

## **CHAPTER 3**

### **METHODS AND VALIDATION OF FOOD FREQUENCY QUESTIONNAIRE**

#### **3.0 INTRODUCTION**

This chapter describes the research methods of the study and validation of the food frequency questionnaire.

#### **3.1 STUDY DESIGN**

##### **3.1.1 A CROSS-SECTIONAL STUDY**

A cross-sectional study, also known as a descriptive study, reveals the pattern of disease in human populations and provides general observations about the relationship between the disease and population characteristics. These observations are often confined to a geographic location for a given time frame. However, cross-sectional studies cannot prove causality, they only have power for associations. Only with longitudinal, prospective data or that from an intervention study can causality be inferred (Friedman, 1980).

Under a cross-sectional design, this study aimed to collect base-line information on health, food intake and lifestyle in elderly Greeks living in Melbourne, Australia and Spata, Greece and subsequently to look at associations between these variables. This study did not seek data to facilitate a cause-effect relationship interpretation. The cross-sectional design, limits the interpretation of the data in relation to changes with increasing age. Any differences between age groups may reflect cohort effects, changes within individuals and selective mortality. The available data do not allow these effects to be evaluated separately.

##### **3.1.2 SAMPLE SIZE**

The International Union of Nutritional Sciences (IUNS) committee on Nutrition and Ageing, in conjunction with the World Health Organization (WHO) global program for the

elderly, embarked on a program in 1987 to test key hypotheses in relation to food habits, health status and lifestyle in the elderly in developed and developing countries. The study of elderly Greeks in Greece and Australia is part of the wider international study. In 1987 a workshop was held in Wageningen, Netherlands to design a European study on the food habits and health of elderly people in Europe; the study was to be called Euronut-Seneca (de Groot et al., 1991).

The candidate was invited as coordinator of the IUNS study. At this workshop, it was decided the European study should have both a first stage cross-sectional design and a second stage mixed longitudinal design (for study centres able to take part). The cross-sectional study was conducted in 1988-89 on 2600 elderly people aged 70-75 years, living in 19 small towns of 12 European countries. It included a randomly selected sample of 30 men and 30 women (cohort of 1913-1914) giving a total sample of 60. The longitudinal design included a randomly selected sample of 30 men and 30 women born in 1913-1914, 35 men and 35 women born in 1915-1916, 45 men and 45 women born in 1917-1918, giving a final sample of 110 men and 110 women.

The IUNS committee on Nutrition and Ageing decided that the IUNS study should begin with a cross-sectional design of at least 60 people to allow comparison with the Euronut data, but preferably more, between 100-200 subjects if possible. The sample size of Greeks in Melbourne and Spata was therefore based on the recommendations of the IUNS committee and the Euronut workshop.

### **3.1.3 CHOICE OF ENTRY CRITERIA**

This study included individuals of Greek ethnicity, aged 70 years and over. This age cutoff was chosen to define 'elderly' because most mental and physical functions do not begin to deteriorate until age 70-75, rather than at retirement age of 65 (Heikkinen et al., 1983; Chebotarev, 1982). The elderly can be seen to belong to two broad age groups: the young elderly aged 70-79 and the old elderly aged 80+ (Davies, 1990). In this study, subjects were recruited from both age groups. Data was analysed and presented according to these age groups.

Individuals unable to answer questions independently were excluded as were psychogeriatric patients in nursing homes. The 'Greek ethnicity' is a self-imposed identification and may include individuals with one non-Greek parent or grandparent. All individuals living in the same household e.g spouse, were included, provided they met entry criteria.

This sampling method is unlikely to induce selection bias due to gender or age clustering since epidemiological data is usually analysed and presented for men and women separately with age adjustment. In the Melbourne sample, households in the outer fringe of the city were excluded since access to these households usually required more than one hour of transportation time (one way). In the Spata sample, all selected households were included since most households were within walking distance. The 'target populations' for this study were individuals who met these eligibility criteria.

### **3.2 POPULATION SELECTION AND RECRUITMENT**

In order to obtain representative samples of elderly people in Greece and Australia it was decided to use the best possible recruitment method available in each country that will give the most complete information for that age group. There are many sampling methods to obtain representative samples for epidemiological studies. Such methods include: a) population registers b) multistage probability sampling in private censuses (Pensabene and Kabala, 1986) c) social network methods in geographically defined and ethnically homogeneous areas (Powles et al., 1990b) d) electoral rolls and e) telephone directory (Lavrakas, 1987).

Population registers are not available in Australia or Greece. Multistage probability sampling is very expensive and the social network method suffers from uncertainty in ascertaining a representative sample. They were therefore considered unsuitable for this study. In countries with high telephone usage such as Australia, telephones have been extensively employed to define a study sample.

Some ethnic groups are distinguished by their family names. Rutishauser and Wahlqvist (1983) exploited this feature for a study of food intake patterns of Greek migrants in Melbourne, using systematically selected names from the membership list of a large Greek welfare organisation to define a study population from the telephone directory. This selection correlated well with the distribution of the Greek population within the Melbourne municipalities and their sociodemographic characteristics, as identified in the national census taken before the survey. In contrast, in countries with low telephone usage, such as Greece, it is preferable to use electoral rolls. Thus, electoral rolls were considered suitable for use in Greece and the telephone directory in Australia (see below).

### **3.2.1 Spata**

Spata was chosen for two reasons:

- 1) Due to the maintenance of the traditional Greek diet by most families in this town, which would act as a good standard by which to determine the degree of dietary change made on migration by elderly Greeks in Melbourne.
- 2) Most families have resided in Spata for many generations, with households being multi-generation and still very traditional in their way of life, thus making it an ideal set-up for the study of traditional Greek culture, food habits and beliefs.

The electoral rolls were chosen to sample the elderly because less than 80% of all households have a telephone in Greece. At the 1981 census, the total population of Spata was 6398. At the time of the study (June-October 1988), the total population was about 10 000. Electoral rolls were obtained from the Spata council as well as the names of people who had died since the last revision of the electoral rolls - these names were subsequently removed.

A total of 640 (M 284, F 356) people aged 70+ were identified from the electoral rolls, which formed the sampling base of the study. Names were randomly selected from the electoral rolls and interviewed until the required sample size was achieved. A smaller sampling base was not further selected given uncertainties with response rates and accessibility. Mobility of the elderly could have been a problem at the contact stage since the study period coincided with grape harvesting and holidays.

All subjects in the household, aged 70+ and who claimed to be of Greek ancestry, were eligible for the study. Eligible spouses were also included in the study once the housecall was made (as in the Melbourne study). No attempt was made to achieve equal numbers of men and women. The representativeness of the sample was checked against the electoral rolls for Spata by comparing the gender ratios and age group distribution (70-79, 80+) (see Chapter 4).

### **3.2.2 Melbourne**

In Melbourne, it was not possible to study Greeks from Spata because only a few families had left Spata to migrate to Melbourne. Alternatively, a cross-section of the Greek-speaking community in Melbourne was taken. In contrast to the sampling method used in

Spata (electoral rolls), the telephone directory was chosen as the sampling method because more than 95% of all households in Melbourne are telephone subscribers. The Melbourne electoral rolls were not chosen as a sampling method because the likelihood that most elderly Greeks would be listed there was doubtful. Greek sounding surnames were randomly selected from the 1989-1990 Melbourne telephone directory, with reference to a list of commonly used Greek surnames subscribing to a popular Greek newspaper. This list included presumptive Greek surnames which had been abbreviated or Anglicised on migration e.g Gregoriou changed to Gregory or the direct translation of Triantafilou to Rose. Non-residential listings (i.e business listings), where identified, were excluded.

A total of about 30 000 telephone connections were identified to form the sampling base of the study. In order to obtain a representative sample of about 200 subjects, 500 names were randomly selected, their addresses and telephone numbers transcribed. This larger initial sample list was necessary because not all households contacted would have an elderly person living with them. This point is particularly relevant given the low numbers of elderly Greeks in Melbourne at the 1986 census - 2712 aged 65-75, 1594 aged 75+, representing 4% and 2.4% respectively of those claiming Greek ancestry.

This sampling list was updated in 1990 when the '1990 Telecom White Pages' became available. All individuals selected lived in the urban parts of Melbourne. All subjects in the household, aged 70 and over and who claimed being of Greek ancestry, were eligible for the study. Similarly to Spata, representativeness was checked against gender ratio and age structure of the sampling base (ABS 1986 census). Additionally, representativeness was also checked geographically by comparing the percentage of elderly Greeks in the sample living in various regions in Melbourne to data obtained from the 1986 census on the geographic distribution of all Greeks aged over 70 (see Chapter 4).

### **3.3 RESPONSE RATE & REFUSALS**

The non-responders were registered and asked why they did not wish to participate in the study. Where possible, information was collected from them about their age and health. If subject did not wish to have blood taken, they were given the option of only completing the questionnaire and having anthropometry undertaken. This helped to improve the overall response rate since many elderly people did not feel the need to have a blood test.

### 3.3.1 Spata

A total of 104 elderly Greeks were interviewed with the questionnaire. Similar to the Melbourne study, the mean age of the sample was 77 years for both men and women and almost equal numbers of men (51) and women (53) were obtained by chance. The distribution of men and women across the age groups was as follows: 70-79 M 31%, F 30%; 80+ M 18%, F 21%). Only 13 subjects (M 7, F 6) refused to be in the study giving a *questionnaire response rate of 89%*.

Of the 104 subjects interviewed, 67 subjects (M 40, F 27) agreed to come to the local health centre to have anthropometry undertaken as well as their blood tested giving a *biological measurement response rate of 57%*. Similar proportions of men and women aged 70-79 and 80+ had their blood tested (65%, 35% respectively). In many cases, more than two housecalls were necessary to complete the questionnaire, for the following reasons: a) the holiday season from August to September b) elderly men being at the local coffee shop (cafeneion) drinking coffee and playing cards c) elderly helping during the grape harvest in September. As a result, only 104 subjects were interviewed in the time available (three months) to do the study.

### 3.3.2 Melbourne

A total of 189 elderly Greeks (M 94, F 95) were interviewed with the questionnaire. Similarly to Spata, almost equal proportions of men and women were sampled by chance (see also chapter 4). The mean age of the sample (78 years) was similar to the Spata sample (77 years). The distribution of men and women was also similar across the age groups and strikingly similar to the Spata sample: 70-79 (M 35%, F 31%) and 80+ (M 15%, F 19%).

Only 37 elderly (M 19; F 18) refused to take part in the study giving a *questionnaire response rate of 83.6%*. Of the 189 subjects interviewed (most of whom also had their anthropometry undertaken), 109 (M 60, F 49) agreed to have their blood tested giving a *biological measurement response rate of 48%*. A similar proportion of men and women aged 70-79 and 80+ had their blood tested (72%, 28% respectively).

Due to the small numbers of elderly Greek people living in Melbourne (2% of Greek community were aged 70+) it was very time consuming to find subjects for the study - there was only one Greek-speaking researcher (candidate) doing both the recruitment and interviewing. Often, after a whole week of phone calls, only a couple of subjects

would be found. Thus, after two years of recruitment and interviewing 189 subjects had been studied instead of the intended 200 - time and financial constraints did not allow further recruitment.

### **3.4 CONDUCT OF SURVEY AND DATA COLLECTION**

This section describes the survey methods, including the socio-demographic questionnaire, dietary assessment and biological measurements (anthropometry, blood tests, blood pressure, skin microtopography).

#### **3.4.1 GENERAL**

The aim was to achieve a participation rate of at least 60% (60% of those invited). Hospitalized subjects were also included by contacting them after discharge. If subjects were away on holiday, they were contacted at a later date (if within months of study). At least three attempts were made to contact subjects not at home when visited or contacted by phone. Once contact was made, it was emphasized that the information provided would be kept in strict confidence and that it would be in no way possible to identify individuals in the final results. The interviewer was not introduced as a dietitian/doctor as this might have influenced the subject's response. The interviewer was referred to as 'researcher' or 'interviewer'.

The subjects were told that this study was part of a wider international program looking at the health, lifestyle and diet of elderly people in many countries and that their participation will contribute in making future recommendations to the population at large about how to live a long and healthy life. The interviewer avoided communicating her views and was careful not to make assumptions about the respondents. The interviewer stressed that she did not have any intention of telling the subject what to do but that she was interested in their experiences.

The questionnaire was administered by an interviewer because literacy limitations as well as failing eye sight made it difficult to have the questionnaire self administered. It was desirable that the questionnaire be answered by the participant with no help from anyone else. However, in some cases it was impossible to exclude the involvement of others completely. The cooperation of an informant was desirable if the subject was unable to

answer basic questions relating to recall of year, month or day of the week (questions MA7-MA9). The interviewer made a note of contributions of others to the interview.

### **3.4.1.1 Spata**

The questionnaire designed for the study was first used on the Greeks in Spata from August-October 1988 (Wahlqvist et al., 1988; Kouris et al., 1989). Rapid Assessment Procedures (Scrimshaw and Hurtado, 1987) were used to get an overall picture of the community and to ensure that the questionnaire was sensitive to areas that may have been overlooked. This was achieved by having the director of the Spata Community Health Centre, researchers from the Athens School of Public Health and Spata community leaders, check the questionnaire.

The questionnaire was also piloted on 10 elderly inhabitants of Spata before using it on the elderly selected for the study. The candidate, fluent in Greek and familiar with Greek culture and tradition, found an apartment in Spata which would facilitate rapport and acceptance of the study. There was no need to announce the study, as word of mouth in such a tight-knit community rapidly spread the news about the study.

Electoral rolls were obtained from the Spata council which unfortunately did not include the phone numbers of voters, only incomplete addresses (street name but not the number). As a result, it was not possible to send a letter to elderly subjects describing the study nor was it possible to make a phone call prior to the housecall. The elderly selected were located by word of mouth i.e by asking locals for directions.

The candidate made two home visits. The first visit was to inform the participants of the study, to ascertain their desire to participate and complete part one (non-nutritional section) of the questionnaire which took about 60 minutes. Due to literacy problems and cultural apprehension in signing papers, consent forms were not used. Nevertheless, detailed explanation was given to both participants and relatives before verbal consent was obtained.

The second visit involved completing part two (nutritional section of the questionnaire) which took about 90-120 minutes. Interviews were conducted from 9am-1pm and from 5pm-9pm, so as to coincide with the daily routine of the elderly subjects (from 1-5pm lunch was eaten and the afternoon nap was taken).

In the morning, about four non-nutritional sections of the questionnaire were completed and in the afternoon two dietary sections of the questionnaire were completed. A Greek researcher (Dr Evangelos Polychronopoulos) from the Athens School of Public Health assisted by doing 30 interviews with the non-nutritional section of the questionnaire. The candidate did the remaining 74 interviews with part one of the questionnaire and all of the dietary interviews, in order to avoid inter-observer variation.

In October 1988, all participants were contacted and asked to attend the Spata Community Health Centre on certain days (19-26/10/1988) in order to have their blood collected, blood pressure and anthropometry measured as well as the skin microtopography. It was explained that they would have their blood results after 1-2 months from Australia which would be given to them by the health centre. About 15-20 subjects were asked to come on each day in the morning after an overnight fast. Data coding and entering into a computer data base (DBase 3) was only possible on returning to Australia in November 1988.

#### **3.4.1.2 Melbourne**

The questionnaire which was trialled in Spata, was also used in Melbourne, with some minor alterations to tailor to the lifestyle of Greeks in Australia. The study was announced in a local Greek paper and a popular Greek radio station in December 1989. Greek sounding surnames were selected (randomly) from the telephone directory and contacted in 1990 and 1991.

If a household was rung and subjects were not eligible or if they refused to participate, the subsequent name was contacted, and so on, until the required sample size was achieved. There was no attempt to ensure that equal numbers of men and women were recruited. The study was briefly described on the phone and inquiry was made about individuals in the household aged 70 and over. If no such individual lived there, we asked for a name and phone number of someone they knew that was aged over 70, such as a relative. This system of referral was limited to no more than two names from each contact made.

All subjects aged 70+ in a household were eligible for the study, including both husband and wife if married. If subjects were unable to answer most questions independently they were excluded or if they did not identify themselves as Greek they were also excluded (eg Slavo-Macedonians). Due to literacy problems and cultural apprehension in signing

papers, consent forms were not used. Nevertheless, detailed explanation was given to both participants and relatives before verbal consent was obtained.

Individuals who agreed to be interviewed and have their blood tested were seen on two occasions. On one occasion in their home where the questionnaire (nutritional and non-nutritional questions) was administered and where interview time often ranged between 2-4 hours (food frequency questionnaire took 90-120 minutes; lifestyle questions 30-60 minutes).

Only 1-2 subjects could be studied per day. A few weeks later, the subject would come to the hospital to have anthropometry and blood pressure measured as well as the skin and blood tested. Transport by taxi was arranged to the hospital where this was a problem, at no cost to the subject. About 2-4 subjects were studied per week due to difficulties in finding eligible subjects. Blood test results were briefly explained in a personalized letter in Greek to each participant along with a print out of their results in English, which they were instructed to show their doctor for interpretation.

Individuals who did not wish to have their blood tested were seen only once in their homes. The questionnaire would be administered followed by anthropometry, blood pressure measurement and skin test. The food intake section was by far the most difficult and tiring for the subject. Food photographs were used to assist in the estimation of serving size.

### **3.5 INTERVIEWER ADMINISTERED QUESTIONNAIRE**

Survey research relies on a very basic assumption, which rarely is evaluated, that persons report accurately in response to questions asked during an interview. Evidence does not support consistently the common sense notion of an age-related decrease in reporting accuracy (Herzog and Dielman, 1985). Rodgers and Herzog (1987) interviewed 1491 subjects, with an oversampling of persons aged 60+. Questions were asked about the respondent's car, driver's licence, voting behaviour, birth date, distances to neighbourhood facilities, characteristics of neighbours and house value and property taxes. Responses were validated against publicly accessible records.

For many measures, no age differences were detected, and for those where age differences were observed, the older respondents were sometimes more accurate than the younger respondents. Overall the correlation between the respondents answers and

the validating information was in the high 0.7s. While many older people are quite capable of completing a self-administered questionnaire, some cannot do so because of language or literacy problems or because of various handicaps. In order to obtain a higher rate of response, personal interview is therefore recommended (Fillenbaum, 1984).

Two general approaches were used to elicit nutritional and non-nutritional information from the elderly respondents (see Appendix 2 for questionnaire):

**a) Quantitative approach**

Questionnaire with coded answers for scoring, to obtain information on health, lifestyle, demography and food habits.

**b) Qualitative approach**

Anthropological method, Rapid Assessment Procedures (RAP), with open ended questions to elicit information on food and health beliefs. Anthropologists, Scrimshaw and Hurtado (1987) have developed guides which allow rapid assessment of beliefs, perceptions regarding health, the prevention and treatment of illness and utilization of traditional and biomedical health resources.

These guides have been modified to develop a set of open ended questions to obtain information on food and health beliefs for the purpose of exploring *reasons* for food habits and dietary choices to complement the quantitative food intake data (Wahlqvist et al 1991a; Kouris et al., 1991).

### **3.5.1 DEMOGRAPHY, HEALTH AND LIFESTYLE QUESTIONS**

This section of the questionnaire was adapted from a number of previously trialled questionnaires on the elderly (see below). In order to meet the objectives of this study, questions were tailored to the Greek populations surveyed (Kouris et al., 1988). Unfortunately, these questionnaires were not suitable for use in their entirety (Fillenbaum 1984; Kane and Kane, 1981).

Questions were adapted from the following questionnaires (see also Chapters 5 and 6):

1. Multi-level Assessment Instrument for the Elderly (MAI), Philadelphia Geriatric Centre, USA (Lawton et al., 1982)
2. World Health Organisation, Ageing in the Western Pacific: A Four Country Study, (Andrews et al., 1986)
3. World Health Organisation, Elderly in Eleven Countries: A Sociomedical Survey (Heikkinen E et al., 1983).
4. Older Americans Resources and Services (OARS) questionnaire, (Fillenbaum and Smyer, 1981)
5. Euronut-Seneca, Nutrition and the elderly in Europe, (de Groot et al., 1991)

Scores were developed for all variables. Scores were computed by summing numbers in front of question responses (see Appendix 2). In all cases a higher score was a better score.

The questionnaire for the study included the following sections (together with the source of the questions) (see Appendix 2 for questionnaire):

**a) Demography**

Questions on demography included gender, date of birth, marital status, birthplace, ancestry, education, past and present employment, income, rural/urban background, living arrangements and household members. These questions were adapted from the WHO instrument used in the Western Pacific (originally taken from the WHO 11 country study instrument, Heikkinen et al., 1983).

**b) Self Reported Health**

The relationship of food intake and lifestyle to health is a central theme in this study. Thus a valid and reliable method of measuring health was required. Fillenbaum (1984) reviews all available questionnaires measuring the health status of the elderly and concludes that the Multi-level Assessment Instrument (MAI) designed by Lawton et al (1982) is one of the most valid and reliable measures to use on such populations. It is carefully constructed and has been tested for reliability and validity.

The health section of the Multi-level Assessment Instrument (MAI) (Lawton et al., 1982) appeared well suited for describing the health status of the elderly due to the existence of a physical health domain index, composed of subindices measuring self rated health,

health behaviour and health conditions. The reliability and validity of these indices have been affirmed by several different approaches whereby a physician is asked to rate the subject for the various subindices. The subindices can be scored by counting or summing and can be used in isolation from each other and from the rest of the questionnaire. A higher score in all cases indicating better health. The health subindices are based upon 'subjective' reports from the interviewee.

***The health questions and subindices include:***

- i) Self rated health subindex (score 4-13):*** 4 questions (e.g how would you rate your overall health at present).
- ii) Health behaviour subindex (Use of Medical Services) (score 3-9):*** 3 questions (e.g frequency of physician visits).
- iii) Self Reported Health conditions subindex (score 25-50):*** 23 item check-list of common health conditions (e.g diabetes, high blood pressure, question on eyesight and hearing and question on whether arms or legs are missing/handicapped).
- iv) Non Index Item (score 1-2):*** use of a wheel chair.
- v) Total Health Score =*** self rated health + health behaviour + health conditions + non index = 33-74

Questions were also asked on vitamin supplements and use of various health aids (cane, hearing aid etc.). Questions on ***self reported medication-use*** consisted of a 21 item check-list. The source for these questions was from the OARS questionnaire. A score was devised ranging from 21-42.

***c) Well-being***

Self reported status of well-being included questions WB11-19 and SAR101-102 in the questionnaire. A total of 7 questions WB11-17 e.g feelings of worry, depression, tiredness, loss of interest and sleeplessness. These questions were modified from the WHO Western Pacific Study (Andrews et al., 1986) and a score was created ranging from 0-7. Questions WB17a, WB18, WB19 and SAR101-102 were created for the study. These questions described contentedness with life, tendency to laugh, enjoyment of music, feeling lonely, and feelings of acknowledgement and respect by friends and relatives.

**d) Memory**

Memory was assessed with 5 questions (MA7-MA10, WB17) e.g ability to recall correct year, month and day of the week, including their address and whether they feel they forget names of people more often. Modified from the WHO Western Pacific Study, (Andrews et al., 1986) and a score created ranging from 0-5.

**e) Social and leisure activity (time use)**

Consisted of a 22 item check-list (SAR92a-u, DC23b) of ways of spending time either with others (social) or alone (leisure) e.g meetings, church, hobbies, gardening. Modified from the Multi-level Assessment Instrument and score created ranging from 22-176.

**f) Social networks (relations)**

A total of 12 questions (SAR93-102) regarding contact with friends and relatives, feelings of loneliness or degree of support. Modified from the Multi-level Assessment Instrument and WHO Western Pacific Study and score created ranging from 12-46.

**g) Activities of daily living**

Questions regarding degree of difficulty in coping with basic bodily functions (e.g using the toilet, eating) and with performing basic tasks e.g cooking, housework, walking between rooms etc (ADL88a-n2, ADL88O, ADLP). The unmodified 14 item check-list was taken from the WHO 11 country study questionnaire (Heikkinen et al., 1983) and a score created ranging from 15-62. The Euronut-Seneca study of elderly in Europe have used the same questions (Osler et al., 1991).

**h) Exercise**

A total of 2 questions (EX84, EX86) e.g how often do you go out of this house or building and how many minutes/hours spent per day/week doing various activities. Based on these questions, the subject was scored by the interviewer on a scale of 1-7. These questions and score were created for the study.

**i) Sleep**

A total of 5 questions (SL89a-d, SL89DY) created for the study e.g time of waking & sleeping, hrs/night, naps during day.

**j) Smoking & alcohol**

Questions on past and current smoking (SM90a-d) and alcohol intake (DH76a-e), created for the study.

### **3.5.2 FOOD HABITS & DIETARY ASSESSMENT**

In the absence of preexisting dietary data, the dietary method for epidemiologic and nutritional studies of adults aged 65 and over should be designed to estimate the usual food and nutrient intakes over an extended period of time, such as one year. To meet this objective, the quantitative food frequency questionnaire (FFQ) has been recommended as the method of choice (Hankin, 1989). Additionally, the FFQ method is appropriate for assessing mean group intake or in placing individuals into broad categories (i.e tertiles) of nutrient intake, or when the focus is on describing patterns of food intake.

FFQs are not appropriate where the aim is to accurately determine absolute nutrient intakes for individuals. In such cases, multiple days or weeks of diet recording would be necessary. However, many investigators have provided evidence of FFQ reproducibility and validity. Correlations of observed nutrient intakes from FFQs and from other dietary assessment methods are in the same range as those seen for repeated measures of various physiologic variables (Horwath, 1990).

In the past, researchers often included only selected food items in the FFQ that were major sources of nutrients hypothesized to be associated with risk of disease. More recently, there has been a trend toward including sufficient foods to assess total dietary intakes because of the uncertainty concerning the role of particular nutrients, non-nutrients and foods per se in the aetiology of diseases (Willet et al., 1985; Block et al., 1986).

Age and memory loss have been mentioned as possible limiting factors in the ability to recall past dietary intakes using FFQs (Dwyer et al., 1987; Krall et al., 1988). However, there is no firm evidence that long-term memory of diet is affected adversely during

ageing, particularly if the FFQ is interviewer administered and not self administered. Hankin et al (in press) recently conducted a reproducibility study among older Japanese men in Hawaii and found that those aged 70+ performed as well as those aged <70 years in recalling their past diets. Similar findings were reported by Byers et al (1983). The ability to recall past dietary intakes has been associated with the person's awareness of what is consumed than age per se. Women, who usually plan, prepare and serve the meals tend to give more accurate reports of food intake (Byers et al., 1983). Hankin (1989) suggests that if interviews with men are conducted at home, it may be helpful to have the spouse present, particularly for information on home-prepared items.

The length of the FFQ is a potential limitation. Some investigators have placed priority on designing instruments that may be completed in less than 30 minutes (Willet et al., 1985; Byers et al., 1985). These generally provide only frequency data and omit the precise information needed for evaluating nutritional status or testing associations of diet and disease. Hankin et al. (1975, 1989) needed 1.5-2 hours to administer the FFQ to Japanese men in Hawaii of all ages. In most cases, cooperation was very good. The interview was enhanced by using food photographs which enabled the subject to be an active participant during the interview.

### **3.5.2.1 Food Frequency - food intake in past year**

***The dietary method chosen for this study was based on the following objectives:***

- 1) To describe usual food intake of the whole diet (i.e not only selected foods, rich in certain nutrients) over an extended period of time e.g one year
- 2) To report on study group intakes rather than for individuals.
- 3) To obtain data on actual portion sizes with the aid of photographs

To meet these objectives, the interviewer administered quantitative FFQ (with food photographs) was the method of choice. The majority of Greek elderly had not finished primary school, therefore it would have been inappropriate to design a self administered FFQ. The quantitative FFQ from the Australian Polyp Prevention Project (Macrae et al., 1989) was adapted so that foods related to the Greek culture were encompassed (see Table 3.5.2.1 and FFQ in Appendix 2).

Popular Australian foods were retained in the FFQ so that possible changes in food habits on migration could be observed. Australian Food Composition Tables were used

(NUTTAB 1991). For most Greek dishes, the nutrient composition was obtained from Greek Food Composition Tables (Athens School of Public Health). Some Greek dishes were composed in NUTTAB using proportions of major ingredients in mixed dishes (see also section 3.7.1.2 and Appendix 3). For single ingredient foods, such as Greek cheese, the closest food in NUTTAB was taken.

The food frequency was aimed at discovering the variety and quantity of foods consumed over the past year in order to account for seasonal variation (e.g number of times per week or month or year).

The following symbols were used to indicate frequency:

1W or 2W or 3W etc.	= once a week, twice a week etc.
1M or 2M or 3M etc.	= once a month, twice a month etc.
3Y or 6Y or 12Y etc	= 3, 6 or 12 months of the year

The questionnaire consisted of a total of 250 foods. However, on many occasions it was not necessary to ask every single food item in the FFQ. For example, it was faster to ask the subject what kind of legumes they ate. For fruit and vegetables (also soups, yoghurt and ice-cream), each subject was asked to specify number of months of the year they were eaten. Since assumptions were not made about seasonality, the problems with overestimation of vegetable and fruit intake were reduced - particularly since most fruit and vegetables are available all year round in Australia and in Greece (see Appendix 4).

Pensioners with limited income tend to buy when in peak season, when the prices are lower. This is particularly relevant in Greece, where prices 'sky rocket' when fruit and vegetables are out of season and grown in green houses. Additionally, many Greeks in Australia rely solely on their own gardens for some vegetables and in the Greek culture, certain traditional vegetable dishes are associated with the seasons, for example brian (ratatouille) is eaten in summer and lahanorizo (cabbage rice) in winter. Food items consumed only a couple of times a year have little effect on the usual daily intakes (Hankin, 1989). Such foods were therefore not recorded.

Table 3.5.2.1

### Typical Greek Foods and Dishes Added to Food Frequency Questionnaire

Food/Dish Greek name	Description/major ingredients	Source for Nutrient Composition Aust. NUTTAB = A Aust. NUTTAB used to compose dishes = AK Athens School Public Health = GK
1. Stifado	Stewed rabbit - rabbit, onion, tomato, wine	GK
2. Gouvarlakia avgolemono	Mince rissoles with sauce - beef mince, garlic, bread, onion, egg, lemon, olive oil	GK
3. Pastichio	Spaghetti, beef mince, onion, tomato, white sauce (flour, margarine, milk, eggs)	GK
4. Moussaka	Eggplant, beef mince, tomato, onion, garlic, olive oil, white sauce (flour, margarine, milk, eggs)	GK
5. Souvlaki	Souvlaki - lamb, onion, tomato, pita bread	A
6. Kalamari	Squid - fried	A
7. Htapodaki	Octopus in vinegar and oil	GK
8. Spanakopita	Spinach pie - spinach, feta cheese, dill, onion, egg, olive oil, filo pastry	GK
9. Tiropita	Cheese pie - feta cheese, egg, olive oil, filo pastry	A
10. Spanakorizo	Spinach rice - spinach, rice, onion, dill, olive oil, garlic	GK
11. Prasorizo	Leek rice - leeks, rice, carrot, celery, onion, tomato, olive oil, garlic	AK
12. Lahanorizo	Cabbage rice - cabbage, rice, carrot, onion, tomato, celery	AK
13. Fasolakia prasina ladera	Green string beans, onion, tomato, potato, garlic, parsley, olive oil	AK
14. Melitzanes laderes	Eggplant casserole - eggplant, tomato, onion, garlic, parsley, olive oil	AK
15. Gemista	Stuffed vegetables - tomatoes, capsicum, zucchini, rice, onion, garlic, parsley, olive oil	GK
16. Bamies laderes	Okra casserole - okra, tomato, onion, garlic, parsley, olive oil	AK
17. Aginares avgolemono	Artichoke casserole - artichoke, peas, broad beans, onion, lemon, egg	AK
18. Briam	Ratatouille - potato, zucchini, tomato, eggplant, onion, garlic, parsley, olive oil	GK
19. Lahanodolmades avgolemono	Cabbage dolmas - cabbage, rice, onion, mince, egg, lemon, olive oil	GK
20. Dolmadakia avgolemono	Vine leaf dolmas - grape vine leaves, rice, egg, lemon, flour, onion, dill, olive oil	GK
21. Gigandes plaki	Lima bean casserole - lima beans, tomato, parsley, onion, olive oil	GK
22. Fakes	Lentil soup - lentils, onion, tomato, garlic, olive oil	GK
23. Revithia	Chickpea soup - chickpeas, onion lemon, olive oil	GK
24. Fasolada	Haricot bean soup - haricot beans, onion tomato, garlic, parsley, olive oil	GK

Table 3.5.2 - continued

## Typical Greek Foods and Dishes Added to Food Frequency Questionnaire

Food/Dish Greek name	Description/major ingredients	Source for Nutrient Composition Aust. NUTTAB = A Aust. NUTTAB used to compose dishes = AK Athens School Public Health = GK
25. Fava	Split pea soup - yellow split peas, onion, garlic, olive oil	A
26. Trahana soupa	Pasta soup - Semolina, yoghurt, onion, tomato, olive oil	GK
27. Psarosoupa	Fish soup - fish, carrot, onion, celery, potato, rice, lemon, egg, olive oil	GK
28. Kotosoupa	Chicken soup - chicken, carrot, onion, potato, rice, lemon, egg, olive oil	GK
29. Kreatosoupa	Meat soup - meat, rice, lemon, egg, olive oil	GK
30. Taramosalata	Dip - Fish roe, bread, olive oil, vinegar	GK
31. Skordalia	Dip - Potato, garlic, lemon, olive oil	GK
32. Melitzanosalata	Dip - Eggplant, vinegar, onion, parsley, olive oil	GK
33. Tzatziki	Dip - Yoghurt, cucumber, garlic, vinegar	GK
34. Bobota	Cornmeal boiled with added oil	AK
35. Horta	Wild greens/chicory/endives, boiled	A
36. Feta Tiri	Feta cheese - Australian	A
37. Kaseri Tiri	Kaseri cheese - Gouda	A
38. Haloumi Tiri	Haloumy cheese	A
39. Kefalotiri	Kefalotiri cheese - romano	A
40. Provio Giaourti	Sheep yoghurt	GK
41. Koulourakia	Sweet biscuits made with olive oil	GK
42. Kourambiedes	Shortbread with icing sugar	GK
43. Baklava	Filo pastry, nuts, honey, butter	A
44. Diples	Fried pastry with honey and nuts	GK
45. Galaktobureko	Custard, filo pastry, honey	A
46. Gliko koutaliou	Stewed quince with syrup	A
47. Halva	Tahini, honey, oil - seed/nut honey bar	A
48. Kafes Elinikos	Greek coffee - percolated coffee	A

Where relevant, foods in the questionnaire were listed in various cooked states e.g. boiled, stewed, grilled etc. For example, if a subject indicated that beef was eaten in all possible ways (boiled, casseroled, fried), then the quantity consumed was divided equally between the different cooking methods. Otherwise, if the beef was mainly boiled, then it was coded only for this cooking method and other methods were left blank.

### 3.5.2.2 Food photos & actual portion sizes

In the absence of amounts consumed, some investigators have assigned *standard portion sizes* to each item so that quantitative estimates of food and nutrient intakes may be computed (Caster, 1986; Cummings et al., 1987; Willet et al., 1985; Horwath, 1987). This procedure reduces interview time, decreases respondent burden and the cost of data collection. This practice, however, assumes either that all persons in a study consume the same amount of each food, or that amounts are highly correlated with frequencies, or that individuals do not have and cannot specify a usual portion size.

Also several researchers have compared dietary data based on standard portions with estimated portions from the same subjects. Bias has been observed; it appears that the use of standard portion size information seriously underestimates the intake of nutrients (Samet et al., 1984; Chu et al., 1984; Hankin 1989), especially retinol, carotene, vitamin C and folate (Clapp et al., (1991). It has been recommended that large-scale studies proposing to evaluate the classification of subjects intake and their risk of disease, collect *actual portion sizes* from study subjects with the aid of photographs depicting different serving sizes of foods (Hankin, 1989).

Furthermore it has been shown that the accuracy of food eaten in quantifiable terms without the aid of measuring devices, is poor (Guthrie, 1984; Rapp et al., 1986). Food photographs in conjunction with the FFQ have been used successfully by several researchers (Samet et al., 1984; Byers et al., 1985) including the large epidemiological study of dietary habits in men of Japanese ancestry in Hawaii (Chu et al., 1984; Hankin et al., 1975) and the Finnish Lung Cancer Study on 27,000 men aged 55-69 years (Pietinen et al., 1988).

***In this study, actual portion size information was obtained in:***

- a) household measures
- b) natural units of the food e.g one apple, one slice
- c) portions identified in the food photographs

Standard household measures included 'cup', 'glass', 'bowl', 'tablespoon' and 'teaspoon' which were subsequently converted to grams by referring to the standard gram conversion of these measures in the FFQ. Food items which had natural units (e.g one slice) were also converted to grams. Photos were obtained from the Athens School of Public Health for typical Greek dishes (Trichopoulou et al., 1988) and from the Australian

Polyp Prevention Project for other foods (Maclennan et al., unpub.). Of the 250 foods in the FFQ, servings of 60 foods were available in photographs (25 foods from Greece). If a photo was not available for a certain food, another photo which depicted a similar food was used e.g the photo of cheese pie could also be used for spinach pie. In the FFQ, if a food had a photograph it was labelled with an '\*' (see FFQ in Appendix 2). The food photographs included large and small servings of certain food items in the questionnaire which were hard to describe in household measures (e.g meat, cheese). A photo of the medium serving size was deliberately excluded in order to avoid biased responses towards this serving. For the majority of the foods in the FFQ, the small serving was '1/2 the medium serving' and the large serving was '1 1/2 times the medium serving'. Similar proportions for food servings have been calculated from NHANES II data (Block et al., 1986).

***There were 5 possible serving sizes when using the 2 photos for each food:***

- 1) smaller than the small serve photo;
- 2) equal to the small serve photo (1/2 medium serving);
- 3) medium or between the small and large serve photo ;
- 4) equal to large serve photo (1 1/2 X the medium serving);
- 5) greater than large serve photo.

The corresponding gram weight of the small, medium and large servings of the photographed food items were included along side these foods in the FFQ for the interviewer's convenience and for coding (see Appendix 2). Foods which had a natural unit (e.g slice of bread) or had household measures (e.g glass of milk) had the grams equivalent in the FFQ (Briggs and Wahlqvist, 1984). To avoid misunderstandings, the use of the terms small, medium and large serves were avoided. Instead, individuals were asked to indicate, using the food photos, what fraction or amount they actually ate. For example, if a subject ate half of the small serving photo, grams corresponding to this photo were halved. Where possible, the person in charge of meal preparation was also consulted to cross-reference responses to questions on food intake.

***The reference serving sizes have been based on typical servings:***

- 1) consumed by elderly Greeks living in Greece (obtained from the Athens School of Public Health)
- 2) consumed by elderly Greeks living in Australia (obtained from Australian Anti-Cancer Council).

On migration, food portions of certain foods (e.g. meat) tend to become more generous. Applying the reference servings from Greece to Australia may have led to underestimation of food intake by elderly Greek migrants. The Athens School of Public Health participated in a European study on the food habits and health status of elderly people (de Groot et al., 1992). In 1989, 33 men and 27 women aged 75 living in Markopoulo (near Spata) were studied in Greece. Typical food portions consumed by these subjects were determined using the 3 day estimated food records, which included one day of the weekend. Portion sizes were recorded in household measures and checked by weighing (Trichopoulou et al., personal communication). These portion sizes were used with the elderly Greeks studied in Spata (see Table 3.5.2.2 and FFQ in Appendix 2 for a more complete list).

**Table 3.5.2.2**  
**Differences in reference food serving sizes between elderly Greeks in Greece and Melbourne**

	<b>EURONUT study 1989</b> Athens School of Public Health N=60 medium serving (g)	<b>HEALTH 2000 pilot study, 1990-91</b> Australian Anti- Cancer Council M=64 medium serving (g)
Chicken roast/boil	170	180
Lamb/mutton roast	180	210
Beef roast/boil	120	180
Pastichio (spaghetti pie)	300	360
Fish	240	195
Spinach pie	150	170
Cheese pie	150	165
Cauliflower	200	180
Lettuce	120	95
Spanakorizo (spinach rice)	200	400
Fasolakia ladera (green bean casserole)	350	310
Bamies Laderes (okra casserole)	350	320
Briam (ratatouille)	350	320
Dolamdakia avgolemono (vine leaf dolmas)	180	270
Fasolada (haricot bean soup)	300	365
Fakes (lentil soup)	300	300
Revithia (chickpea soup)	300	280
Trahana (semolina and yoghurt soup)	250	350
Psarosoupa (fish soup)	250	330
Kotosoupa (chicken soup)	250	330
Kreatosoupa (meat soup)	250	280

The Anti-Cancer Council is conducting a 20 year longitudinal study (Health 2000) on the food habits and cancer prevalence of Greeks, Italians and Anglo-Celtic Australians living in Melbourne aged 25 and over. Weighed food records for eight days have been collected on a subsample in 1991-92 to determine typical serving sizes consumed by these ethnic groups. Data is available on typical food servings consumed by Greek Australians aged 60+ (M 37, F 26) (Giles et al, unpub. data). These portion sizes were used with the elderly Greeks living in Melbourne (see Table 3.5.2.2 and the FFQ in Appendix 2). Table 3.5.2.2 shows how portion sizes of elderly Greeks increase on migration for meat and meat/chicken soups and decrease for fish and certain vegetable dishes.

### **3.5.2.3 Food patterns & modified diet history**

The diet history method was modified so that it highlighted only the type of foods and beverages consumed across the day, including the time of eating, to establish the pattern of eating. There was no attempt to quantify foods - this was left for the FFQ. For example, questions were asked about whether a cooked meal was eaten for lunch and dinner, the number of cooked meals per day or week, whether fruit was eaten with or after meals, whether bread was eaten with meals, whether coffee, tea were drunk between meals etc.

### **3.5.2.4 Distant past food intake**

Current eating habits are relevant for assessing excess or deficient intake. The level of current consumption may be of interest as an outcome measure, in relation to subject characteristics and environment, or as a predictor of future disease risk. However, food consumption in the past may have had a greater impact on current health status than current diet. Practical and reliable methods for obtaining distant past food intake are needed.

There is a tendency for people to report their current rather than their distant past intakes. Many investigators have come to the conclusion, that a more valid method of assessing food consumption retrospectively may be found if more attention is paid to *changes* in food consumption occurring over the time period in question (Moller Jensen et al., 1984; van Staveren et al., 1986; Metzner et al., 1988; Thompson et al., 1987; Byers et al., 1987).

**The objectives for collecting distant past food intake in this study included the following:**

- a) To obtain base line information on the diet of elderly Greeks before migrating to Australia
- b) To detect major shifts in food patterns from early adulthood to later life and upon migration.

Distant past food intake was qualified by asking the subjects whether *frequency* of intake was *more, less or the same in the past*, for each food currently reported to be consumed in the FFQ. Each subject compared current intake with their diet just prior to the 2nd world war.

**This period (just prior to 2nd World War) was chosen for the following reasons:**

- a) It was a period which the subjects distinctly remembered and it was important to keep the time frame constant when interviewing each subject.
- b) The majority of subjects would have been aged in their late twenties and mid thirties; at these ages food habits begin to stabilize and the potential for chronic disease risk increases.
- c) Melbourne subjects would still have been living in Greece (there was a wave of migration in the 1950's).

The intervening period was *generally* enquired about using RAP procedures to detect food intake changes that may have occurred, for example, in the first decade after migration. Due to the qualitative nature of the data, it was not possible to express distant past intake in food or nutrient quantities.

**The following symbols were used:**

M = frequency more in the past, compared with current frequency

L = frequency less in the past, compared with current frequency

S = frequency same in the past, compared with current frequency

### **3.5.2.5 Food habits**

Questions on food habits extended individual dietary assessment beyond the frequency and quantity of food consumption. In particular, the dietary habits and practices, which could modify food consumption and could be associated with cultural and traditional values were assessed.

***The following food habits were questioned:***

1. Types of fats used in cooking and for spreading. Subjects were asked to quantify fat intake in terms of spoonfuls of oil consumed daily.
2. Fats eaten on meat and chicken.
3. Salt added to cooking and at the table
4. Preferred cooking methods
5. Cooking facilities
6. Eating environment - eating with others, eating out
7. Shopping
8. Appetite
9. Food avoidance
10. Dental status
11. Eating difficulties

**3.5.2.6 Food Beliefs**

The purpose of these questions was to get descriptive information about the past as well as prevailing food beliefs amongst Greek elderly. For example, they were asked about food intake during hardship, what foods they were fed when they were young, foods believed to be good or bad for health, and why they thought they had survived to later life and others had not.

Additionally, this information provided insight into the reasons for observed food habits and dietary choices which complemented the quantitative food intake data (Wahlqvist et al 1991b; Kouris et al 1991). By using Rapid Assessment Procedures (see section 3.5), it was not necessary to question each and every participant to get this information. It was indeed sufficient to ask these questions to certain reliable individuals and verifying information by triangulation e.g acknowledged village elders and leaders.

***The questions on food and health beliefs included:***

1. What advice would you give your children and grandchildren about how to stay healthy and live a long time?
2. Are there any foods you think are good for health? How do you know?
3. Are there any foods you think are bad for health? How do you know?
4. What traditional health practices are you aware of that can be used to treat or prevent certain illnesses, injuries? How do you know?
5. What do you think has contributed to your longevity?
6. What foods are good or bad for children? What were you fed as a child?
7. What foods are good or bad for people your age?
8. What hard times can you remember where there was a shortage of food eg war, famine?
9. What foods did you eat and for how long? How did it affect your health?. What was a typical week's food intake when you were in your early twenties and how do you think this has affected your health? Which foods have been detrimental or good for your health?

### 3.6 VALIDATION OF FOOD FREQUENCY QUESTIONNAIRE

There is no gold standard in the assessment of individual dietary intake methodology (Bingham, 1991; Block, 1982). Food records for a few days or 24 hour recalls are commonly used to estimate short-term food intake of individuals. These methods are expensive and unrepresentative of usual intake and therefore are not appropriate for the validation of long-term intake as measured by FFQ or diet histories, where the denominator is usually the 'past year' (Willett, 1990). There is a great need for independent external validation of dietary measurement techniques.

More appropriate methods for validating food intake methods, especially if measuring long-term intake, include (Black et al., 1991; Bingham, 1987; Hsu-Hage & Wahlqvist, 1992):

- a) comparison of reported energy intakes with minimal energy requirements (MER)
- b) comparison of nutrient intake estimates with appropriate biochemical markers

#### 3.6.1 REPORTED ENERGY INTAKES & MINIMAL ENERGY REQUIREMENTS

Bias in energy intake will lead to false conclusions if the aim of the study is to establish the mean population intake and the probability of malnutrition, particularly relevant in elderly studies. In the past, the incidence of undernutrition has been overestimated due to the combination of a biased (low) mean for energy and artefactual extension of the range towards low intakes (Black et al., 1991). Bias on the measurement of energy intake is likely to lead to bias on measurement of other nutrients closely correlated with total energy such as the macronutrients and B vitamins.

##### a) ***Basal Metabolic Rate***

Basal metabolic rate (BMR) is a measurement of the energy expended for maintenance of normal body functions and homeostasis, plus a component for activation of the sympathetic nervous system. BMR accounts for 60 to 75% of total energy expenditure (TEE). The thermic effect of exercise represents the cost of physical activity above basal levels and ranges from 15-30% of TEE in a moderately active individual. The thermic effect of food accounts for 10% of TEE and is the energy expended to digest, transport, metabolize and store food. The Schofield equations for predicting BMR from body weight, age and sex are shown in Table 3.6.1a (Schofield et al., 1985). The normal range of

variation in BMR is such that most healthy individuals are expected to have measured BMRs which fall within about 10% of predicted values, although the range of variation is greater in infants and elderly (Schofield et al., 1985; Warwick 1989). Shah et al. (1988), concluded that the equations could be applied to normal weight women who had never been obese, but that they overestimated BMR in a group of normal weight but 'post-obese' women. Foster et al. (1988), reported that the equations were not appropriate for moderately or severely obese patients.

Table 3.6.1a

**Equations for estimating basal metabolic rate  
in Mj/day from body weight (kg)**  
(Schofield et al, 1985)

	<b>AGE</b>	<b>EQUATION</b>
<b>MALES</b>	10-18	$(0.074 \times \text{wt}) + 2.754 = \text{BMR}$
	18-30	$(0.063 \times \text{wt}) + 2.896$
	30-60	$(0.049 \times \text{wt}) + 3.653$
	60+	$(0.049 \times \text{wt}) + 2.459$
<b>FEMALES</b>	10-18	$(0.056 \times \text{wt}) + 2.898$
	18-30	$(0.062 \times \text{wt}) + 2.036$
	30-60	$(0.034 \times \text{wt}) + 3.538$
	60+	$(0.038 \times \text{wt}) + 2.755$

**b) BMR + Sedentary activity = Minimal Energy Requirements**

The physical activity level or PAL is defined as TEE divided by BMR. Expressing expenditure as a multiple of BMR provides a useful index by which the activity level of all individuals can be directly compared. The 1985 FAO/WHO/UNU report calculated minimal energy requirements (MER) for maintenance or survival to be at least 1.27 X BMR for both men and women. This allowed for minimum movement not compatible with long term health and made no allowance for the energy needed to earn a living or prepare food. The same report used factorial calculations to estimate the **MER associated with a sedentary lifestyle to be 1.55**. This covers the cost of processes essential to life (BMR), the thermic effect of food, plus the cost of 'minimal' activity (Goldberg et al., 1991).

**Other multiples of BMR include (Black et al., 1991):**

Bed-rest 1.2	Light-moderate M 1.7, F 1.6
Very sedentary 1.3	Moderate M 1.8, F 1.7
Sedentary 1.4	Heavy M 2.1, F 1.8
Light 1.5	Very heavy M 2.3, F 2.0.
*MER 1.55	

A cut-off limit of PAL = 1.55 for MER is recommended when validating energy estimates derived from FFQ or diet histories reflecting long-term/habitual intake. A dietary method is regarded as invalid if the population mean habitual energy intake is less than the MER calculated from BMR and an activity factor (Goldberg et al., 1991; Black et al., 1991).

There appears to be conflicting data on what is appropriate for the elderly (Warwick, 1989; Vernon, 1992). As a general guide, various expert groups have judged the average energy requirement in healthy elderly men to be about 1.55 times the BMR (National Research Council, USA 1989; Dept. of Health, UK 1991).

To validate the FFQ in this study, the MER was calculated for each subject by firstly calculating BMR using the Schofield equations for the 60+ age group (see Table 3.6.1.a) and then multiplying by an activity factor of 1.55 (sedentary lifestyle) as recommended by Black et al. (1991), Goldberg et al., (1991) and WHO/FAO/UNU (1985).

Wilcoxon signed rank test was performed to examine whether or not mean estimated energy intake from the FFQ was the same as the mean expected MER (Table 3.6.1b). Subjects were also categorized into two groups; those whose energy intake estimates derived from the FFQ were higher than the expected value derived from MER, and those who had a lower estimate. Differences in energy intakes were then tested (Table 3.6.1c).

**Results:** The mean MERs were not significantly different from those estimated from the FFQ for men, but were for the women ( $p=0.05$ ). The percentage of subjects falling above and below 10% of their MER is shown in table 5.3.2.4c. There were striking similarities in the percentage of men and women in both sites above and below their MER. Overall, 40%-50% of the men and women in Spata and Melbourne fell within 10% of their MER.

About 40% of the women in both sites were below their MER when compared to energy intake estimated from the FFQ. Since the majority (two thirds) of women not meeting their MER were obese, this suggests either under-reporting or negative energy balance to lose weight. Alternatively, the PAL factor of 1.55 may be too high for elderly Greek women who are not as active as their male counterparts (mean exercise score: M 3.6; F 2.8). However, when a PAL factor of 1.4 was used, the percentage of women not meeting their MER dropped only to 30%.

Table 3.6.1b

**Comparison of mean reported energy intakes (Mj/d) (FFQ)  
and expected minimum energy requirements (BMR x 1.55)**

	SPATA		MELBOURNE	
Mean energy intake Mj/day (Kcal/day)	Reported FFQ	MER BMR x 1.55	Reported FFQ	MER BMR x 1.55
<b>MEN</b>				
N	51	41	94	92
Mean	9.3 (2211)	9.4 (2236)	10.0 (2374)	9.6 (2277)
SD	1.8 (435)	1.0 (234)	2.2 (533)	0.8 (201)
Minimum	3.8 (907)	7.4 (1775)	6.0 (1437)	6.9 (1639)
Maximum	13.6 (3246)	12.2 (2901)	20.0 (4764)	11.7 (2783)
FFQ - MER	-0.1 Mj/d (-35 kcal/d)		+0.2 Mj/d (+48 kcal/d)	
<b>WOMEN</b>				
N	53	29	95	94
Mean	7.2 (1708)	8.0 (1919)*	7.8 (1863)	8.2 (1953)*
SD	1.9 (463)	0.6 (140)	1.7 (405)	0.8 (180)
Minimum	4.0 (967)	7.0 (1677)	4.8 (1145)	6.2 (1487)
Maximum	12.0 (2841)	9.5 (2262)	12.2 (2895)	10.6 (2529)
FFQ - MER	-0.5 Mj/d (-129 kcal/d)		-0.4 (-85 kcal/d)	

\* p=0.05 Wilcoxon signed rank test comparing MER (minimal energy requirement) with FFQ (energy intake calculated from food frequency questionnaire)

Table 3.6.1c

**Reported energy intakes (FFQ) falling above and below 10% of the MER  
with reference to body mass index**

	SPATA		MELBOURNE	
	% above BMR x 1.55(+10%)	% below BMR x 1.55(-10%)	% above BMR x 1.55(+10%)	% below BMR x 1.55(-10%)
<b>MEN</b>				
N	41	41	92	92
all subjects	27%	24%	31%	25%
subjects with BMI > 28	5%	19%	13%	19%
<b>WOMEN</b>				
N	29	29	94	94
all subjects	24%	45%	21%	40%
subjects with BMI > 28	14%	31%	14%	29%

Compared to the women, half as many men (25%) were not meeting their MER, of which more than two thirds were obese. This also suggests under-reporting by obese Greek men in both study sites. Obesity was more prevalent overall in the women than the men (BMI  $\geq$ 30: F 42%, M 27%) which may explain the greater percentage of under-reporting (or attempts to lose weight by decreasing food intake) by elderly Greek women.

Overweight and obese subjects have been reported to underestimate food intake in other studies (Lansky and Brownell, 1982; Zegman, 1984).

**Comparisons with reported data:** There is increasing evidence to suggest that energy intake data tend to underestimate what is actually consumed, especially by elderly women (Moreiras et al., 1991) and obese subjects (Westerterp et al., 1991; Lichtman et al., 1991; Lissner et al., 1989; Prentice et al., 1986; Black et al., 1991). This conclusion is supported by studies of food intake observed under controlled conditions (Hallfrisch et al., 1982; Lissner et al., 1989). However, the diet history and FFQ method are less prone to underestimation (Bingham, 1987).

In the European study on 2000 elderly people aged 75 (de Groot et al., 1991) in 19 centres located in 12 countries, women in seven centres had group means below the MER (BMR x 1.4) (Moreiras et al., 1991). The chief investigators of this study (de Groot et al., unpublished data) argue that such low values suggest underestimation of energy intake. However, recent data from their institute (Wageningen Agricultural University) showed an overestimation of predicted versus measured BMR by some 9-22%. This raises questions about the applicability of the Schofield equation for use in the elderly.

Black et al. (1991) stress the importance of such validation of dietary instruments because *'it is important that the possibility of bias is acknowledged and that the data are examined with this possibility in mind and interpreted accordingly .....the common assumption that carefully collected dietary data are valid is no longer tenable... this view had propagated many misleading hypotheses and will continue to do so until corrected'*.

Black et al. (1991) provide suggestions in determining whether data collected is biased/valid: *'In any survey some individuals will be studied in a period of low intake and others in a period of high intake. This is part of the normal random variation and does not produce bias, and the mean intake should reflect the habitual intake of that group. If the overestimates cancel the underestimates, then no bias is observed and the mean intake reflects the habitual intake of that group - the error of the measurement of individual intakes, however, is increased and the range of reported intakes may be widened'*. For the Greek men (but not the women), the overestimates cancelled the underestimates thus explaining why the differences between the reported mean energy intake from FFQ and MER were not significantly different.

**When interpreting the data on elderly Greek women, the following possibilities should be kept in mind. Either:**

- a) under-reporting is operating bringing down the means for nutrient intake (actual intake > reported intake) OR
- b) negative energy balance is operating in an attempt to lose weight (actual intake = reported intake) OR
- c) the Schofield equation and the physical activity factor of 1.55 overestimate total energy expenditure (actual intake = reported intake)

### **3.6.2 REPORTED PROTEIN INTAKES & URINARY NITROGEN EXCRETION**

Urine was collected from participants in Melbourne in order to validate the food frequency questionnaire by comparing protein intake calculated from urinary nitrogen excretion to protein intake calculated from the FFQ. Urine could not be collected in Greece for technical reasons. Urine was collected over 24 hours, the day prior to blood collection, so that the urine could be brought to the hospital. Urine was collected using the following procedure: subjects were instructed to discard the first urine on the morning of collection and to start collecting from the second urine onwards till the following morning. The urine was kept in a cool place and brought to the hospital/research centre on the day of blood collection. The urine was stored at -20°C.

Urinary total nitrogen was analysed by the Kjeltex Kjeldahl technique (Tecator Ltd). Single measurements were performed at the Monash Medical Centre, Department of Clinical Biochemistry on a small subsample of Greek subjects in Melbourne (M 20, F 10). The cooperation of subjects to collect urine correctly was fraught with difficulties.

Of the 30 urine samples, only 21 were collected properly, which were subsequently used for the validation exercise (M 14, F 7). In the subsample, 71% of the women and 86% of the men were aged <80 which was similar to the age distribution of the whole sample (62% and 70% respectively).

Expected protein intake was calculated by using the following equation (Gibson, 1990) :

***estimated protein intake = (24 hr urinary nitrogen (g) + 4g faecal/dermal loss) x 6.25***

Expected mean protein intake calculated from urinary nitrogen was compared with mean reported protein intake derived from the FFQ using Wilcoxon signed rank test (table 3.6.2a). The mean protein intakes of men n=14 (110g/day) and women n=7 (94g/day) in

the subsample were not significantly different to the sample means shown in the table in brackets (M 110g/day n=94, F 84g/day n=95).

Subjects were also categorized into two groups; those whose reported protein intake derived from the FFQ was higher than the expected value derived from urinary nitrogen, and those who had a lower estimate. Differences in protein intakes were then tested (Tables 3.6.2b).

**Table 3.6.2a**

**Comparison of mean reported protein intakes (FFQ) of a subsample of elderly Greeks in Melbourne and expected protein intake (urinary nitrogen output)**

	MEN		WOMEN	
	Reported FFQ	Expected urinary nitrogen	Reported FFQ	Expected urinary nitrogen
N	14 (94)	14	7 (95)	7
Mean subsample	110.0* (110)	90.0*	94.0 (85)	84.0
SD	26.8 (11.2)	17.0	23.0 (23.4)	27.0
Minimum	74.7 (65)	58.6	73.4 (38.2)	55.2
Maximum	149.0 (188)	117.0	142.3 (160)	135.0
FFQ protein - expected	+21		+10	

\*p=0.05 Wilcoxon signed rank test; FFQ = food frequency questionnaire .

**Table 3.6.2b**

**Reported protein intakes (FFQ) of a subsample of Melbourne Greeks falling above and below their expected protein intake (urinary nitrogen)**

	MEN	WOMEN
N	14	7
% ABOVE (+10%)	57	43
% BELOW (-10%)	7	14

For the women in the subsample, the mean protein intake estimated from 24-h urinary nitrogen output was not significantly different to the mean protein intake estimated from the FFQ. In contrast, the mean protein intake estimated from the FFQ for the men was higher than that estimated from urinary nitrogen (P=0.05).

The validation of habitual protein intake using FFQ or diet history with a short term biochemical measurement such as 24 hr urinary nitrogen, has been reported to be a poor marker of long-term or habitual protein intake (Hsu-Hage and Wahlqvist, 1992). Bingham and Cummings (1985) showed that single 24 hr urine collection can be substantially in error. In healthy individuals with normal western diets, an 8-day 24 hr urine nitrogen collection was necessary to obtain habitual protein intake.

It has also been shown that individuals with a higher protein intake have a lower urinary nitrogen output than expected (Alpers et al., 1983; Bingham and Cummings, 1985). In summary, single 24 hr urinary nitrogen output is not appropriate for the individual validation of protein intake because the steady state condition is rarely achieved in free-living individuals and large day-to-day fluctuations in protein intake exist. The single 24 h urinary nitrogen output, however, provides a good 'ball-park' figure for the validation of group mean intake in the case where urinary nitrogen does not exceed the estimate of dietary intake over a short period of time (Bingham and Cummings, 1985). Therefore, the FFQ used in this study gives a reasonable estimate of group mean protein intake.

## **3.7 BIOLOGICAL MEASUREMENTS**

### **3.7.1 ANTHROPOMETRY**

Anthropometry included height, weight, electrical impedance, skinfolds, circumferences at the level of the umbilicus, gluteal maximus, and mid-upper-arm. These measurements were then used to obtain body mass index, percent body fat, percent lean mass, as well as fat distribution. To avoid inter-observer variation, these measurements were made by the candidate only (except where indicated) who had been trained by the Body Composition Laboratory at Monash University. The measurement procedures adopted match those used in the Euronut-Seneca study (de Groot et al., 1991) and those recommended by a North American Consensus Conference (Lohman et al., 1988). All measurements were made in duplicate and the means of paired values were used in analysis.

**a) Spata**

Anthropometry was performed on 70 subjects aged 70-79 (M 26, F 20) and 80+ (M 15, F 9). A trained Greek researcher measured the height and weight of each subject and the candidate measured the tricep skinfold (only one skinfold was measured), all circumferences and hip length. Equipment was not available to do electrical impedance.

**b) Melbourne**

Anthropometry was performed on 186 subjects aged 70-79 (M 64, F 59) and 80+ (M 28, F 35). The candidate measured weight, height, four skinfolds, all circumferences, hip length and electrical impedance.

**3.7.1.1 Height and Weight**

The standing height was measured using a microtoise fixed to the wall. The subject stood (without shoes) on a horizontal platform with his heels together and with the Frankfurt plane horizontal. The subject then drew himself to full height without raising the shoulders, with hands and arms hanging relaxed, with feet flat on the ground and while breathing in deeply. If subject was only seen once at home, their height was measured by asking them to stand against a wall looking straight ahead while a household measuring tape was used to measure their height. Heights were taken to the nearest centimetre.

Participants were weighed in socks, stockings or bare feet and light clothing on calibrated scales of 120kg capacity to the nearest 0.5 kg. Weight was measured before blood collection (for which the subject was fasting) or alternatively after breakfast. The body mass index was calculated as weight in kilograms divided by height squared in metres (weight kg/ height m<sup>2</sup>).

***Body fat and lean mass were estimated from:***

- 1) Skinfold measurements using the Durnin equation (Durnin & Womesley, 1974; see appendix 5)
- 2) Height, weight and age using the Deurenberg equations (Deurenberg et al. 1991b):  
*Fat free mass (FFM kg)*= 0.282\*H + 0.395\*W + 8.4\*sex - 0.144\*age - 23.6  
H=height cm, W=weight kg, sex=1 men & 0 women, age in years.

$$\begin{aligned} \text{Total body fat (TBF; kg)} &= \text{weight} - \text{FFM} \\ \text{Percent body fat (\%BF)} &= (\text{TBF}/\text{weight}) * 100 \end{aligned}$$

### 3.7.1.2 Arm circumference & arm muscle area

The following procedure was used to measure arm circumference (AC). The subject was in standing position. The left arm hung relaxed, just away from the trunk. The middle of the upper-arm was located by measuring the midpoint between the olecranon process (point of the elbow) and acromion process (shoulder) and marked with a pen. At this point, the circumference was measured twice with a tape measure to the nearest millimetre.

The arm muscle circumference (AMC) was calculated using the following formula (Gibson, 1990):

$$AMC = AC - (3.14 \times \text{triceps skinfold})$$

The arm muscle area (cm<sup>2</sup>) was calculated using the following formula (Gibson, 1990):

$$AMA = \frac{[(AC \text{ cm}) - (3.14 \times (\text{triceps skin fold mm}) / 10)]^2}{12.56}$$

### 3.7.1.3 Skinfolts

Skinfolts were measured and recorded in triplicate and averaged. A caliper with constant pressure was required (e.g Harpenden). Successive measurements had to agree within 4mm. Lean body mass and fat mass were calculated from the four skinfolts using the Durnin and Womersley tables (see Appendix 5). A modified version of these tables have been created by the Body Composition Laboratory at Monash Medical Centre to calculate lean body mass and fat mass if only the triceps skinfold is measured. This table was used for the Greeks in Spata since they only had the triceps skinfold measured (see Appendix 5). Greeks in Melbourne had all four skinfolts measured.

The following procedures were used to measure these skinfolts:

#### a) *Triceps*

The triceps skinfold thickness was measured in the posterior midline of the arm, parallel to its long axis, midway between acromial and the olecranon. The skinfold was picked up between thumb and forefinger, 1 cm above the level marked on the skin for the mid upper-arm circumference, parallel with the axial line of the upper arm. The caliper jaws were then applied exactly at the level marked for the circumference measurement. The tissue was pinched gently until only the soft tissue (fat) was between the thumb and

forefinger. The fingers were not removed even after the callipers had been applied. After the full pressure of the caliper jaws was applied, the actual measurement was read at the time the readings started to stabilize (usually about 2 seconds). This procedure was then repeated. Values were recorded to the nearest 0.2mm. Tricep skinfolds were measured in Melbourne and Spata.

**b)        *Biceps***

Following the procedure described for the tricep skinfold, the biceps skinfold was picked up on the front of the arm directly above the centre of the cubital fossa. The callipers were then applied at the same level as the triceps skinfold. The biceps skinfold was only measured in Melbourne Greeks.

**c)        *Subscapular***

Following the procedure described for the tricep skinfold, the subscapular skinfold was measured just posterior to the left scapula, or shoulder blade. The skinfold was in line from the inferior angle of the left scapula to the left elbow. This skinfold was only measured in Melbourne Greeks.

**d)        *Suprailiac***

Following the procedure described for the tricep skinfold, the suprailiac skinfold thickness was measured just above and parallel to the left superior iliac spine (hip bone). This skinfold was only measured in Melbourne Greeks.

**3.7.1.4    *Waist and Hip circumferences***

The subject was in standing position with the feet about 12 to 15cm apart with weight equally distributed between them. Subjects were in light clothing when these measurements were made. Waist circumference was measured at the level of the umbilicus to the nearest 0.1cm at the end of normal expiration, using a tape measure. The hip circumference was measured to the nearest 0.1cm as the maximum circumference over the buttocks usually at the level of the greater trochanters but not lower than the pubic symphysis (Bjontorp and Smith 1987; Lapidus et al., 1984).

The waist hip ratio was then calculated by dividing the waist circumference by the hip circumference as an index of body fat distribution i.e 'apple' or 'pear' shape.

### 3.7.1.5 Hip length

Hip length was measured in women in order to calculate loss of height and maximum height (Walqvist and Flint, 1988). The participant was in standing position. The left superior iliac spine and left knee joint space were located and the distance measured between these two points with a tape measure, making sure to measure parallel with the axial line of the left leg. This measurement was performed twice and values averaged.

*Maximum height and loss of height* were calculated using the following equations:

maximum height =  $1.096 + 1.185 \times \text{hip length (metres)}$

loss of height = max height - current height

### 3.7.1.6 Electrical Impedance

Electrical impedance was used to calculate lean and fat mass and to compare to values obtained using skin folds. A calibrated machine was used in the Body Composition Laboratory at Monash University. Electrodes were placed on the right hand and foot and the resistance and reactance recorded. These values were then entered into a computer programme to calculate percentage body fat mass, lean mass and water using the Lukaski equation (Lukaski et al., 1985).

## 3.7.2 BLOOD PRESSURE

Blood pressure was measured on 70% of subjects in Spata and 92% of subjects in Melbourne. Subjects were seated in a chair and allowed to rest for 5 minutes. Whilst seated, systolic and diastolic blood pressure were measured using a calibrated sphygmomanometer on the left arm. The measurement was repeated after 10 minutes. Readings were recorded and averaged. A larger cuff was used on obese subjects.

### a) *Spata*

Blood pressure was measured on 70 subjects aged 70-79 (M 26, F 20) and 80+ (M 15, F 9) by a trained Greek nurse.

### b) *Melbourne*

Blood pressure was measured on 183 subjects aged 70-79 (M 63, F 57) and 80+ (M 28, F 35) by the candidate.

### 3.7.3 CUTANEOUS MICROTOPOGRAPHY

As opposed to chronological age, biological age is a measure of physical age or how well the body is ageing. However, there is no evidence that a general factor of biological age exists. There appear to be many biological ages each related to a specific performance or organ. It is well known that the appearance and properties of the skin change with age. These changes are due to a combination of the ageing processes and the environment (e.g. to sun exposure). The skin, being the largest organ in the body, may be a useful index for obtaining a global measure of biological age (Steen, 1993).

A silicone rubber impression material has been utilized in Australia to measure skin texture changes in relation to sun exposure, known as skin microtopography (Holman et al., 1984). In this study the silicone rubber material was placed on areas of the body which had limited sun exposure (e.g. forearm) and on areas that had maximum sun exposure (back of hand). It was hypothesised that if the degree of wrinkling on the hand was similar in Spata and Melbourne then one could assume that sun exposure was equivalent. Any differences in the less sun exposed sites could therefore be assumed to be caused by other factors, such as ageing.

Skin microtopography was done whilst taking the anthropometric measurements. It was simple, fast and painless. Participants were told that the test showed how old their body was and were reassured that it would be painless. A skin imprint was taken of the back of the left hand (as a measure of actinic exposure) and of the palmar aspect of the left forearm midway between the wrist and elbow (as a measure of skin ageing).

The procedure used to obtain the skin imprints was as follows:

1. A silicone rubber impression material was used (Optosil Flussig, Bayer Leverkusen, West Germany) which is a viscous white liquid setting in 3-5 minutes after addition of a catalyst.
2. The silicone white liquid was poured into the small plastic holder provided to the marked line.
3. 6-10 drops of the catalyst (red liquid) was then added to the silicone liquid. The silicone would then thicken and set almost immediately.
4. The silicone was stirred quickly with the spatula provided for about 3 minutes or until it began to thicken. It was not stirred for too long because it would become hard and difficult to spread.
5. Using the spatula, the silicone was spread on the back of the left hand and on the palmar aspect of the left forearm midway between the wrist and elbow. The size of a 20 cent coin was spread on the skin.
6. When the rubber set in a couple of minutes, it was stripped slowly and steadily from the skin surface and stored in labelled envelopes. It did not cause any pain to the subjects.

The skin imprints were then graded from 1-6 by referring to published photos in the paper by Holman et al. (1984) and the accompanying table developed by Beagley and Gibson (see table 3.7.3). A dissecting microscope (x10 magnification) was used. The skin imprints were also graded by a second rater to determine inter-observer variation.

**a) Spata**

Skin microtopography was performed on 70 subjects aged 70-79 (M 26, F 20) and 80+ (M 15, F 9) by a trained Greek nurse. Two trained researchers graded the skin imprints.

**b) Melbourne**

Skin microtopography was performed on 177 subjects aged 70-79 (M 59, F 56) and 80+ (M 27, F 35) by the candidate. Two trained researchers graded the skin imprints.



**Table 3.7.3**

**The Beagley-Gibson (1980) grading of cutaneous microtopographs**

<b>Grade</b>	<b>Features</b>
1	Primary lines are all of the same depth. Secondary lines are all clearly visible, are nearly the same depth as the primaries, and often meet to form an apex of triangles ("star formation").
1.5	Stars intact but enlarged
2	Some flattening and loss of clarity of the secondary lines. Star formations are still present, but often one or more of the secondary lines making up the configuration are unclear.
2.5	Stars not intact and enlarged.
3	Unevenness of the primary lines. Noticeable flattening of the secondaries with little or no star formation.
3.5	Enlarged and more disorganized.
4	Macroscopic deterioration in texture. Coarse, deep primary lines. Distortion and loss of secondary lines.
4.5	Enlarged version of grade 4.
5	Noticeable flat skin between the primary lines. Few or no secondary lines.
5.5	Enlarged version of 5.
6	Large deep and widely spaced primary lines. No secondary lines.

### 3.7.4 BLOOD TESTS

Venous blood was collected for the following tests:

1. albumin
2. total lymphocyte count (TLC), white blood cells (WBC)  
TLC=  $\frac{\% \text{lymphocytes} \times \text{White blood cells}}{100}$
3. haemoglobin, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), haematocrit.
4. serum iron, ferritin, iron binding capacity, iron saturation
5. vitamin B12 & folate
6. total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, LDL:HDL
7. glucose

If the subject refused to give blood they were not excluded from the whole study. Blood sampling was preceded by a fast overnight (no food or drinks after 10pm, except water). In Melbourne, all blood was collected between 8-10 am at Prince Henry's Hospital or Monash Medical Centre. In Spata, blood was collected at the local community health centre. Before the venepuncture was made, the participants had been sitting for 10 minutes with their arm on the table. Subjects were asked which arm was better for collecting blood. If necessary, hand veins were used. Five and 10ml vacutainers were used to collect a minimum of 35ml of blood (4 tubes). Any left over serum was stored at -70°C for future analyses.

tube 1	10ml blood	serum----->haematology
tube 2 + EDTA	5ml blood	plasma----->haematology (kept in darkness)
tube 3	10ml blood	serum----->chemical pathology
tube 4	10ml blood	serum----->research (Lp(a), vitamin E, carotenoids)

#### a) **Spata**

Fasting blood was collected from 67 subjects aged 70-79 (M 25, F 18) and 80+ (M 15, F 9). Dr Evangelos Polychronopoulos withdrew 20ml of blood, which was immediately centrifuged and divided into three tubes to be taken to Australia for the biochemical tests. About 1ml of serum was removed to measure a) serum glucose (measured immediately after blood was drawn using the laboratory procedure 'glucose oxidase method') b) hepatitis B (stipulated by the permit to import biological specimens into Australia). The serum samples were initially kept on ice at <-4 °C for a few hours and then transferred to -34°C. The centrifuged blood was sent to a major Greek hospital to have haematological tests performed eg haemoglobin, platelet, lymphocyte counts, haematocrit.

**b) Melbourne**

Fasting blood was collected from 108 subjects aged 70-79 (M 47, F 32) and 80+ (M 14, F 15) at Prince Henry's Hospital and Monash Medical Centre. Laboratory procedures used at the hospital comply with standardised methods and are subject to strict quality control.

### **3.8 DATA PREPARATION AND ANALYSIS**

This section describes the processing of questionnaires and statistical methods used to analyse the data. All questions in the questionnaire were precoded prior to commencing the study. The majority of questions had numeric codes (e.g 1=Yes 2=No).

#### **3.8.1 DATA BASE PREPARATION**

##### **3.8.1.1 Health, lifestyle & biological measures**

The questionnaires were coded and information entered into a data management programme (DBase 3 plus).

***The Dbase files were named as follows:***

SPATAQMD.dbf	=	demographic questions, well-being, memory
SPATAQHT.dbf	=	health questions
SPATAQL.dbf	=	lifestyle, social activity and network questions
TOTSCORE.dbf	=	biological and anthropometric measurements

Each DBase file contained all study subjects. Questionnaires were entered at the end of every week to avoid back log and to minimise human error. Questionnaires from Greece were entered in Australia over a period of three months. All files in DBase were converted to SAS files using the command PROC DBF. In SAS, the Melbourne and Spata data were put into separate files (prefix OG denotes Melbourne and SP Spata).

***The SAS files were named as follows:***

OG/SPDEMO.ssd	=	demography
OG/SPHLTH.ssd	=	health
OG/SPMISS.ssd	=	wellbeing, memory, other variables
OG/SPLIFDIS.ssd	=	lifestyle and disability
OG/SPSOCIAL.ssd	=	social support and networks
OG/SPBIOAGE.ssd	=	biological age
OG/SPBIOMKR.ssd	=	anthropometry and blood tests

The frequency distribution or cross-tabulation were performed in SAS (PROC FREQ) to cross-check the data. In the case of continuous variables, attributes of the sampling

distribution were examined (PROC MEANS) to detect possible human errors induced in the data process. Data files were edited to ensure quality of data.

### 3.8.1.2 Current & distant past food intake

The questionnaires were coded and information was entered into a data management programme (DBase 3 plus):

***The Dbase files were named as follows:***

SPATAQNT.dbf	=	food habits
DIETHIST.dbf	=	diet history
SPATFOD1-9.dbf	=	food frequency questionnaire (FFQ)
NUT2.dbf	=	NUTTAB plus added Greek dishes

Questions on food habits such as appetite, eating environment, food purchase and preparation were entered into a DBase file (Spataqnt.dbf). The foods reported to be eaten in the diet history were coded as Y (=yes eaten) or N (=no not eaten) and entered into a DBase file (diethist.dbf). Dbase was also used to enter food intake data (Spatfod1-9.dbf). The FFQ contained 250 foods. Each food required 5 fields (a total of 9 DBase files were necessary to accommodate these foods):

e.g	chickenQ	chicken quantity in grams
	chickenF	chicken frequency
	chickenM	chicken months per year eaten
	chickenQWEEK	weekly quantity in grams = QxFxM
	chickenP	past intake of chicken compared to current intake M=more, L=less, S=same

The quantity of food consumed in the food frequency was entered in grams (e.g chickenQ), the frequency as a fraction of a week (e.g once a week as '1', once a month as '0.25') (e.g ChickenF), the months per year as a fraction of a year (e.g all year round as '1', 6 months of the year as '0.5') (chickenM). A programme was written in DBase to calculate grams eaten per week by multiplying : quantity x frequency x months per year (e.g ChickenQWEEK) (see Appendix 6).

Past intake of each food item was entered as M = more in the past compared to current intake, S=same in the past compared to current intake, L = less in the past compared to current intake. Nutrient intake data were obtained using the new Australian Food Composition Tables (NUTTAB 1991) which was set up firstly in DBase. To simplify SAS usage, the foods in the FFQ were renamed F1--F250 and a nuttab code allocated to each food (see Appendix 3).

Ethnic dishes recorded in the updated version of the Australian food tables (Cashel et al. 1989) include mainly foods served in restaurants and based on popular recipes in Australian cookbooks, which were found to differ from traditional recipes. Therefore, Greek dishes were composed in NUTTAB by the candidate using proportions of major ingredients in mixed dishes (see Appendix 3). Alternatively they were taken from Greek Food Composition Tables (Athens School of Public Health, Department of Nutrition & Biochemistry, Trichopoulou et al., 1988) (see table 3.5.2.1).

For single ingredient foods, such as Greek cheese, the closest food in NUTTAB was taken. The nutrient values of these foods were inserted directly into NUTTAB in DBase. The data source for most foods in the Australian food composition tables come from analysis of Australian foods, otherwise they have been taken from United Kingdom McCance and Widdowson food composition tables (Paul and Southgate, 1978). The food composition tables from Greece have also used United Kingdom food tables when analysing typical Greek recipes. All files in DBase were converted to SAS files using the command PROC DBF. In SAS, the Melbourne and Spata data were put into separate files (prefix OG denotes Melbourne and SP Spata).

The sas files were named as follows:

OG/SPFDHBT.ssd	=	food habits
OG/SPDIET.ssd	=	diet history
OG/SPFDDAY.ssd	=	grams/day of each food (a total of 250 foods)
OG/SPNUT2.ssd	=	NUTTAB plus added Greek dishes
OG/SPPAST.ssd	=	distant past food intake

The frequency distribution or cross-tabulation were performed in SAS (PROC FREQ) to cross-check the data. In the case of continuous variables, attributes of the sampling distribution (PROC MEANS) were examined to detect possible human errors induced in the data process. Data files were edited to ensure quality of data. The food intake file in SAS (OG/SPFDDAY.ssd) was converted to nutrients using the NUTTAB file (NUT2.ssd) with the command PROC TRANSPOSE AND PROC SCORE (see Appendix 7).

## 3.8.2 DATA ANALYSIS

### 3.8.2.1 Health & lifestyle scores

Apart from the general health score (Lawton et al., 1982), all other scores were developed for the study. All scores are obtained by summing numbers in front of responses, and in all cases, a higher score was a better score. Judgements have not been made on the importance of various questions over others i.e weighting of questions comprising a score has not been performed. The relative importance of one question over another may be different upon migration, so in order to retain robustness, it was not deemed appropriate to have 'weighted' scores. Programmes were written in DBase to calculate the various scores (health, medication, memory, well-being, exercise, activities of daily living, social activity and social networks) (see Appendix 8 and section 3.5.1).

The health and lifestyle scores can be summarised as follows:

1. **Total Health score (33-74)**
  - a) **Self rated health subindex (score 4-13):** Questions H34, H35, H36, H37 (e.g how would you rate your overall health at present, is your health better, same or not as good as people your age).
  - b) **Health behaviour subindex (score 3-9):** Questions H38, H39, H40 (e.g frequency of physician visits, days spent in hospital, days spent in bed because of illness).
  - c) **Self Reported Health conditions subindex (score 25-50):** Question H43 (23 item check-list of common health conditions e.g diabetes, high blood pressure), question H41 and H42 on eyesight and hearing and question on whether arms or legs are missing/handicapped (H46).
  - d) **Non Index Item (score 1-2):** use of a wheel chair (H47c).
  - e) **Total (General) Health Score = self rated health + health behaviour health conditions + non index item = 33-74**
2. **Self reported medication score (21-42)**  
Question H44 - 21 item check-list.
3. **Memory score (0-5)**  
Questions MA7, MA8, MA9, MA10, WB17 e.g ability to recall correct year, month and day of the week, including their address and whether they feel they forget names of people more often.

4. **Wellbeing score (0-7)**  
 Questions WB11, WB12, WB13, WB14, WB15, WB16, WB17A e.g feelings of worry, depression, tiredness, sleeplessness, contentedness with life.
5. **Activities of daily living score (15-62)**  
 Question ADL88a-n2, ADL88O, ADLP i.e degree of difficulty with coping with basic bodily functions and with performing basic tasks e.g using toilet, eating, walking between rooms etc. For each item, the level of competence was measured on a 4-point scale. Grades of difficulty were assigned to categories defined in terms of the ability to perform an activity (without difficulty, with difficulty but without help, with help only, unable to complete). A total ability score (ADL) was calculated as a sumscore over all items; higher score indicating better performance.
6. **Exercise score (1-7)**  
 Questions EX84 (e.g how often do you go out of this house or building), and question EX86 (e.g how many minutes/hours spent per day/week doing various activities). Based on the answers to these questions the subject was scored by the interviewer on a scale of 1-7 (see figure 3.8.2).

**Figure 3.8.2**

**Grading of the Exercise score**

**What exercise score would you give the subject from 1 to 7 based on the answer to EX86?**

- 1 = inactive, in bed or seated all day
- 2 = inactive, seated most of the day
- 3 = inactive, seated most of day with few hours of pottering
- 4 = active, walks or gardens (about 1 hr) or does few hours house work at least 3-4 times/week, on feet most of day
- 5 = active, walks or gardens (about 1 hour) or few hours of housework daily and on feet most of day
- 6 = active, heavy gardening or farming or plays aerobic sport or few hours walking 3-4 times a week
- 7 = very active, heavy gardening or farming or plays aerobic sport or few hours walking daily

7. **Social activity or time use score (22-176)**  
 Questions DC32B (currently working), SAR92a-u (21 item check-list of ways of spending time e.g meetings, church, hobbies).
8. **Social networks score (12-46)**  
 Questions SAR93, SAR94, SAR95, SAR96, SAR97A-D, SAR98, SAR100, SAR101, SAR102 (e.g contact with friends and relatives, feelings of loneliness and respect by family, and degree of support).

## 9. **Later life status score (0-80)**

For multivariate analyses, all the lifestyle and health scores were standardised (i.e each score was divided by its highest score and X 10) so that they all had a common denominator (10). These scores were then summed to form the Later life status score which ranged from 0-80. A higher score representing better 'quality of life'.

**Later life status score (standardised)** = *memory score + wellbeing score + general health score + medication score + activities of daily living score + exercise score + social activity score + social networks score = 0 - 80*

### 3.8.2.2 **Food groups**

#### **A. Specific food groups**

The 250 foods in the FFQ were collapsed into 54 food groups according to their biological source (Briggs and Wahlqvist, 1984). The aim of this grouping was to retain some detail about the types of foods consumed but making it more manageable for analysis (see Appendix 9). Similar types of foods were grouped together, but still retaining major characteristics of the food.

For example, milk, cheese and yoghurt were separated (although they are all dairy products) to avoid grouping solids with liquids. Mixed vegetable dishes were grouped separately (mixed vegetable dish and vegetable and rice dish). The 'flower' vegetables (cauliflower and broccoli) were grouped together. These food groups were then reported as grams/day (percentile distributions).

#### **B. Broad food groups**

The 54 food groups were collapsed in to 10 broad food groups. The purpose of this grouping was to lose the detail of types of foods consumed in order to get a broader picture. For mixed dishes, the main ingredient in the dish was used for grouping. For example, the mixed dish spinach rice 'spanakorizo' was grouped under 'vegetables' and not 'cereal' since spinach was the main ingredient and not rice. Eggs, water, tea, coffee, alcohol and fats were not grouped but kept separate.

**The broad food groups included the following** (see Appendix 9):

1. **Meat group** - included beef, lamb, chicken, turkey, game, bird, rabbit, pork, offal, processed meat, chicken/meat soups.
2. **Fish group** - fish, shellfish, fish soup, fish roe dip.
3. **Dairy group** - milk, cheese, yoghurt, custard, milk puddings, custard pastry
4. **Vegetable group** - all vegetables, garlic, olives, including mixed vegetable and rice dishes (e.g moussaka, spinach rice casserole, eggplant and garlic/potato dip) and nuts.
5. **Legume group** - all legume soups, salads and casseroles, chickpea felafel, green peas and split peas.
6. **Cereal group** - bread, rice, noodles, pasta (including mixed pasta dishes like pastichio, lasagna), breakfast cereals, polenta, trahana (flour and sour milk pasta), cakes, sweet and dry biscuits.
7. **Fruit** - all fresh and dried fruit
8. **Alcohol** - beer, wine, spirits, liqueurs
9. **Sugar products** - all foods where sugar is major ingredient e.g softdrinks, juices, sugar, jam, honey, confectionery, jelly, halva (tahini paste and sugar), chocolate, Turkish delight.
10. **Fats** - butter, margarine, oils, peanut butter, tahini paste.

Of the 10 food groups, 7 were further collapsed into **2 very broad groups** (Appendix 9):

1. ***Animal foods group*** - meat + fish + dairy
2. ***Plant food groups*** - vegetable + legume + cereal + fruit

Food groups were reported as percentile distributions:

- a) grams/day
- b) calories consumed per day from each food group
- c) ratio plant:animal foods

### **C. *Traditional foods***

In order to determine the degree of acculturation upon migration or 'westernization' of the Greek diet, 70 foods were identified as being 'traditional' or culture specific foods (see Appendix 10). The grams/day (percentile distribution) consumed of each of these foods was reported separately.

### **3.8.2.3 Food scores**

#### **A. *Food group variety and total food variety***

Previous studies of food variety have determined a variety score representing the total number of foods consumed in a given time period (Reid and Miles, 1977; Kronl et al., 1982; Horwath, 1987; Wahlqvist et al., 1989; Hage, 1992; Hodgson et al., 1991; Hodgson et al., in press). Variety indices for specific food groups or nutrients have also been used (Fanelli and Stevenhagen, 1985; Randall et al., 1985; Horwath 1987). For this study, variety scores were computed for the broad food groups and for the diet as a whole. Food group variety scores were constructed in order to describe variety of foods consumed within a food group (see Appendix 11). The broad food groups described in section 3.8.2.2b were used. Alcohol and fats were collapsed into one group 'other foods variety score', giving a total of 9 food group variety scores.

#### ***Food group variety scores:***

1. *Meat variety score (0-24/month)* - consisted of 24 foods from the meat group.
2. *Fish variety score (0-19/month)* - consisted of 19 foods from the fish group.
3. *Dairy variety score (0-30/month)* - consisted of 30 foods from the dairy group.
4. *Cereal variety score (0-34/month)* - consisted of 34 foods from cereal group.
5. *Vegetable variety score (0-48/month)* - 48 foods from vegetable group.
6. *Legume variety score (0-13/month)* - consisted of 13 foods from legume group.
7. *Fruit variety score (0-33/month)* - consisted of 33 foods from the fruit group.
8. *Sugar products variety score (0-17/month)* - 17 foods from sweets group.
9. *Other foods variety score (0-20/month)* - consisted of 20 foods from the alcohol and fats group, including tea, coffee and water.

### **Broad food groups and total variety scores:**

1. *Animal variety score* (1-73/month) : meat + fish + dairy variety
2. *Plant variety score* (1-128/month) : vegetables + legumes + fruit + cereals
3. *Total food variety score* (1-238/month) : animal + plant + 'other' food score

The sugar products and other foods variety score were not included in the plant and animal variety scores. A *medium serving* of a food or mixed dish within a food group had to be consumed at least *once a month* or more to score. Since all foods had been converted to grams/day, a new SAS file was created (og/spfdgrp) and foods were converted to grams/month by multiplying each food by 30 (30 days). For multivariate analyses, the food group variety scores were standardised (i. each score was divided by its highest score and x 10) so that they all had a common denominator (10). These scores were then summed to form the total food variety score which ranged from 0-90.

<b><i>Total food variety score (standardised)</i></b> = meat variety + fish variety + dairy variety + cereal variety + vegetable variety + legume variety + fruit variety + sweets variety + other foods variety = 0-90/month
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### **B. Traditional food score**

The 70 foods described in section 3.8.2.2C (see also Appendix 10) were used to construct a traditional food score (0-70). If the food was consumed at least once in a year in any quantity, then it received a score of 1.

#### **3.8.2.4 Nutrient intake**

Nutrient intake data was expressed in percentile distributions for absolute amounts, as percentage of total energy intake (mainly macronutrients), nutrient density (per megajoule) and percentage achieving two thirds of the recommended dietary intakes (see Appendix 7 for SAS programmes). For the nutrient conversion of coffee beverage, 10g of coffee powder was used and 200ml water added for instant coffee and 50ml water for Greek coffee. Fat intake was quantified by asking subjects to estimate the number of tablespoons of oil consumed daily which was added to food at the table, or eaten with bread or in salads. Oil added to cooking was not to be included in their estimation. However, elderly subjects found it difficult to separate oil consumed

in cooking and at the table, and tended to give an 'all inclusive' answer to oil intake. Since most of the Greek dishes in the Food Composition Tables included oil, this would have resulted in an overestimation of fat. Therefore, 70% of a tablespoon was taken to prevent overestimation.

### 3.8.3 STATISTICAL METHODS

#### 3.8.3.1 Descriptive analysis

The Statistical Analysis System (SAS, 1993) was used for statistical analyses. The descriptive analysis is used to report sampling distribution and its attributes for confounders, antecedent factors, and outcome variables for men and women. SAS procedures included PROC FREQ (frequency and percentage for discrete variables) and PROC UNIVARIATE (mean, standard error of means, range and percentile distribution) for continuous variables (see Appendix 12 for programmes written in SAS).

Percentile distribution included the following: 5%, 10%, 25%, 50%, 75%, 90%, 95%. Discrete variables included yes/no answers and fixed responses in the questionnaire. Continuous variables included food and nutrient intake, all scores (health, lifestyle, food) and biological measures. The results are presented by gender for two age groups: the young elderly aged 70-79; old elderly aged 80+. Even though the number of observations are small for some variables, particularly for Spata Greeks, statistically it still warranted the separation of these age groups as they appeared to show quite different trends which would not have been obvious if results had been reported collectively for 70+.

**For continuous variables**, nonparametric statistics, namely Wilcoxon rank sum test (PROC NPAR1WAY WILCOXON see Appendix 12) was used to look for:

- a) gender differences by age group, denoted in the tables by the superscript a,b,c or d
- b) age group differences by gender, denoted in the tables by the superscript e,f,g or h
- c) significant differences between centres by age group and gender, denoted in the tables by the superscript i,j,k or l.

A summary of significant results is presented beneath each table.

**For discrete variables**, Chi-Square (PROC FREQ/ALL see appendix 12) was used to test the significance of differences in question responses between gender, age groups

and centres. Rather than identifying the significance of a particular response, this test identified an *overall or general association* of question responses. For simplicity, superscripts were not used in the table; significant findings were summarised below the table. The significance level reported in the tables was set at 5% ( $p < 0.05$ ).

### **3.8.3.2 Univariate and Multivariate Analyses**

The aim of these analyses was to determine the interactions between food culture, health and lifestyle and to identify food and nutrient predictors of later life status. A later life status score was created which encompassed all facets of an elderly persons life including health, social activity, exercise, disability, medication, well-being and memory. The lifestyle and health scores were standardised to produce the standardised later life status score for use in multivariate analyses (see Chapter 12). Regression techniques allowed examination for the relationship between outcome variables and antecedent factors, adjusting for confounders. Regression analysis, particularly step-wise regression techniques, was used to determine food and nutrient predictors of later life status (see Appendix 12 for programmes written in SAS).

### **3.8.3.4 Statistical Conventions**

#### **a) Significance levels**

The significance level is set at 5% ( $p < 0.05$ ), unless otherwise specified: \*\*\*\*\*,  $p < 0.00001$ ; \*\*\*\*,  $p < 0.0001$ ; \*\*\*,  $p < 0.001$ ; \*\*,  $p < 0.01$ ; \*,  $p < 0.05$ ; NS, not significant

#### **b) Abbreviations**

The following statistical abbreviations have also been adopted: SD or sd, standard deviation; SEM or sem, standard error of the mean; N or n, number of subjects

### **3.8.4 REPORTING TEST RESULTS TO THE PARTICIPANT**

Results of anthropometry, blood pressure and blood tests were mailed to the subject; normally two weeks after the interview. Participants were instructed to show these results to their doctor for interpretation.