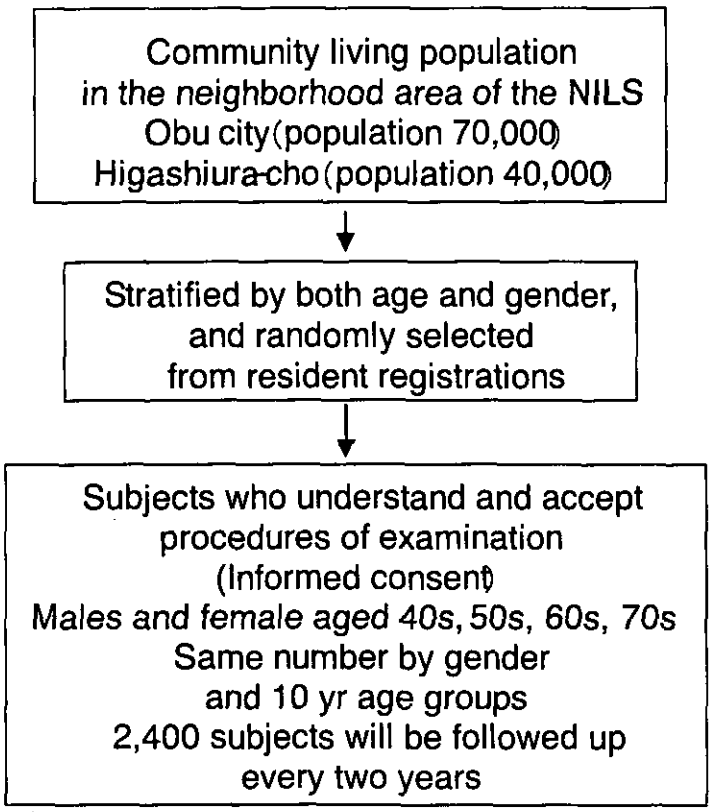

Fig. 3 Selection of the subjects in the NILS-LSA.

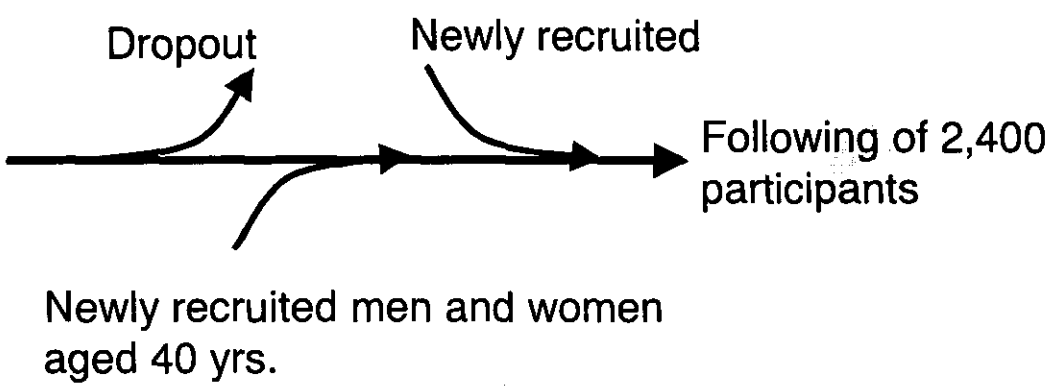

Fig. 4 NILS-LSA as a dynamic cohort

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. Number of males and females who participated both the first and second wave examinations

	Male	Female	Total
First wave	1,139	1,128	2,267
First and second wave	944	869	1,813
Percentage	82.9%	77.0%	80.0%

Table 4. Age and gender distribution of the third wave participants until September, 2004

Age	Male	Female	Total
40 – 49	172	195	367
50 – 59	216	189	405
60 – 69	227	197	424
70 – 79	200	202	402
80 –	35	24	59
Total	850	807	1,657

6) Implementation of the study

Selected males and females who were assigned to the examination were invited by mail to an explanatory meeting that was held (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 8:30 am to 5 pm at a special examination center within a facility at the Chubu National Hospital located next door to 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 a 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. The follow up period is to be up to 30 years, but we hope to get initial significant longitudinal results within 5 to 10 years.

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

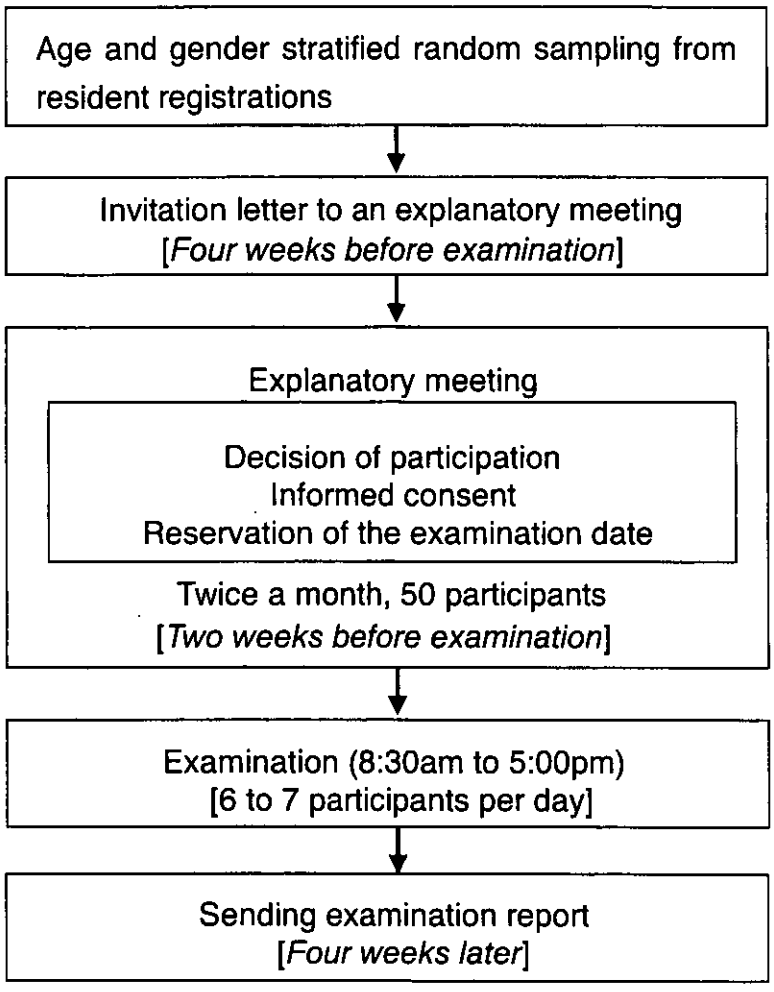

Fig. 5 Examination schedule in the NILS-LSA.

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 to a written form to participate in the study (informed consent) were included. This informed consent included; (1) purpose of the study; (2) detailed procedures for each examination; (3) gene analysis; (4) preservation of blood, urine 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 Chubu National Hospital 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 4).

The NILS-LSA is a longitudinal study to observe age related changes of various examination and tests. Thus examinations and tests of the third wave were basically same with the first and second wave except oral examinations including assessment of dental status, periodontal status, denture wearing habit, and tongue coating status.

Table 4. The third wave examinations and tests in the NILS-LSA

Health related questionnaire

Self-rated Health (SRH), Medical history, Clinical symptoms, Life-style, Personal history (job, marriage, education, etc.), Family history, Environment, Smoking, Social and economical back ground

Routine clinical evaluations

Physical examination

Blood pressure

Blood chemistry (fasting)

GOT, GPT, gamma-GTP, Total protein, Albumin, LDH, Alkaline phosphates, Chorine esterase, Uric acid, Creatinine, Calcium, Total cholesterol, Trigrlyceride, HDL-cholesterol, Lipid peroxide, Fasting glucose, HbA1c, Insulin, Vitamin A, Serum sialic acid, Fe, Cu, Mg, free T3, free T4, TSH, DHEA-S, DHA, EPA, arachidonic acid, Dihomo-gamma-linolenic acid

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

Urine analysis: Protein, Sugar, Urobilinogen, Ketone, pH, Occult blood, Hemoglobin, Nitrite

Sensory examinations

Visual system

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

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

Head MRI (Magnetic resonance imaging system)

Thoracic and lumbar radiography

Dual energy X-ray Absorptiometry (DXA)

Lumbar spine, Right and left femur neck, Total bone density, Body fat (total and segmental fat)

High Quality Peripheral Quantitative CT (pQCT)

Radial bone mineral density

Resting metabolic profiles (Computerized indirect calorimetry system)

Resting energy expenditure and Respiratory exchange ratio

Oral examinations

Dental status

Periodontal status

Denture wearing habit

Tongue coating status

Aging and geriatric disease related genotypes

Alzheimer disease and dementia related genotypes

Osteoporosis related genotypes

Obesity and diabetes related genotypes

Parkinson disease related genotypes

Cardiovascular disease related genotypes

Anthropometry and body composition

Anthropometric measurements

Body fat measurement

Dual energy x-ray absorptiometry (DXA)

Body fluid measurement (Bioimpedance spectroscopy)

Intra- and Extra-cellular fluid

Thickness of fat and muscle tissue (Ultrasonic tomography)

Intra-abdominal fat, Muscle thickness, Subcutaneous fat thickness

Abdominal fat distribution (Computed tomography)

Intra-abdominal and Subcutaneous fat area

Physical function

Exercise test system

Grip strength, Sit-up, Trunk flexion, Static 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 (four 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 (MMSE, WAIS-R), Life events, Stress, Social relations, Basic ADL (Katz Index)

Questionnaire

Depression (CES-D, GDS), Personality (Rosenberg Self-Esteem Scale, Locus of control, Scale of Attitude toward Death), Social relations, Subjective well-being (LSI-K), Stress checklist, Stress coping scale, Instrumental ADL, Basic ADL (Katz Index)

Nutrition analysis

Food and nutrition intake

Three-day dietary record using scale and disposable camera

Dietary supplement frequency interview

Beverage frequency questionnaire

1. Routine clinical evaluations

First of all, physical examinations including history taking, 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 and urine samples were collected early in the morning after at least 12 hours' fasting.

Life-style, personal history (job, marriage, education, etc.), family history, environment, smoking, social and economical back ground, 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 and urine analysis including renal and liver functions, serum protein and lipids, minerals, glucose, HbA1c, Insulin, Vitamin A, sialic acid, lipid peroxide, fatty acid fractions, thyroid hormones, DHEA-S, and complete blood count were also examined. DNA of the first visit participants was stored in deep freezers for future examinations. As for DNA analysis, genotypes related geriatric diseases such as Alzheimer's disease, arteriosclerosis, osteoporosis, benign prostate hypertrophy and diabetes mellitus were examined with the agreement of the participants.

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 was assessed via a computerized trace of the MRI. Electrocardiograms are assessed by computerized automatic diagnosis and Minnesota codes of the diagnosis were stored in a database. Cardiac functions and intima-media thickness of the carotid artery were assessed by ultrasonic tomography. Blood pressure was measured by a physician as well as with an automatic blood pressure manometer.

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. Moreover, bone density was also measured by high quality peripheral quantitative computed tomography (pQCT, Dinsiscan 1000).

Resting metabolic profiles were presented as resting energy expenditure,

oxygen intake and carbon dioxide excretion, and respiratory exchange ratio on resting using a computerized, open-circuit, indirect calorimetry system.

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. An anterior eye segment analysis system is used for the assessment of cataracts. 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 and endothelial cell density were obtained with the Topcon SP-2000 specular microscope.

Auditory function assessed by pure-tone audiometry (Audiometer RION AA-73A), 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 examined in participants with elevation of air conduction thresholds. Middle ear function was evaluated by impedance audiometry.

4. Oral examinations

A calibrated dentist asserted the participator's oral health conditions, including decayed, missing and filled teeth, periodontal status, denture wearing habit, and tongue coating status. The numbers of intact, filled, caries observation, decayed, and missing teeth were counted. The periodontal status was evaluated according to the Community Periodontal Index (10 index teeth: 17/16, 11, 26/27, 36/37, 31, 47/46), based on the World Health Organization standard. Behavior of denture wearing of the participants was asked. The tongue coating status was also assessed.

5. Anthropometry and body composition

For anthropometry measurements, height, weight, abdominal sagittal diameter, circumferences of waist, hip, thigh and upper arm and other parameters were taken. Using ultrasonic tomography, intrabdominal and subcutaneous fat thickness and muscle thickness were evaluated. Intra- and extra-cellular fluid was measured via bioimpedance spectroscopy. Body fat was assessed by DXA. Abdominal fat

distribution was evaluated as intra-abdominal and subcutaneous fat areas at the level of umbilicas using a computed tomography

6. Exercise examinations

Grip strength, leg extension power, sit-up and static balance, reaction time, and trunk flexion are measured with a computerized automatic diagnosis system. Step length, pitch, and velocity of walking are assessed by the 10m walking test using four video cameras and two force plates. 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.

7. 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. At lunchtime on the day of the examination, 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.

8. 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 and stress coping were also assessed by interview. Basic ADL was checked via the Katz index.

Depressive symptoms, personality, subjective well-being, social relations, hassles, stress-related factors 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 NILS-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.

9) Future of the NILS-LSA

We will continue the NILS-LSA to investigate the natural course of aging and the changes that lead to disease. The second wave examination was completed in May 2002. The participants will be examined every 2 years. The cohort of the NILS-LSA is a dynamic cohort, that is, new subjects participate in the study instead of those who do not attend their next examination. Participants who move out of the area are to be followed up by telephone interview or postal questionnaire. Medical records of the participants who die during follow-up will be checked to find out the cause of death.

We are collaborating with other research facilities in Japan and other countries as shown in Fig. 5. Extensive tests and examinations should be repeated in longitudinal studies on aging. However, it is actually impossible to repeat many tests and examinations in multiple research facilities with the same protocols and methods. There are no comprehensive longitudinal studies on aging that have been followed up for a long period by multi-center collaboration in the U.S. or other countries.

However, cohort studies with common end points such as dementia and disturbance of ADL are also important for aging studies. For these studies, a relatively large number of subjects and cases during follow-up will be required to get significant analysis results.

Comparative studies of the aging process accounting for regional and cultural differences between northern and southern areas, or between urban and rural areas, are also important. In these comparative studies, the number of common examinations and tests should be limited and measuring errors of each test and examination should be small. The study design should be a cross-sectional or short-term longitudinal study, considering the difficulties involved continuing and repeating the examinations in all facilities with same protocols. An international comparative study collaborating with the Baltimore Longitudinal Study of Aging (BLSA) at the National Institute on Aging (NIA) in the U.S. is also planned.

We are going to make the data of this study public through the Internet. We hope that the results from this large longitudinal study of aging can serve the development of health science on aging.

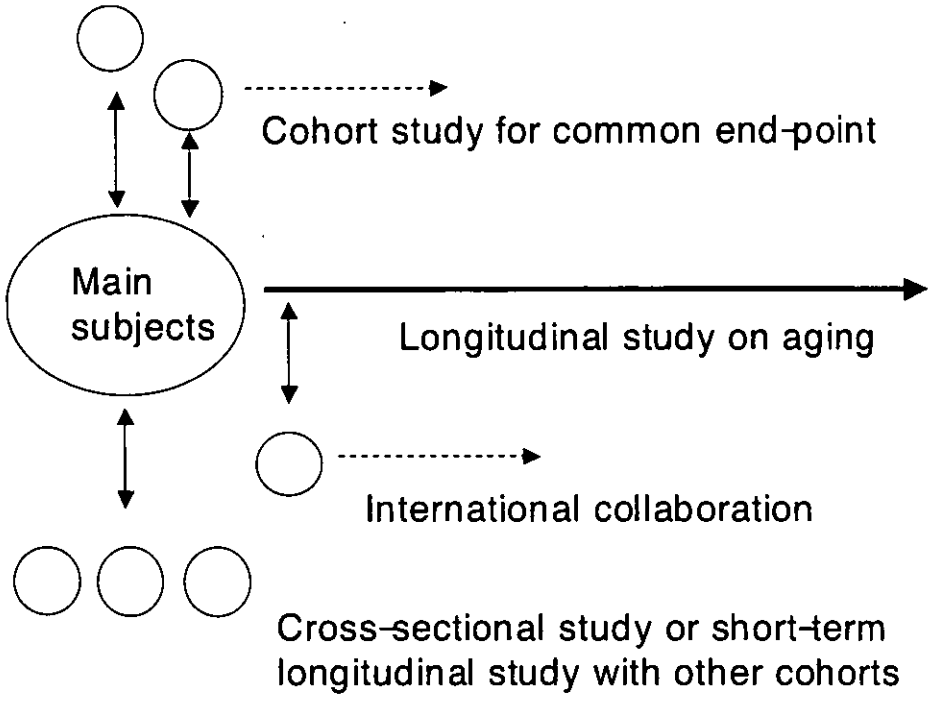

Fig. 6 Design of the longitudinal study by multi-center collaboration

10) Staff of the second wave examinations

Department Head

Hiroshi Shimokata, MD, PhD (Gerontology, Geriatrics and Epidemiology)

Chief, Section of Long-term Longitudinal Study

Fujiko Ando, MD, PhD (Gerontology, Geriatrics and Epidemiology)

Chief, Section of Preventive Epidemiology

Naoakira Niino, MD, PhD (Gerontology, Epidemiology and Public Health)

Researchers

Yukiko Nishino, BS (Radiology)
Yasuyuki Fukukawa, MS, Clinical Psychologist (Psychology)
Tomoko Imai, PhD, Registered Dietician (Nutrition)
Wataru Doyo, MS (Exercise Physiology)
Keiko Mori, PhD, Registered Dietician (Nutrition)
Satoshi Iwao, MD,PhD (Sports Medicine,Epidemiology)
Nobuko Iwao, PhD (Body Composition, Metabolism)

Research residents

Michiko Fujisawa, MD (Internal Medicine)
Tomohiro Okura, PhD (Body composition and anthropometry)
Chiori Nakashima, MS (Psychology)
Rumi Kozakai, MS (Exercise Physiology)
Yukiko Nishita, MS (Psychology)
Itsuko Kitamura, MD (Internal Medicine)

Visiting fellows

Satomi Tsuboi, MS, Clinical Psychologist (Psychology)
Kiyoshi Takekuma, MD (Internal Medicine)
Michiko Koda, MS, Registered Dietician (Body composition and anthropometry)
Taeko Kajioka, PhD (Body Composition, Metabolism)
Takako Kuno, MS, Registered Nurse (Nursing)
Naoko Shimizu, MD (Ophthalmology)
Chikako Tange, MS (Psychology)

Jiro Kanie ,MD (Internal Medicine)

Hospital Researchers

Hidetoshi Endo, MD, PhD (Gerontology, Geriatrics, Care-giving)
Hisayuki Miura, MD,PhD
Marie Takemura, MD
Yasue Uchida, MD (Otorhinolaryngology)
Hideki Nomura, MD, PhD (Ophthalmology)
Kazuko Asano, MD (Ophthalmology)
Makiko Iwano, MD (Ophthalmology)
Hideki Honda, MD
Yasunori Sumi,MD
Kouji Arai,MD
Shousuke Satake,MD
Yasumoto Arai,MD

Research Assistant

Kiyoji Toyama (Radiology)
Takashi Nakamura (Radiology)
Osamu Ueda(Radiology)
Saeko Toda(Radiology)
Yuko Goto (Blood Chemistry, DNA)
Chika Nagata (Nutrition)
Michiyo Tanaka (Nutrition)
Michiyo Kamae (Nutrition)
Hitomi Suzuki (Nutrition)
Masako Matsukawa (Nutrition)
Mieko Torii (Nutrition)
Junko Jinno (Nutrition)
Keiko Okamoto (Nutrition)
Satoko Yata (Nutrition)
Masako Taneda (Nutrition)
Namie Yamada (Nutrition)
Miyuki Sanada (Nutrition)
Sayaka Kitagawa (Nutrition)
Naomi Yamagishi (Clinical examinations)

Yumiko Hirose (Clinical examinations)
Yuki Iwata (Clinical examinations)
Chie Nakagawa (Clinical examinations)
Keiko Katogi (Clinical examinations)
Kazumi Sibata (Clinical examinations)
Kumiko Tomita (Clinical examinations)
Yoshiko Honda (Clinical examinations)
Naomi Tsurusaki(Clinical examinations)
Kazuyo Asano(Clinical examinations)
Keiko Maeba (Anthropometry)
Yoko Suzuki (Exercise examinations)
Eriko Takeuchi (Exercise examinations)
Hiromi Yamamoto (Exercise examinations)
Rumi Harada (Exercise examinations)
Ai Iriguchi (Exercise examinations)
Eri Iwaki (Exercise examinations)
Toshie Saito (Exercise examinations)
Taki Machiya (Exercise examinations)
Saori Furukawa (Exercise examinations)
Yuko Watanabe (Exercise examinations)
Naoko Suzuki (Exercise examinations)
Setsuko Saizuka (Exercise examinations)
Harumi Kuroda (Exercise examinations)
Shinji Yamamoto (Exercise examinations)
Shinichi Omiya (Exercise examinations)
Shiho Kitayama (Exercise examinations)
Mie Shimizu (Exercise examinations)
Miki Motousu (Exercise examinations)
Norio Hotta (Exercise examinations)
Emi Odaka (Exercise examinations)
Pei Oyan (Exercise examinations)
Sina Nishimura (Exercise examinations)
Tokio Takagi (Exercise examinations)
Tsutomu Jinji (Exercise examinations)
Kei Sasakawa (Exercise examinations)
Kenta Itou (Exercise examinations)

Ayumu Inoue (Exercise examinations)
Rieko Kimura (Clinical psychology)
Junko Aida (Clinical psychology)
Akie Miyata (Clinical psychology)
Hironori Kusajima (Clinical psychology)
Hiroko Ito (Clinical psychology)
Kayoko Ueno (Clinical psychology)
Yoshihiro Maruyama (Clinical psychology)
Naoko Izawa (Clinical psychology)
Yoko Watanabe (Clinical psychology)
Machi Tsuchiya (Clinical psychology)
Noriko Kubo (Clinical psychology)
Yuki Nakagami (Clinical psychology)
Hiromi Hanaoka (Clinical psychology)
Madoka Hotta (Clinical psychology)
Masako Arimoto (Clinical psychology)
Ayumi Isono (Clinical psychology)
Kaori Miyata (Clinical psychology)

Administration Staff

Hitomi Ogasawara
Eriko Kamiya (Secretary)
Masako Takahashi (Secretary)
Manami Iwao (Secretary)
Kanae Yamabe (Accounting)
Yukiko Matsuda (Accounting)
Yukie Takenaka (Administration)
Emi Hattori (Administration)
China Momose (Administration)

II. Background Examinations