APPENDICES

IUNS STUDY PROTOCOL AND QUESTIONNAIRES

INTERNATIONAL UNION OF NUTRITIONAL SCIENCES

FOOD HABITS IN LATER LIFE- A CROSS CULTURAL STUDY

1. GENERAL DESCRIPTION

1.1 INTRODUCTION

There is currently a great deal of concern regarding the aged because it has been estimated that by the year 2000 there will be an increase from 258 to 396 million in the elderly population (>65 years) of the world, with the life span increasing by as much as 25%. Relatively more of these will be in developing countries. This phenomenon has created massive social, political and economic problems due to associated high risk of morbidity, disability and need for medical services.

To date, most studies on the elderly have been descriptive in nature with the purpose of providing information on the health and functional ability of the elderly and their use of health services. Little information is available regarding the food habits of elderly people in different countries and the relationship of such life long eating habits to longevity and health in later life. Such information would make an invaluable contribution to the formulation of national health policies for the public at large so that their longer years can at least be spent in relatively greater health, allowing a continued active role in society.

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 to test key hypotheses in relation to food habits and health status in the elderly in developed and developing countries.

1.2 OBJECTIVES

An important rationale for cross-cultural collaboration lies with trying to study food cultures that are as disparate from each other as possible because such variations in dietary patterns may

influence life expectations and morbidity and mortality patterns in the elderly. Current dietary recommendations suggest that there is one certain way of eating which is healthy and desirable. Cross-cultural studies of this nature may indicate that there are a number of desirable food patterns conducive to good health.

The general objectives of the IUNS study are both descriptive with regard to the food habits, health status and lifestyle of the elderly as well as hypothesis generating and testing by looking at relationships between nutritional and non-nutritional variables. To meet these objectives the study will be performed in population groups with different dietary patterns in different geographical settings in developed and developing countries. Research will be focused on obtaining both nutritional and non-nutritional information, and looking at the relationships between health, food and lifestyle. The study has a cross sectional design. However, a 5 year mortality follow-up study will also be performed.

1.3 SIGNIFICANCE

Such a cross-cultural study will:

- make available background information about nutrition and the elderly
- advise on possible dietary guidelines for the elderly and their younger peers in disparate cultures and to contribute to national health policies
- further research on nutrition and ageing
- develop methodology to define health-food-lifestyle relationships which can be applied in different communities to allow for community specific programs

1.4 AIMS

1) To describe health status, lifestyle and the range of food habits (present and past) among the aged in developed and developing countries.

2) To determine to what extent food habits and lifestyle variables (social activity, social networks, exercise, activities of daily living, mental function, well being, etc.) predict health status in the elderly.

1.5 HYPOTHESES

1) That people following quite different food cultures could achieve comparable levels of health.

2) That a given health status would be accounted for better by food intake descriptors than by nutrient descriptors of nutritional status.

3) That there would be offsets within and between cultures in components of lifestyle which in aggregate would produce comparable health status.

4) That a community with a greater variance in food intake would have better health status than one with little variance.

1.6 DESIGN

1) Cross-sectional ——> Mortality follow-up after 5 years

2) Subjects 70 years and over, 100 men and 100 women

3) Elderly subjects are not representative for country but only for community being studied.

4) Subjects are randomly selected from telephone directory, register, electoral roll or all elderly in the community are taken. The method chosen will depend upon community being studied.

5) Interviewer administered questionnaire (health, lifestyle, food habits), 1-2 home visits, 2-3 hours in total needed to complete interview.

6) Perform anthropometry, blood pressure and blood collection (40 ml) at a second visit (at hospital, or local health centre).

1.7 SUBJECTS

Representative samples obtained and cell sizes of about 100 men and 100 women, 70 years and over, in each location. Where this is not possible, the sample will be the corresponding percentage (upper decile) of the age pyramid. This problem will be encountered in communities with low life expectancies. The feasibility of cross-national comparison, based on biological and chronological age, will also be investigated.

In principle all elderly subjects 70 years or older are eligible except for:
- psycho-geriatric patients in nursing homes
- (foreign) people not fluent in the country's language
- people that are not able to answer questions independently

A participation rate of at least 60% is desirable. The total number of addresses in the sample must amount to 340 (170 men, 170 women). Taking non-eligibility and non-participation into account, a number of about 200 subjects (100 men , 100 women) will remain in the study population. The lower limit is set at 100 subjects (50 men, 50 women).

1.8 QUESTIONNAIRE

For all variables, scores have been developed to give a global picture for that particular variable for each subject. These scores will also be used in statistical analysis. Apart from the health score, all other scores have been developed for the study and need to be validated. In all cases a higher score is a better score. All scores are obtained by summing numbers in front of responses (see Questionnaire in Appendix).

1.8.1 Demography

Demography includes education, work, income, rural/ urban background, living arrangements.

1.8.2 Health

The Multi-level Assessment Instrument [1] has been recommended as one of the most valid and reliable measures of the health status of elderly people [2]. It includes a 30 item check-list of common self-reported health conditions in the elderly, as well as 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 was 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 indicates better health. However, the health subindices are based on "subjective" reports from the interviewee i.e. self reported health conditions.

(i) Self rated health subindex (score 4-13): 4 items (e.g. how would you rate your overall health at present).

(ii) Health behaviour subindex (Use of Medical Services) (score 3-9): 3 items (e.g. frequency of physician visits).

(iii) Self Reported Health conditions subindex (score 25-50): 23 item checklist 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 item = 33-74

(vi) Self Reported Medication Use (score 21-42): 21 item checklist;

Source: OARS questionnaire [3].

Questions are also asked on vitamin supplements and use of various health aids (cane, hearing aid, etc.).

1.8.3 Well-being

A well-being score (score 0-7) was developed for the study. It included 7 questions e.g. feelings of worry, depression, tiredness, sleeplessness, contentedness with life. Source: Modified from WHO Western Pacific Study [4].

1.8.4 Cognitive function & memory

Cognitive function is assessed using the mini-mental state questionnaire e.g. serial 7's, write a sentence, repeat statement "no ifs, ands or buts" [5]. This test may not be used in all centres because it presumes a certain level of education and literacy which does not make it cross-culturally robust. Alternatively, memory can be assessed (score 0-5) with 5 questions 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. Source: Modified from WHO Western Pacific Study [4] and score created .

1.8.5 Food habits

1.8.5.1 Food Frequency Questionnaire

The food frequency questionnaire (FFQ) is aimed at discovering the variety and quantity of foods consumed over the past year (e.g. number of times/ week/ month/ year). If a certain food is eaten at certain times of the year, this will be recorded as months/ year eaten. Food intake will be quantified mainly in the food frequency with the aid of food photos and reference portion sizes.

Foods/ dishes that are culture specific and not already listed in the FFQ should be added along with their typical serving sizes. The list of foods and reference serving sizes used for the study of elderly Greeks in Greece and Australia are included in the Questionnaire in the Appendix as an example. This FFQ was adapted, using Rapid Assessment Procedures [6] from a core list of biologically distinct foods (see Table A1.1) to incorporate all Greek foods and dishes most likely to be consumed by elderly Greeks. The serving sizes used also reflect typical servings of these foods consumed by these communities.

Food photographs depicting different serving sizes of Greek foods were also developed for use in these communities [7].

1. SMALL SERVE OF SPINACH RICE

2. LARGE SERVE OF SPINACH RICE

3. SMALL SERVE OF PASTICHIO (SPAGHETTI, MEAT, WHITE SAUCE)

4. LARGE SERVE OF PASTICHIO (SPAGHETTI, MEAT, WHITE SAUCE)

5. SMALL SERVE OF BOILED CHICORY

6. LARGE SERVE OF BOILED CHICORY

The reference serving sizes should be used by the interviewee to determine their serving size, by referring to either the household measures or to the food photographs. Fat is quantified by asking weekly household consumption of oil/ butter/ margarine and whether fat on meat and chicken is eaten.

Table A1.1 Biologically distinct foods used in the development of the Food Frequency Questionnaire [8].

Animal	Plant
	Vegetables
Eggs	Root, white (potatoes, turnip)
Milk(plain, flavoured custard)	Root, yellow (carrots, pumpkin)
Cheese (all types)	Dark Leafy (spinach, silverbeet)
Yoghurt (all types & desserts)	Light Leafy (lettuce, cabbage)
Fish (sea, fresh)	Marrow (zucchini, cucumber)
Shellfish (mussels, oysters)	Flowers (broccoli, cauliflower)
Crustaceans (prawns, lobster)	Stalks (celery)
Ruminants (sheep, cattle)	Onion-like (spring, brown, leeks)
Monogastric (pig)	Parsley
Poultry (chicken, duck, turkey)	Tomato (fresh,
Game (rabbit, bird, kangaroo)	Peppers (capsicum, chillies)
Giblets (heart, kidneys)	Legumes (peas, dried beans, lentils)
Liver, brain	Fungi (mushroom)
	Yeast (vegemite)
	Cereals & Grains
	Wheat cereal (weet bix)
	Corn (cornflakes, polenta, oil)
	Barley (bread, barley cereal)
	Oats (porridge, oat cereal, bran)
	Rye (bread)
	Bread (white wheat flour)
	Bread (wholemeal wheat flour)
	Pasta (all types)
	Rice (grain, bran)
	Cake, pastry, biscuits
	Seeds (whole, oil, margarine)
	Nuts (whole, oil, peanut butter)
	Fruit
	Citrus (oranges, lemons, juice)
	Tropical(mango, banana, melon)
	Stone (peach, cherry, plums, juice)
	Apples (whole, juice)
	Pears
	Berries (strawberries)
	Grapes
	Olives (fruit, oil)

1.8.5.2 Validation

About 20% of the subjects will be asked to collect 24 hour urines in order to measure total nitrogen excretion and thus protein intake as estimated from the food frequency questionnaire (FFQ). The FFQ should also be validated by comparing reported energy intake from the FFQ with minimal energy requirements (MER) calculated from basal metabolic rates (BMR) and the physical activity level (PAL) (MER=BMR x PAL). The MER is calculated for each subject by firstly calculating BMR using the Schofield equations for the 60+ age group: Men (0.049 x weight) + 2.459; Women (0.038 x weight) + 2.755 [9] and then multiplying by an activity factor of 1.55 (sedentary lifestyle for elderly) [10].

1.8.5.3 Dietary History

The dietary history is brief and not quantitative, aimed at highlighting the type of foods eaten across the day, including the time of eating .

1.8.5.4 Past Food Intake

Due to difficulties associated with remembering frequency of consumption of particular foods in the distant past, past food intake will be qualified by asking the subject whether they are eating more, less or the same amount of each food currently reported to be consumed in the food frequency, compared to a specific point in time in earlier life (i.e. just prior to the second world war). This method has been successfully used by others [11]. This method does not assess past intake in precise quantities. However, less precise methods which locate individuals on the distribution in broad categories of low, medium, and high intake still permit the examination of nutritional hypotheses.

1.8.5.5 Food Beliefs

The elderly are 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 think they have survived to later life and others have not.

1.8.5.6 Other

Cooking methods & facilities, eating environment, shopping, appetite, food avoidance, dental status, eating difficulties.

1.8.5.7 Food intake analysis

Food intake data will be described in terms of:

a) Foods - absolute intake; total plant & animal food intake; consumption of different food

types e.g. fruit (total), vegetables (total), meat, cereals etc.

b) Nutrients - absolute intake; % achieving Recommended Dietary Intakes

c) Food variety scores

d) Traditional foods score - (e.g. number of times traditional foods/ dishes are eaten)

1.8.6 Lifestyle

1.8.6.1 Social Activity

Social activity (time use) was described using a 22 item checklist of ways of spending time e.g. meetings, church, hobbies. Source: Modified from Multilevel Assessment Instrument [1] and score created (score 22-176).

1.8.6.2 Social networks

Social activity (relations) was described with 12 questions regarding contact with friends and relatives, feelings of loneliness or degree of support. Source: Modified from Multilevel Assessment Instrument [1] and score created (score 12-46) .

1.8.6.3 Disability

The level of disability is measured using 14 questions on Activities

of daily living (ADL) 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. Source: Adapted from the WHO 11 country study [12] and Euronut-Seneca study [13]; ADL score created (score 15-62) for measuring degree of disability. These questions were originally adapted from the validated instrument developed by Katz & Apkom [14].

1.8.6.4 Exercise

The level of exercise was described with two questions 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 and based on this answer subject is scored by the interviewer on a scale of 1-7. Source: nil; score developed (score 1-7).

1.8.6.5 Sleep

A total of 5 questions created for the study e.g. time of waking & sleeping, hrs/ night, naps during day.

1.8.6.6. Smoking & alcohol - past and current

Questions on past and current smoking and alcohol intake created for the study.

1.8.7 Biological

1.8.7.1 Anthropometry

- weight; height; body mass index (BMI)
- hip circumference (largest diameter below the umbilicus or maximal gluteal circumference)
- waist circumference (taken at level of umbilicus)
- waist hip ratio to describe body fat distribution
- mid arm muscle area (using mid upper arm circumference and triceps skin fold)
- triceps, biceps, suprailiac, subscapular skin folds to obtain percentage body fat and lean mass
- hip length from anterior superior iliac spine to knee joint space to calculate loss of height in women [15].
- electrical impedance to obtain percentage body fat and lean mass

1.8.7.2 Blood Pressure

Systolic and diastolic blood pressure to be measured using a calibrated sphygmomanometer.

1.8.7.3 Blood Tests

Where possible, fasting blood samples to be taken from allindividuals in the study and analysed by standardised laboratories that conduct quality control.

1. Haemoglobin (MCHC, MCH, HB), platelets, haematocrit
2. Immune function - total lymphocyte count (% lymphocytes x white blood cells/ 100)
3. total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, LDL: HDL, Lp(a)
4. Glucose & fructosamine
5. Kidney function (urea, creatinine)
6. Serum iron, ferritin, iron binding capacity, iron saturation
7. Liver function (AST, ALP, GGT, BILI)
8. Serum sodium, potassium, calcium, phosphate, bicarbonate
9. Albumin and total protein
10. Vitamin B12 & folate
11. Carotenoids and retinol
12. Vitamin E (vitamins)
13. Uric acid
14. Dehydroepiandrosterone sulphate (DHEA SO4)

1.8.7.4 Biological Age

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. Plasma concentration of dehydroepiandrosterone sulphate (DHEAS) declines quite drastically in both men and women after the age 50, to values at age 70 years that are 20% of those at age 20 years. DHEAS is measured with radioimmunoassay from venous blood. DHEA levels have been recommended as good biomarkers of ageing [16,17].

A silicone rubber impression material has been utilised in Australia to measure skin texture changes in relation to sun exposure, known as cutaneous microtopography [18]. It is hypothesised that if this silicone rubber material is placed in areas of the body which have limited sun exposure (e.g. forearm) and actinic damage accounted for by comparing to sun exposed sites (back of hand), then any changes that have occurred in the former are due mainly to the natural ageing process rather than accelerated ageing caused by the sun.

1.9 STATISTICAL ANALYSES

a) Descriptive

Discrete variables e.g. yes/ no answers, fixed responses (questionnaire) - expressed as %

Continuous variables e.g. food & nutrient intake, health & lifestyle & food scores, biological measures - expressed as percentiles (5%, 10%, 25%, 50%, 75%, 90%, 95%), means, min-max, SEM, SD.

b) Multivariate Analyses

$y = a + bx1 + cx2 + dx3 +$ _____

health, food & lifestyle

Which lifestyle and food variables can explain/ contribute to the variance of Y (health)?

Continuous variables (e.g. scores) or discrete variables (e.g. individual questions) can be tested in this equation.

Example of model to be tested:

Dependent variable y = total health score or health subindices e.g. self rated health or well

		being score or skin test grade (biological age) or Dehydroepiandrosterone(biological age)
Independent	=	food intake (variety, grams, traditionalfood)
Variables x 1, 2, 3 etc.	=	lifestyle variables (social activity, social networks, ADL, exercise, memory)

1.10 RAPID ASSESSMENT PROCEDURES

At first, especially with non-literate communities, a general anthropological methodology may be required, as described by Scrimshaw and Hurtado [6]. Group inquiry may be more valuable than individual inquiry at this stage, especially with certain groups e.g. Australian Aborigines [19] and when developing the food frequency questionnaire (FFQ) for the community being studied. RAP can be very useful when developing the FFQ because it enables the interviewer to become familiar with local menus, standard recipes and commonly eaten foods [20]. A questionnaire approach for individuals may later be applied. RAP can also be very useful when trying to describe common food and health beliefs in the community being studied, (e.g. focus groups; triangulation by interviewing key elderly subjects, community leaders, health workers etc.) [21].

1.11 GENERAL INFORMATION ON THE COMMUNITY

General descriptive information should be obtained on the community and include the following if available using RAP procedures [6] and official Census/ Mortality/ Morbidity statistics on the community being studied (otherwise may need to resort to country statistics).

1.11.1 Geographic Characteristics

a) community type - urban, suburban, semi-rural, rural, dispersed
b) type, availability and cost of public transportation (train, bus, waterway, other)
c) distance from home to urban centres (school, market)
d) topography, climate, seasons
e) map (if possible) -area, boundaries, roads, features
f) history of the community

1.11.2 Demographic and epidemiological characteristics

a) population size ("usual" at time of study)
b) adult mortality, morbidity - major causes of death, life expectancy at age 70
c) ethnic groups (identify)
d) sex and age distribution

e) economically active population (per cent by age and sex)
f) migration patterns (seasonal for work, rural-urban, etc.)
g) languages spoken and written; religious groups

1.11.3 Socio-economic characteristics

a) community organisation - local authorities, leaders, groups) religious, clubs, occupational), sanitation, water supply
b) domestic/ household organisation - nuclear versus extended, residence patterns, land sharing by family members
c) economic characteristics - major employers, major activities (male/ female), unemployment and underemployment, per capita income, distribution of wealth, landownership, access to land.

1.11.4 Health resources

a) health resources used by the community transportation, distance to resource, facilities, cost.

1.11.5 Housing conditions

a) type of housing - single family dwelling, multiple family dwelling, public housing.
b) description of residences - tent, brush hut, mud.
c) house, materials (walls, roof, floor), compound area (type of surrounding wall), number of rooms.
d) kitchen facilities - gas/ electric range, hot plate, wood fire, refrigerator, means of food storage, disposition of garbage.
e) water source, disposition of human waste, electricity, inventory of key possessions (radio, television, bicycle) garden and local (near household) crops.

2. WORKING PLAN & MANUAL OF OPERATIONS

2.1 Subjects

2.1.1 Selection Procedure

Obtain information on the town's inhabitants that are 70 years and older by consulting the electoral rolls or civil registration services or telephone directory. Choose the method that will give the most complete information for that age group. Randomly select 340 names from the list in order to take into account non-participation and non-eligibility. Aim for a sample size of about 200 subjects (100 men and 100 women). The lower limit is set at 100 (50 men and 50 women).

2.1.2 Recruitment

After the announcement of the study either in local papers or in community organisations for elderly, the selected elderly may be approached by means of a letter, telephone or home visit or combination of these. Choose method most appropriate to that town and population.

When the contact is made, the following should be mentioned:

a) Reasons for the study: the IUNS subcommittee on Nutrition and Ageing is interested in the elderly and that this study focuses on the health of elderly in different countries; the town has been chosen as an example for the country and their participation will contribute in making future recommendations to the population at large about how to live a long healthy life.

b) The place and time that the study will be carried out

c) The elements of the study i.e. what it involves:

   One session will take about 60 minutes where information will be obtained on their health and lifestyle (part 1 of questionnaire, non-nutritional questions). The other session will take about 120 minutes where information will be obtained on the type of foods consumed (part 2, nutrition questions). If more appropriate, the nutrition questions can be asked first. Indicate that blood also needs to be collected for analysis with their consent and that they will be informed of the results (posted in mail). Arrange to have blood collected at one of the sessions. If subject refuses to have blood taken, they can still participate in the study i.e.. be interviewed and have anthropometry taken. If transport to the research centre can be subsidised, one could arrange to do anthropometry, take blood and also do part 1 and 2 of the questionnaire in one session (about 3 hours).

d) Emphasise that the information is completely confidential and that it will be in no way possible to identify individuals in the final results. If individuals would like to know the results of the study they will be provided.

e) Avoid introducing interviewers as dieticians/ doctors as this may influence the subject's response. Refer to interviewers as researchers or interviewers.

f) Interviewers should avoid communicating their views and should be careful not to make assumptions about the respondents.

Interviewers should stress that they do not have the intention to tell the subject what to do but that they are interested in their experiences.

Try to achieve a participation rate of 60% (60% of those who are invited). Include hospitalised people in the study as well; if possible contact them after hospitalisation (if within months of study). If subjects are away on holiday, contact at a later date (ifwithin months of study). Go three times for subjects that are not at home, preferably at different times of the day. Exclude

elderly that are:

- psycho-geriatric patients in nursing homes
- subjects not fluent in the country's language
- people that are not able to answer questions independently

2.1.3 Non-Responders

The non-responders should be registered and asked why they do not wish to participate in the study. If possible, try and get information from them about their age and health. If subject does not wish to have blood taken, they can still participate in study.

2.2 INTERVIEW

2.2.1 General

The dietary section requires about 120 minutes to complete and the remaining questions, including anthropometry and blood collection should take another 60 minutes. A total of 3 hours per subject is required. If a minimum of 5 subjects are interviewed a week, 200 can be studied in 10 months. Preferably the general questionnaire should be answered by the participant with no help from anyone else. It may however be impossible to exclude the involvement of others completely.

If there are two or more mistakes with the first few questions the co-operation of an informant may even be meaningful. The interviewer must make a note of contributions of others to the interview. If a subject is in hospital, the interviewer should wait to see if he or she is likely to return home during the survey period. If not, they will be excluded from the study. Record the answers using the indicated numbers. In some cases this may not be feasible, e.g. some questions are open-ended in an attempt to get as much information as possible.

2.2.2 Food Intake Data

2.2.2.1 Diet History

The interviewer should start with obtaining a very general diet history in order to record the following:

a) times of eating
b) kind of foods generally eaten at each meal, e.g. cooked meal, usually meat, for lunch and dinner, fruit always in afternoon and serving size of main meal, not individual food items.

The diet history should be brief - leave all the detail for the food frequency. Do not attempt to quantitate at this stage. Clarify the number of cooked meals eaten a day because subjects often

indicate that they eat chicken for example once a week but actually eat a small serve for both lunch and dinner on the same day. Therefore, this should be coded as a small serve twice a week or a medium serve once a week.

2.2.2.2 Food Frequency

The food frequency aims at capturing types, quantities and frequencies of foods consumed over the past year in order to account for seasonal variation. It may not be necessary to ask every single food item in the checklist. It may be faster to ask the subject for example what kind of legumes they eat. Use the reference serving sizes that are already listed in the food frequency (see Appendix). Add any culture specific foods relevant to the community being studied with their corresponding reference serving sizes.

Where possible, try and quantitate intake with food photographs by taking photos of the large and small servings of the food items in the questionnaire or by using existing scanned photos used in Australia. A photo of the medium serving size is deliberately excluded as responses tend to be biased towards this serving.

There are 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
3) medium or between the small and large serve photo
4) equal to large serve photo;
5) greater than large serve photo.

However to avoid misunderstandings, since in many cultures actual serving sizes for example of meat might be much smaller, avoid using terms such as small, medium and large serves. But rather ask individuals to indicate, using food photos or household measures (if appropriate), what fraction or amount they actually eat. For example, if a subject eats half of the small serving photo, grams corresponding to this photo should be halved.

The way in which foods are eaten i.e.. cooking methods, may be difficult to establish in terms of frequency. If a subject indicates that for example beef is eaten in all possible ways (broiled, casseroled, fried) then divide the quantity consumed equally between the different cooking methods. Otherwise if the subject eats beef mainly broiled, then code only for this cooking method and leave others blank. To minimise interobserver variation, limit the number of researchers collecting dietary information.

The following symbols should be 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.

The number of months per year a food is eaten should also be recorded. For fruit and vegetables (also soups and icecream), most of which have fixed seasons, it is easier to ask each subject if they either eat them only when in season or all year round, rather than asking them to specify months/ year eaten. This puts less strain on the elderly subject's memory and saves time when interviewing. For both the history and checklist it is recommended to consult the one in charge of the preparation of meals.

2.2.2.3 Past food intake and food beliefs

As the current frequency of intake of each food is recorded the interviewer should ask simultaneously whether that particular food was eaten more, less or the same just before the Second World War with the following symbols:

More = M; Less = L; Same = S

Record any foods that were eaten then but not now.

Try and obtain as many food beliefs from each subject as possible i.e. which foods they believe are good or bad for health, which foods are used as medicine or for healing. All the food beliefs obtained will be pooled, compiled and presented in a descriptive fashion in the final report.

It may not be necessary to question each and every participant about what was eaten in the past, during times of hardship, what they ate as children, what is eaten during special occasions and festivities, and food beliefs. It may indeed be sufficient to ask these questions to only a few e.g. the acknowledged village elders and leaders using Rapid Assessment Procedures [6].

2.3 ANTHROPOMETRY

For each individual studied, a data collections sheet has to be filled in immediately after each measurement. It is recommended that the measurer is assisted by a person who records the values. None of the measurements are to be undertaken by untrained personnel. If more than one fieldworker is assigned to the anthropometrical part of the study, it is essential that they are regularly checked for repeatability of their measurements, one against the other, to reduce inter observer variability as much as possible. It is recommended that the measurements be taken in sequence as presented on data collection sheet.

2.3.1 Height

The standing height is measured using a microtoise fixed to the wall. The subject should stand (without shoes) on a horizontal platform with his heels together and with the Frankfurt plane horizontal. The subject draws 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. (An accuracy

of 0.1 cm is required). Only one measurement needed.

2.3.2 Weight

Body weight is measured with the subject clothed only in light clothing, to the nearest 0.5 kg. Weight should be measured before blood collection (for which the subject should be fasting) or alternatively after breakfast. Calibrated scales, fit with a board to allow for measurements on a smooth surface must be used. Weighing scales should be calibrated regularly.

Calculate body mass index BMI = weight kg/ height m^2

2.3.3 Mid upper-arm circumference (MAC) & Mid arm muscle area (MAMA)

Subject should be in standing position. The subject's left arm hangs relaxed, just away from the trunk. With a tape measure locate the middle of the upper-arm by measuring the midpoint between the olecranon process (point of the elbow) and acromion process (shoulder) and mark with a pen. At this point take the mid arm circumference (MAC) with the tape measure to the nearest centimetre. This measurement must be done in duplicate.

The mid arm muscle circumference (MAMC) is calculated using the following formula [22]:

MAMC cm = MAC - (3.14 x tricep skinfold)

The mid arm muscle area (MAMA) is calculated using the following formula [22]:

$$\text{MAMA cm}^2 = \frac{[(\text{MAC cm}) - (3.14 \times \text{tricep skinfold mm}/10)]^2}{12.56}$$

2.3.4 Skinfolds

Every skinfold is measured and recorded in triplicate and the average taken. A calliper with constant pressure is required (e.g. Harpenden). All skinfold measurements (i.e. tricep, bicep, suprailiac, subscapular) should be picked up between the thumb and forefinger; pinch the skin and pull it away from the underlying muscle; apply callipers 1 cm from the ridge of skin thus formed; take reading 3 seconds after application of the callipers, to standardise any effects produced by deformation of tissue; do not remove your hold of the skinfold while callipers are applied; record the average of 3 readings.

The actual measurement is read at the time the readings start to stabilise (usually about 3 seconds). Record values to the nearest 0.2 mm. Successive measurements should agree within 4 mm. The skinfolds are summed to calculate % & kg fat mass and % & kg lean mass using the Durnin & Womersley tables [23] (see Table A1.2).

Table A1.2 Durnin & Womersley tables (% body fat), sum of biceps, triceps,

subscapular, suprailiac skinfolds.								
Skinfolds	Men (age in yrs)				Women (age in yrs)			
(mm)	17-29	30-39	40-49	50+	16-29	30-39	40-49	50+
15	4.8	-	-	-	10.5	-	-	-
20	8.1	12.2	12.2	12.6	14.1	17.0	19.8	21.4
25	10.5	14.2	15.0	15.6	16.8	19.4	22.2	24.0
30	12.9`	16.2	17.7	18.6	19.5	21.8	24.5	26.6
35	14.7	17.7	19.6	20.8	21.5	23.7	26.4	28.5
40	16.4	19.2	21.4	22.9	23.4	25.5	28.2	30.3
45	17.7	20.4	23.0	24.7	25.0	26.9	29.6	31.9
50	19.0	21.5	24.6	26.5	26.5	28.2	31.0	33.4
55	20.1	22.5	25.9	27.9	27.8	29.4	32.1	34.6
60	21.2	23.5	27.1	29.2	29.1	30.6	33.2	35.7
65	22.2	24.3	28.2	30.4	30.2	31.6	34.1	36.7
70	23.1	25.1	29.3	31.6	31.2	32.5	35.0	37.7
75	24.0	25.9	30.3	32.7	32.2	33.4	35.9	38.7
80	24.8	26.6	31.2	33.8	33.1	34.3	36.7	39.6
85	25.5	27.2	32.1	34.8	34.0	35.1	37.5	40.4
90	26.2	27.8	33.0	35.8	34.8	35.8	38.3	41.2
95	26.9	28.4	33.7	36.6	35.6	36.5	39.0	41.9
100	27.6	29.0	34.4	37.4	36.4	37.2	39.7	42.6
105	28.2	29.6	35.1	38.2	37.1	37.9	40.4	43.3
110	28.8	30.1	35.8	39.0	37.8	38.6	41.0	43.9
115	29.4	30.6	36.4	39.7	38.4	39.1	41.5	44.5
120	30.0	31.1	37.0	40.4	39.0	39.6	42.0	45.1
125	30.5	31.5	37.6	41.1	39.6	40.1	42.5	45.7
130	31.0	31.9	38.2	41.8	40.2	40.6	43.0	46.2
135	31.5	32.3	38.7	42.4	40.8	41.1	43.5	46.7
140	32.0	32.7	39.2	43.0	41.3	41.6	44.0	47.2
145	32.5	33.1	39.7	43.6	41.8	42.1	44.5	47.7
150	32.9	33.5	40.2	44.1	42.3	42.6	45.0	48.2
155	33.3	33.9	40.7	44.6	42.8	43.1	45.4	48.7
160	33.7	34.3	41.2	45.1	43.3	43.6	45.8	49.2
165	34.1	34.6	41.6	45.6	43.7	44.0	46.2	49.6
170	34.5	34.8	42.0	46.1	44.1	44.4	46.6	50.0
175	34.9	-	-	-	-	44.8	47.0	50.4
180	35.3	-	-	-	-	45.2	47.4	50.8
185	35.6	-	-	-	-	45.6	47.8	51.2
190	35.9	-	-	-	-	45.9	48.2	51.6
195	-	-	-	-	-	46.2	48.5	52.0
200	-	-	-	-	-	46.5	48.8	52.4
205	-	-	-	-	-	-	49.1	52.7
210	-	-	-	-	-	-	49.4	53.0

Durnin JVGA & Womersley J, Br J Nutr 1974; 32: 77-79.

2.3.4.1 Triceps

The triceps skinfold is picked up between thumb and forefinger at the back of the left arm about

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 calliper jaws are then applied exactly at the level marked for the circumference measurement.

2.3.4.2 Biceps

The skinfold is picked up on the front of the arm directly above the centre of the cubital fossa. The callipers should be applied at the skinfold at the same level as the triceps skinfold.

2.3.4.3 Subscapular

The subscapular skinfold is picked up just under the lower angle of the scapular (10 mm above the left superior iliac crest in the midaxillary line and along the horizontal plane). The fold should be taken at a 45^{o} angle in the natural cleavage of the skin.

2.3.4.4 Suprailiac

Suprailiac skinfold thickness is measured just above the iliac crest on the mid-auxiliary line (over the wing of the left scapula, in plane of dermatome).

2.3.5 Waist and Hip circumferences

Subject should be in standing position in light clothing with feet fairly close together (about 12-15 cm apart) with weight equally divided over both legs.

(i) Waist circumference
Measure waist circumference at the level of the umbilicus with a tape. Make two measurements and average.
(ii) Hip circumference

Measure hip circumference as the largest diameter below the umbilicus or maximum circumference over the buttocks, normally at the level of the great trochanters but in all cases not lower than symphyses level. Measure perpendicular on the axial lane of the trunk. Make two measurements and average. Calculate waist-hip ratio.

2.3.6 Hip length

Subject is in standing position. Locate the left superior iliac spine and left knee joint space and measure the distance between these two points with a tape measure, making sure to measure parallel with the axial line of the left leg. Take two readings and average.

Calculate maximum height & loss of height (these equations apply to women only):

max height = 1.096 + 1.185 x hip length (metres)
loss of height = max height - current height

2.3.7. Electrical Impedance

Place electrodes on right hand and foot and record resistance and reactance. Use these values to calculate % & kg fat mass, % & kg lean mass, % & L water.

2.4 CUTANEOUS MICROTOPOGRAPHY

This can be done whilst taking the anthropometric measurements. It is simple, fast and painless. Should not take more than 5 minutes. Indicate to subjects that this test shows how old their body is and reassure them that it will be painless. A skin imprint is 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). A silicone rubber impression material is used to produce cutaneous microtopographs (Optosil Flussig, Bayer Leverkusen, West Germany). It is a viscous white liquid which sets in 3-5 minutes after addition of a catalyst.

a) Pour white liquid in the small plastic holder to the marked line.

b) Then add about 6-10 drops of the catalyst (red liquid) which will begin to thicken and set the white liquid immediately

c) Stir quickly with the spatula provided for about 3 minutes or until white liquid starts to thicken. Do not stir for too long because white liquid will set and become hard to spread.

d) Using the spatula, spread the viscous white material (which should still be slightly runny) 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 is spread on skin.

e) When the rubber sets, strip slowly and steadily from skin surface and store in labelled envelopes. It should not cause any pain to the subject.

f) Label the back of the rubber imprint (i.e. the surface facing away from the skin) from the forearm with the letter A (using a PEN) and the back of the rubber imprint from the hand with the letter H.

Grade the skin imprints from 1-6 (see Table A1.3) using a dissecting microscope 10 x magnification. The skin imprints should also be graded by a second rater to determine inter-observer variation.

Table A1.3 The Beagley-Gibson grading of cutaneous microtopographs taken from

dorsum of hand [18].	
Grade	**Features**
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 disorganised.
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.

2.5 BLOOD PRESSURE

Blood pressure can be taken whilst doing anthropometric measurements and preferably before blood is collected. Seat subject in a chair. Let the subject rest for 5 minutes. Whilst seated, measure systolic and diastolic blood pressure using a sphygmomanometer on the left arm. Repeat measurement after 10 minutes. Record readings and whether subject has taken medication for blood pressure.

2.6 BLOOD ANALYSES

Blood sampling should be preceded by a fast overnight (no food or drinks after 10PM, except water). Clear instructions should be issued that blood should be collected between 7.30 and 9.30 AM. Be sure to be on time, especially for diabetic patients. Ask subject if they have consumed anything since the previous evening or have taken their medication and record on data collection sheet. A single venipuncture is required. However if subject refuses to give blood they should not be excluded from whole study. Record missing values for blood parameters. Samples may be collected either in the homes or at the research centre. If the latter is the case, after anthropometric measurements are done, blood can be collected and the rest of questionnaire completed.

Before the venipuncture the subject should have been sitting for 10 minutes with the arm on the table. Ask subject which arm is better for collecting blood. If necessary use hand veins. Use 5 & 10 ml vacutainers. Collect a minimum of 35 ml of blood (4 tubes).

Tube 1 (10 ml) serum	——>	haematology
Tube 2 + EDTA (5 ml) plasma	——>	haematology (keep in darkness)
Tube 3 (10 ml) serum	——>	chemical pathology
Tube 4 (10 ml) bloodserum	——>	research (Lp (a), vitamin E, carotenoids)

Any left over serum should be stored at -70°C for future analyses.

2.7 URINE COLLECTION

About 20% of the subjects should be asked to collect urine over 24 hours (the day prior to blood collection so that urine can be brought to the hospital/ research centre). Instruct subject to discard first urine on the morning of collection and to start collecting from the second urine onwards till the following morning where they should collect the first urine. The urine should be kept in a cool place and brought to the hospital/ research centre on the day of blood collection. The urine should be stored at -20°C. The urine is analysed for total nitrogen excretion in order to calculate protein intake to validate the food frequency questionnaire. Creatinine should also be measured since nitrogen excretion can be expressed in relation to creatinine excretion in cases where urine has been incompletely collected. Na and K can also be measured.

2.8 DATA ENTRY & REPORT OF RESULTS

The questionnaire structure has been set up on DBase 3 using question numbers as the identifying field name. Anthropometric and biological measurements are also entered into a Dbase file. Each centre is free to publish their own data in any journal. The IUNS committee on "Nutrition & Ageing" will be responsible for analysing and integrating data from all centres for cross-cultural comparisons using a statistics package called SAS. This analysis will take place at the coordinating centre (C/O Professor Mark Wahlqvist, Department of Medicine, Monash Medical Centre, Clayton, VIC, Australia).

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PROTOCOL

FOOD HABITS IN LATER LIFE: A Cross-Cultural Study

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