.]</td>
<td>0 = correct 2 = incorrect</td>
<td>0 2</td>
</tr>
<tr>
<td>(25)</td>
<td>Pretend you are holding a tea (coffee) pot in your hand and you have a cup available. Show me how to pour and stir tea (coffee).</td>
<td>0 = correct 2 = incorrect</td>
<td>0 2</td>
</tr>
<tr>
<td>(26)</td>
<td>Show me how you would thread a needle.</td>
<td>0 = correct 2 = incorrect</td>
<td>0 2</td>
</tr>
<tr>
<td>(27)</td>
<td>Show me how you would use a pair of scissors.</td>
<td>0 = correct 2 = incorrect</td>
<td>0 2</td>
</tr>
<tr>
<td>(28)</td>
<td>Puff out your cheeks.</td>
<td>0 = correct 2 = incorrect</td>
<td>0 2</td>
</tr>
<tr>
<td>(29)</td>
<td>Stick your tongue out as far as possible and keep it there until I ask you to put it back in your mouth. [3-second minimum extension]</td>
<td>0 = correct 2 = incorrect</td>
<td>0 2</td>
</tr>
<tr>
<td>(30)</td>
<td>Stick your tongue out and roll it up.</td>
<td>0 = correct 2 = incorrect</td>
<td>0 2</td>
</tr>
<tr>
<td>Item No.</td>
<td>Description</td>
<td>Quantitative Score</td>
<td>Qualitative Scores</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
<td>--------------------</td>
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<tr>
<td></td>
<td></td>
<td>Criteria</td>
<td>Score</td>
</tr>
<tr>
<td>31</td>
<td>Put your tongue between your upper teeth and upper lip.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 = correct</td>
<td>0 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = incorrect</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>I am going to ask you to make three movements with your mouth. I will show</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>you the movements, then I want you to do them. First, I want you to show</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>your teeth, then stick out your tongue, and third, place your tongue between</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>your lower teeth and lower lip, like this. [Demonstrate sequence.] Now I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>want you to do these three movements. Remember, first show your teeth, then</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>stick out your tongue, then place your tongue between your lower teeth and</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>lower lip.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 = correct</td>
<td>0 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = incorrect</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>Now do these same three movements rapidly several times until I tell you</td>
<td># sequences</td>
<td></td>
</tr>
<tr>
<td></td>
<td>to stop. Remember, first show your teeth, then stick out your tongue, and</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>then place your tongue between your lower teeth and lower lip. [Demonstrate</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>and allow S to practice.] # of sequences in 10 seconds: [_____]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Show me how to chew.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 = correct</td>
<td>0 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = incorrect</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Show me how to whistle. [If S uses fingers, ask: “What is another way to</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>whistle without using your fingers?”]</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>[Present Patient Response Booklet.] Without lifting the pencil from the</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>paper, I want you to draw the best circle you can, as quickly as you can.</td>
<td></td>
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<tr>
<td></td>
<td>[Permit second drawing if pencil is lifted. Time response and allow 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>seconds for each drawing.]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 = correct</td>
<td>0 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = incorrect</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>Quality of drawing</td>
<td>See Manual</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Total time: [_____]</td>
<td>time</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 = 1-3 secs</td>
<td>0 1 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 = 4-5 secs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = 6-16 sec</td>
<td></td>
</tr>
<tr>
<td>Timed</td>
<td>Without lifting the pencil from the paper, I want you to draw the best square you can, as quickly as you can. [Time response and allow 15 seconds.]</td>
<td>Quality of drawing</td>
<td>Total time: [ _____ ]</td>
</tr>
<tr>
<td>---</td>
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</tr>
<tr>
<td>(38)</td>
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<tr>
<td>(39)</td>
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<td></td>
</tr>
<tr>
<td>Timed</td>
<td>Without lifting your pencil from the paper, I want you to draw the best triangle you can, as quickly as you can, and make all three sides equal. [Time response and allow 15 seconds.]</td>
<td>Quality of drawing</td>
<td>Total time: [ _____ ]</td>
</tr>
<tr>
<td>(40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(41)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Card D3</td>
<td>[Present Card D3.] Without lifting your pencil from the paper, copy this figure exactly, as best and as quickly as you can. Make it about the same size. [Time response and allow 15 seconds. See scoring criteria for item 38.]</td>
<td>Quality of drawing</td>
<td>Total time: [ _____ ]</td>
</tr>
<tr>
<td>(44)</td>
<td></td>
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<td></td>
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<tr>
<td>(45)</td>
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</tr>
<tr>
<td>Card D4</td>
<td>[Present Card D4.] Without lifting your pencil from the paper, copy this figure exactly, as best and as quickly as you can. Make it about the same size. [Time response and allow 15 seconds. See scoring criteria for item 40.]</td>
<td>Quality of drawing</td>
<td>Total time: [ _____ ]</td>
</tr>
<tr>
<td>(46)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(47)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(48)</td>
<td>Now, I am going to knock on the table. If I knock once, I want you to knock twice; and if I knock twice, I would like you to knock once. 1: [ _____ ] 2: [ _____ ] 2: [ _____ ] 1: [ _____ ]</td>
<td># errors</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Item No.</td>
<td>Description</td>
<td>Quantitative Score</td>
<td>Qualitative Scores</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
<td>--------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>(49)</td>
<td>Please take my hand. Now, if I say &quot;red,&quot; I want you to squeeze and then relax your hand; if I say &quot;green,&quot; do nothing. red: [<em><strong>] green: [</strong></em>] green: [<em><strong>] red: [</strong></em>]</td>
<td># errors</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 = none</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 = 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = 2-4</td>
<td>0 1 2</td>
</tr>
<tr>
<td>(50)</td>
<td>If I knock once, I want you to raise your right hand. If I knock twice, I want you to raise your left hand. 1: [<em><strong>] 2: [</strong></em>] 1: [<em><strong>] 2: [</strong></em>]</td>
<td># errors</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 = 0-1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 = 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = 3-4</td>
<td>0 1 2</td>
</tr>
<tr>
<td>(51)</td>
<td>If I knock hard, you knock gently; If I knock gently, then knock hard. [Demonstrate hard and gentle knocks.] G: [<em><strong>] H: [</strong></em>] H: [<em><strong>] G: [</strong></em>]</td>
<td># errors</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 = none</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 = 1-2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = 3-4</td>
<td>0 1 2</td>
</tr>
</tbody>
</table>
### General Health Questionnaire (Revised 30 Items)

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 1 | Could not concentrate | Yes | No |
| 2 | Lost sleep |   |   |
| 3 | Restless nights |   |   |
| 4 | Not Busy or occupied |   |   |
| 5 | Not out of house |   |   |
| 6 | Not managing well |   |   |
| 7 | Not doing things well |   |   |
| 8 | Not satisfied with task |   |   |
| 9 | No warmth and affection |   |   |
| 10 | Could not get on with others |   |   |
| 11 | Not chatting with others |   |   |
| 12 | Not playing a useful part |   |   |
| 13 | Could not make decision |   |   |
| 14 | Felt under strain |   |   |
| 15 | Could not overcome difficulties |   |   |
| 16 | Found life a struggle |   |   |
| 17 | Not enjoying activities |   |   |
| 18 | Taking things hard |   |   |
| 19 | Scared or panicky |   |   |
| 20 | Could not face problems |   |   |
| 21 | Felt everything on top |   |   |
| 22 | Unhappy and depressed |   |   |
| 23 | Lost confidence |   |   |
| 24 | Felt worthless |   |   |
| 25 | Felt life hopeless |   |   |
| 26 | Not hopeful about future |   |   |
| 27 | Not feeling happy |   |   |
| 28 | Nervous and strung up |   |   |
| 29 | Felt life worth living |   |   |
| 30 | Nerves feel bad |   |   |
Appendix C  Photographs of Vegetarian Subjects and Foods

1  Chinese New year Banquet for Taoist followers
2  Residents of Taoist old age home
3  Taoist old age home residents at prayer
4  Supper at Taoist old age home
5  Large bedroom for twenty residents
6  Soy “Sausages” with vegetables
7  Soy “Chicken” with potatoes
8  Other Soy foods with vegetables
9  Other Soy foods with vegetables
Chinese New Year Banquet for Taoist followers
Residents of Taoist old age home

Taoist old age home residents at prayer
Supper at Taoist old age home

Large bedroom for 20 residents
Soy "Sausages" with vegetables  Soy "Chicken" with potatoes

Soy foods with vegetables (note lack of green leaf vegetables)
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