

NILS was also reorganized to cover more area of geriatrics and gerontology. The number of department increased from 8 to 13. A new research section, the Section of Nutritional Epidemiology was added to the Department of Epidemiology and the Laboratory of Epidemiology for the Aged was reorganized to the Section of Preventive Epidemiology.

2) Progress of the NILS-LSA

In 1990, projects of “Comprehensive Research on Aging and Health” were started by the Ministry of Health and Welfare to promote longevity sciences in commemoration of the 60th year in the reign of Emperor Showa. A research group for a longitudinal study of aging was organized as one of these projects. Indices of aging were evaluated, the methodology for the longitudinal study was assessed, and many problems in actual longitudinal follow-ups using existing cohorts were analyzed by this research group in order to start a new comprehensive longitudinal study of aging in Japan. A pilot longitudinal study on aging started in 1992. A manual of the many procedures used in the study was published in 1996.

In July 1995, the National Institute for Longevity Sciences (NILS) was established as the leading national research center for aging and geriatrics in Obu city in the suburbs of Nagoya. In 1996, the Laboratory of Long-term Longitudinal Studies was established in the Department of Epidemiology to start a new longitudinal study of aging in Japan.

Various equipments necessary for geriatric research, such as magnetic resonance imaging (MRI) and peripheral quantitative computed tomography (pQCT) were set up in the NILS, and a special examination center for longitudinal study was established in the Chubu National Hospital. Physicians, psychologists, nutritionists, epidemiologists, and exercise physiologists were assigned to the Laboratory of Long-term Longitudinal Studies and the Department of Epidemiology.

In October 1997, a trial run of the examinations was conducted, and in November 1997, the NILS-LSA began as a large-scale and comprehensive longitudinal study of aging in Japan. Every day, seven participants were examined at the NILS-LSA Examination Center. In the first wave of the examination finished in April 2000, 2,267 males and females had completed the examinations. All participants will be examined every two years, but some participants are dropped out. Age and gender-matched random samples of the same number of dropout participants are recruited except the participants over 79 years old. The male and female participants aged 40 years are also newly recruited every year.

The second wave of the examination started in April 2000 and finished in May 2002. Total number of participants of the second wave examination was 2,259. From May 2002, the third wave examination started. The third examination finished in May 2004 and 2,378 participants were examined. The fourth wave examination started in June 2004 (Fig. 1), and finished in July 2006, total 2,383 participants were examined.

The number of examined variables was over 1,000, including various areas of gerontology and geriatrics such as medical examinations, anthropometry, body composition, physical functions, physical activities, psychological assessments, nutritional analysis and molecular epidemiology.

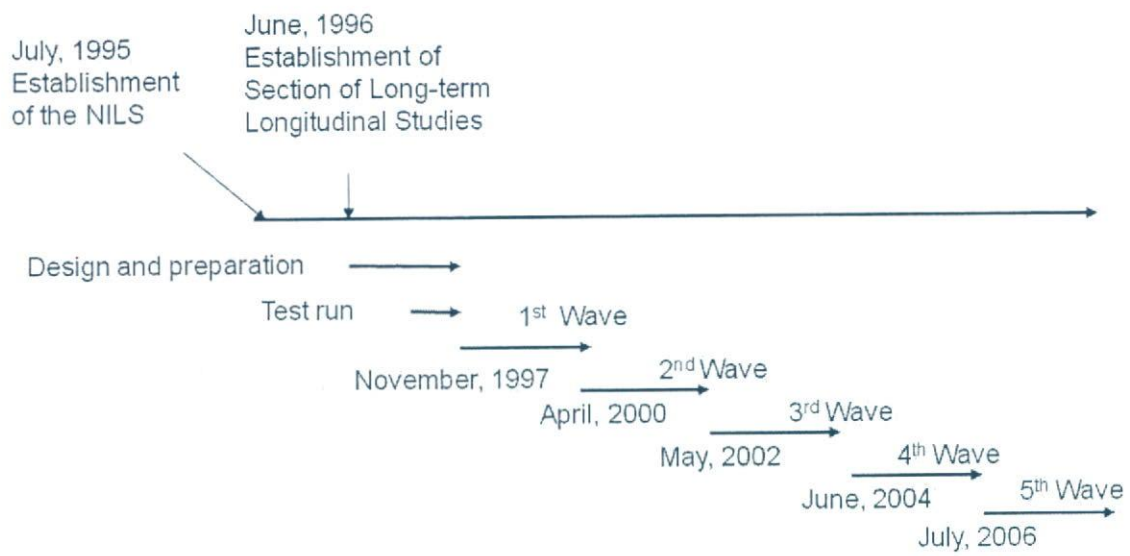


Fig. 1 Progress of the NLS-LSA

3) Objectives of the NLS-LSA

1. Main purpose

Systematic observation and description of the process of normal aging in humans.

- (1) To quantify normal and successful aging
- (2) To determine the reference values in normal aging process by longitudinal observation

2. Additional purpose

- (1) To find out early markers of age-related diseases
- (2) To clarify molecular genetic factors of aging and geriatric diseases
- (3) To find out factors associated with longevity
- (4) To examine the effects of life-style, stress, life events and disease on aging process
- (5) To separate normal aging and age-related disease
- (6) To assess the influence of age on progressive changes of various diseases
- (7) To determine predictors of age at death and risk factors for diseases as well as institutionalization and loss of independence
- (8) To examine race difference by international comparative study
- (9) To assess social and economical changes with age in the elderly
- (10) To develop indices of biological age
- (11) To prepare basic population for the research of clinical and social medicine

4) Research area

The NILS-LSA is a facility-based study using various equipments including MRI, DXA and pQCT for the detailed and comprehensive assessments of aging and geriatric disease. The facility of examinations is located at the NILS. Thus, the research area was determined to be in the neighborhood of the NILS, that is Obu city (population 79,000) and Higashiura town (population 48,000) (Fig. 1). This area is located in the south of Nagoya, and is a bedroom town and also industrial area of the Toyota group, but still has many orchards and farms, having both urban and rural characteristics.

This research area is geographically located at the center of Japan, and the climate is almost Japanese average. We examined the representativeness of the area via national postal questionnaire of prefecture-stratified random samples of 3,000 households from all prefectures in Japan, and found that the life-style of this area was the most typical of all areas in Japan. It is expected that the results of examinations in this area will represent Japan.

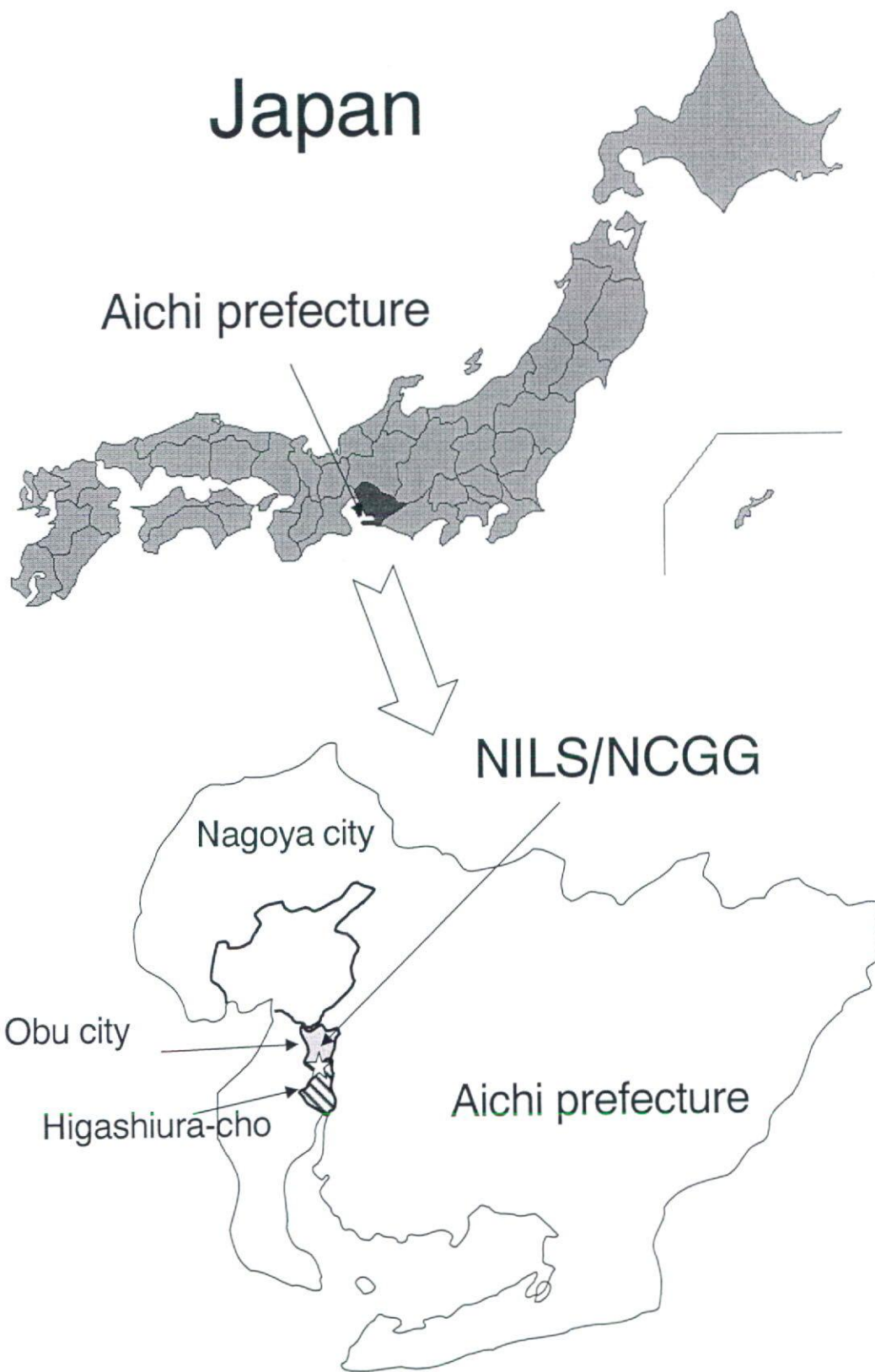


Fig. 2 Research area of the NILS-LSA

5) Subjects

The subjects of baseline examination of the NILS-LSA were males and females aged 40 to 79 years old. The population of Obu city and Higashiura town was stratified by both age and gender, and randomly selected from resident registrations in cooperation with the local governments (Fig.3). The number of males and females was to be equal to test gender difference, and the number of participants in each decade (40s, 50s, 60s, 70s) was also to be equal. The total number of participants was to be 2,400, that is 300 males and 300 females for each decade. They will be followed up every two years. Age and gender-matched random samples of the same number of dropout participants will be recruited except the participants over 79 years old. The male and female participants aged 40 years will be also newly recruited every year (Fig.4). Table 1 shows age and gender distribution of the participants in the first wave examination. Table 2, Table 3, Table 4, and Table 5 also show age and gender distribution of the second, third, and fourth wave participants, respectively. About 60% of the first wave subjects were participated in the fifth wave examination (Table 6), and 86.9% of the fourth wave participants came back for the fourth wave examination (Table 7).

Recruitment and follow up of volunteers would be much easier than with random samples. However, these samples generally tend to be interested in health, and observation of these samples would produce biased results. Examinations in random samples are necessary to observe the aging process of ordinary Japanese who live ordinary lives.

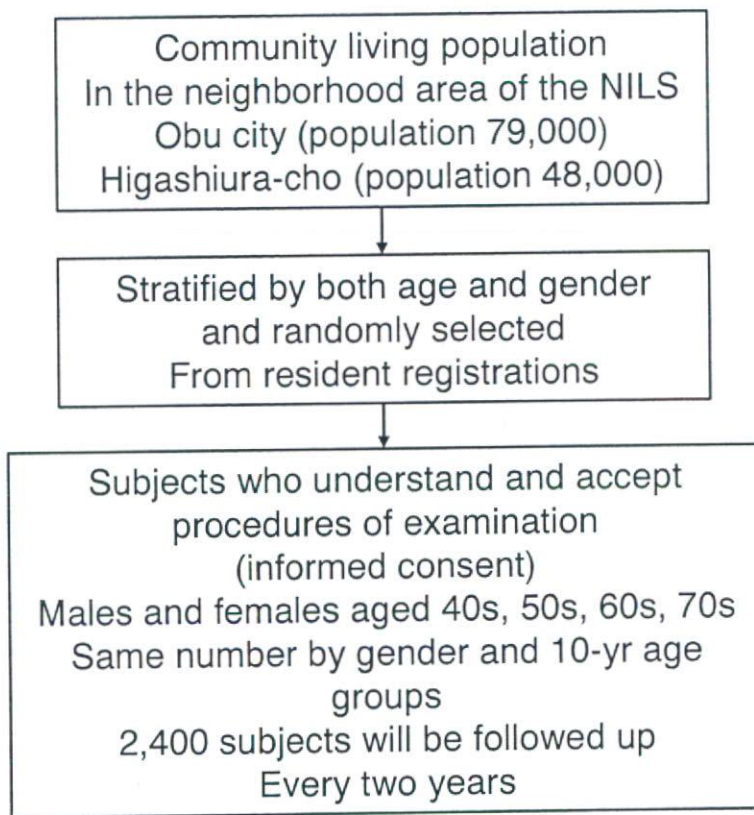


Fig. 3 Selection of the subjects in the NILS-LSA.

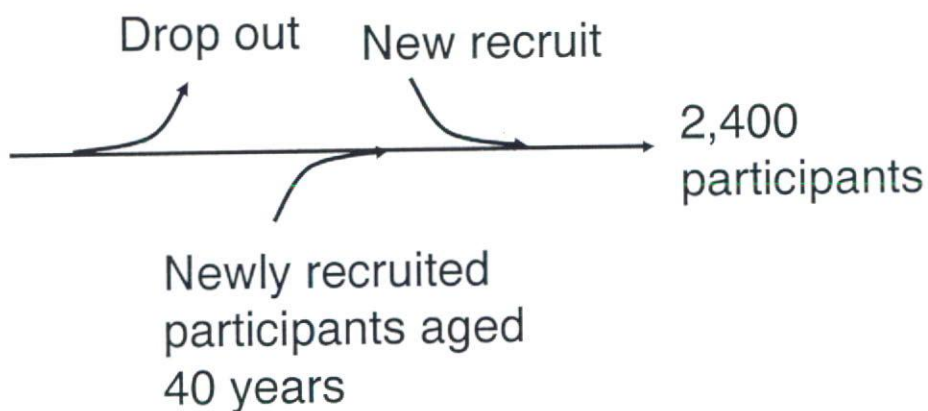


Fig. 4 NILS-LSA as a dynamic cohort

Table 1. Age and gender distribution of the first wave participants

Age	Male	Female	Total
40 – 49	291	282	573
50 – 59	282	279	561
60 – 69	283	285	568
70 – 79	283	282	565
Total	1,139	1,128	2,267

Table 2. Age and gender distribution of the second wave participants

Age	Male	Female	Total
40 – 49	273	261	534
50 – 59	296	284	580
60 – 69	291	271	562
70 – 79	275	269	544
80 –	17	22	39
Total	1,152	1,107	2,259

Table 3. Age and gender distribution of the third wave participants

Age	Male	Female	Total
40 – 49	266	294	560
50 – 59	331	285	616
60 – 69	297	286	583
70 – 79	267	275	542
80 –	43	34	77
Total	1,204	1,174	2,378

Table 4. Age and gender distribution of the fourth wave participants

Age	Male	Female	Total
40 – 49	286	294	580
50 – 59	295	283	578
60 – 69	300	273	573
70 – 79	255	285	540
80 –	53	59	112
Total	1,189	1,194	2,383

Table 5. Age and gender distribution of the fifth wave participants

Age	Male	Female	Total
40 – 49	279	295	574
50 – 59	289	277	566
60 – 69	274	281	555
70 – 79	283	278	561
80 –	75	88	163
Total	1,200	1,219	2,419

Table 6. Number of males and females who participated both in the first and the fourth wave examinations

	Male	Female	Total
First wave	1,139	1,128	2,267
First and fourth wave	708	673	1,381
Percentage	62.2%	59.7%	60.9%

Table 7. Number of males and females who participated both in the fourth and the fifth wave examinations

	Male	Female	Total
Third wave	1,189	1,194	2,383
Third and fourth wave	1,024	1,048	2,072
Percentage	86.1%	87.8%	86.9%

6) Implementation of the study

Randomly selected males and females who were assigned to the examination were invited by mail to an explanatory meeting (Fig. 5). At the explanatory meeting, procedures for each examination and the importance of continuation to follow up were fully explained. Participants were limited to those who accept examination procedures and sign their names on a written form (informed consent).

The Department of Epidemiology of the NILS was taking the initiative for all examinations and investigations. The participants were examined from 9:00 am to 4 pm at a special examination center within a facility at the the NILS. To examine 2,400 males and females in two years, that is, 1,200 males and females per year, six or seven participants were to be examined each day, four days a week from Tuesday to Friday, 200 days (50 weeks) a year. Taking advantage of the fact that all participants can be examined at the center, detailed examinations including not only medical evaluations, but also examinations of exercise physiology, body composition, nutrition, and psychology can be done. Each examination was to be extensive and most up-to-date, aiming at keeping the internationally highest level.

Information from the examinations that would be helpful to manage the health was returned to individual participants as a report from the NILS-LSA.

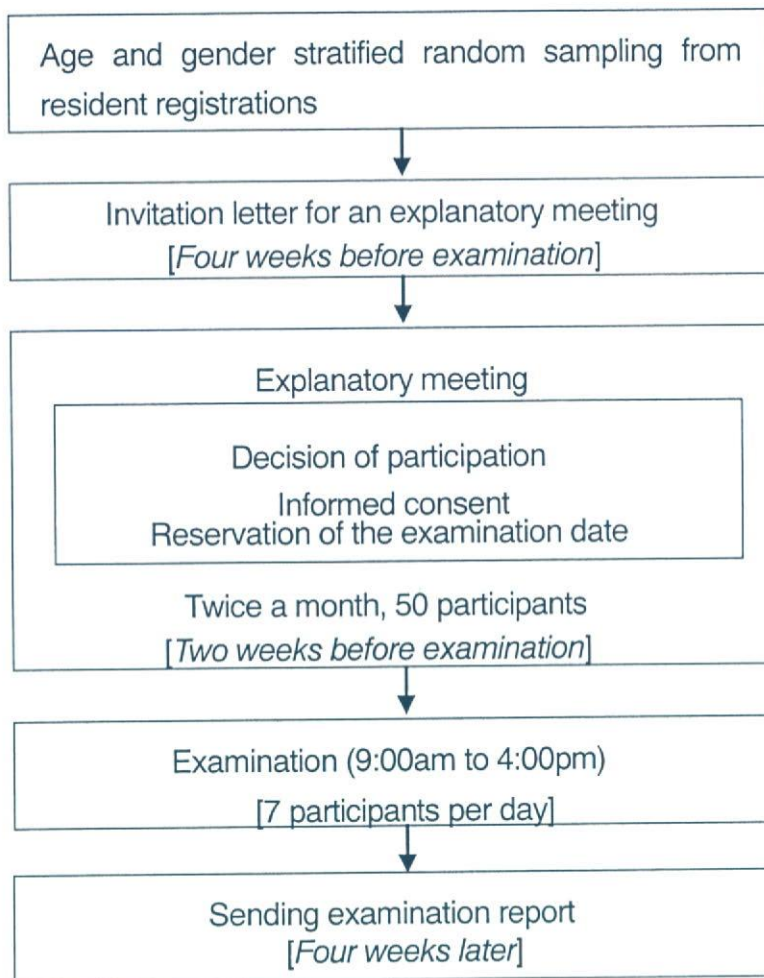


Fig. 5 Examination procedures in the NILS-LSA
(The first examination).

7) Informed consent

Participation in the examinations totally depended on free will, without any enforcement. All participants were fully informed of the following items. Only subjects who understood and accepted examination procedures, and signed their names on a written form to participate in the study (informed consent) were included. This informed consent included;

- (1) purpose and significance of the study
- (2) procedures and methods of each examination
- (3) place of the examination
- (4) gene analysis
- (5) preservation of blood and DNA samples for future examinations
- (5) to send examination report to the participants
- (6) to keep personal data secret.

The Ethical Committee of the National Center for Geriatrics and Gerontology had already approved all procedures of the NILS-LSA.

8) Examinations and tests

The normal aging process was assessed by detailed examinations including clinical evaluation, sensory functions, body composition and anthropometry, physical functions, nutritional analysis, and psychological tests (Table 8).

The NILS-LSA is a longitudinal study to observe age related changes of various examination and tests. Thus examinations and tests of the fifth wave were basically same with the first to the fourth examinations.

Table 8. The fifth wave examinations and tests in the NILS-LSA

Health related questionnaire

Self-rated Health (SRH), Medical history, Clinical symptoms, Family history, Environment, Smoking

Routine clinical evaluations

Physical examination

Blood pressure

Blood chemistry (fasting)

GOT, GPT, gamma-GTP, Total protein, Albumin, LDH, Alkaline phosphates, Choline esterase, Uric acid, Creatinine, Calcium, Phosphate, Total cholesterol, Triglyceride, HDL-cholesterol, Lipid peroxide, Fasting glucose, HbA1c, Insulin, Serum sialic acid, Fe.

CBC: Red cell count, White cell count, Hb, Hematocrit, Platelet count

Sensory examinations

Visual system

Visual acuity: Presenting Visual Acuity, Best-corrected Visual Acuity (5 m), Refraction, Retinal fundus camera, Intraocular pressure, Contrast sensitivity, Corneal thickness

Auditory system

Audiometry (air and bone), Middle ear functions (Single frequency and Multifrequency tympanometry), Video recording of tympanic membrane

Medical examinations

ECG (Automatic ECG analyzer)

Cardiac ultrasonic tomography

Intima-media thickness of carotid artery

Ankle-brachial index (ABI) and pulse wave velocity were (PWV)

Head MRI (Magnetic resonance imaging system)

Knee radiography

Dual energy X-ray Absorptiometry (DXA)

Lumbar spine, Right and left femur neck, Total bone density,

Anthropometry and body composition

Anthropometric measurements

Body fat measurement

Dual energy x-ray absorptiometry (total and segmental fat)

Thickness of fat and muscle tissue (Ultrasonic tomography)

Muscle thickness and subcutaneous fat thickness

Abdominal fat distribution (Computed tomography)

Intra-abdominal and Subcutaneous fat area

Physical function

Physical fitness test system

Grip strength, Sit-up, Trunk flexion, One leg standing balance, Leg extension power,
Isometric knee extension strength, Reaction time, Maximum step length.

10m walking test (pitch, step length, velocity),

3-D motion analysis system (six cameras and two force plates)

Stabilometer (with eye-open and eye-closed conditions)

Physical activity questionnaire

Electric pedometer (7 days average)

Psychological tests

Interview

Cognition

Wechsler Adult Intelligence Scale-Revised Short Forms (WAIS-R-SF)

Short-term Memory Function

WAIS-R digit span test; forwards and backwards

Mini Mental State Examination (MMSE)

Stress-Related Factors

Social network

Basic ADL (Katz Index)

Questionnaire

Depression scale

The Center for Epidemiologic Studies Depression Scale (CES-D)

Subjective Well-being

Life Satisfaction Index-K (LSI-K)

Personality

The Rosenberg Self-Esteem Scale

Neuroticism-Extraversion-Openness Five-Factor Inventory (NEO-FFI)

The Scale of Attitude toward Death (ATD)

Social and Domestic Activities

Social activities

Learning activities

Roles in family

Leisure activities

Work commitment scale

Nutrition analysis

Food and nutrition intake

Three-day dietary record using scale and disposable camera

Dietary supplement frequency interview

Food frequency questionnaire

1. Routine clinical evaluations

First of all, physical examinations including auscultation and blood pressure were taken by a physician, and during the medical examination the physician reconfirms every participant willingness to participate in examinations. Venous blood samples were collected early in the morning after at least 12 hours' fasting.

Medical history, family history, environment, smoking, health status, clinical symptoms, medical history and medication were examined by questionnaires. These questionnaires are checked by a physician at the medical examination. All drugs were to be documented by participants; the physician confirms them by interview and codes drugs used during the last two weeks.

Blood analysis including renal and liver functions, serum protein and lipids, minerals, glucose, HbA1c, Insulin, and complete blood count were also examined. Blood samples for DNA of the first visit participants was stored in deep freezers for future examinations.

2. Physiological examinations

For physiological examinations, a head MRI was taken for the each participant and stored in an image database. Intracranial tumors and vascular lesions are checked and brain atrophy, ventricular dilatation, and white matter lesion were assessed. Electrocardiograms are assessed by computerized automatic diagnosis and Minnesota codes of the diagnosis were stored in a database (NEC KARTIER5500). Cardiac functions and intima-media thickness of the carotid artery were assessed by ultrasonic tomography (Hitachi EUB-550). Blood pressure was measured by a physician as well as with an automatic blood pressure manometer. Ankle-brachial index (ABI) and pulse wave velocity were (PWV) also assessed (Colin).

Osteoporosis is one of the major geriatric diseases. Osteoporosis causes chronic lumbago and bone fracture that disturbs activity in daily life in the elderly. Bone mineral density was measured by dual x-ray absorptiometry (DXA, Hologic QDR-4500). Four scans, including whole body, lumbar spine L2 to L4, right and left femoral bone neck, were taken. Osteoarthritis of the knee was assessed by knee x-ray examination.

3. Sensory examinations

Sensory functions are profoundly associated with QOL in the elderly. Visual and auditory disturbance causes various difficulties in the daily lives of the elderly. Sensory functions, including visual and auditory functions were examined in detail. Distant visual acuity was measured for each eye with a Landolt C letter at 5m. Contrast sensitivity and intraocular pressure were also examined. Fundus photographs were taken with a Topcon fundus camera (TRC-NW5S). Autorefraction was done with the NIDEK-ARK700A. Refractive errors, in the spherical equivalent, were assessed. Corneal

thickness was obtained with the Topcon SP-2000 specular microscope.

Auditory function assessed by pure-tone audiometry (Audiometer RION AA-78), and impedance audiometry (Middle Ear Analyzer, Grason-Stadler model 33, version 2). Air conduction thresholds at 125Hz to 8000Hz were examined in all participants. Bone conduction thresholds at 250Hz to 4000Hz were also examined. Middle ear function was evaluated by impedance audiometry. Video image of tympanic membrane was recorded by CCD camera (MP-5, RF SYSTEM lab) and perforation, calcification and adhesion of tympanic membrane were assessed.

4. Anthropometry and body composition

For anthropometry measurements, height, weight, circumferences of waist, hip, thigh and upper arm and other parameters were taken. Using ultrasonic tomography, subcutaneous fat thickness and muscle thickness were evaluated. Total and segmental body fat and lean body mass were assessed by DXA. Abdominal fat distribution was evaluated as intra-abdominal and subcutaneous fat areas at the level of umbilicus using a computed tomography.

5. Exercise examinations

Grip strength, leg extension power, sit-up, one leg standing balance, reaction time, and trunk flexion are measured with a computerized automatic diagnosis system. Gait performance is assessed by the 10m walking test (pitch, step length, velocity) and using six cameras and two force plates (motion analysis). Physical activities are checked by detailed interview using job-specific questionnaire sheets. Seven-day averages of physical activity are also measured with an electric pedometer.

6. Nutritional survey

Nutritional intakes were assessed by three-day dietary record using a scale. The scale was handed out to each participant to record the weight of each food taken over the recording period. If it was impossible to weigh each food, approximate size and amounts of food were noted. Dieticians explained to each participant how to weigh foods and how to determine the size and approximate amount. For more accurate assessment, disposable cameras were also handed out to all participants. Before and after each meal, participants were asked to take pictures of all dishes to record what kind of foods and how much food were eaten, and how much food was not eaten. Using these dietary records and photographs, dieticians estimate actual food intake. Dietary supplement usage was also assessed by interview by dietitians in addition to three-day dietary record. Beverages and citrus intakes were assessed for previous one year by food frequency questionnaire.

7. Psychological test

All participants were interviewed by psychology specialists. Cognition and intelligence were assessed using the Wechsler Adult Intelligence Scale-Revised Short Form (WAIS-R-SF) in all participants and the Mini-Mental State Examination (MMSE) in participants aged 60 years and over. Life events, hassles, and stress coping were also assessed by interview. Basic ADL was checked via the Katz index.

Depressive symptoms, personality, subjective well-being, social relations, and ADL were assessed using a questionnaire.

Over 1,000 variables, including various areas of gerontology and geriatrics will be checked repeatedly every two years in almost 2,400 participants. The staff of the NLS-LSA were consisted of full time researchers, researchers from hospitals and universities, research assistants such as administrators, clinical technicians, dieticians, psychologists, and radiologists. The total number of staff was about 90.