Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : July 4, 2024
Accepted : August 29, 2024
Published online : September 30, 2024
doi:10.24659/gsr.11.3_121

Original article

Walking-based health promotion intervention and anti-aging medical checkups: A long-term observational study

Iroha Sekimoto [1)], Mari Ogura [1,2)], Masayuki Yagi [1)], Akari Yoshimura [1)], Saki Yokota [1)], Yoshikazu Yonei [1)]

1) Anti-Aging Medical Research Center and Glycative Stress Research Center,
Graduate School of Life and Medical Sciences, Doshisha University, Kyoto, Japan
2) Kyoto Bunkyo Junior College, Kyoto, Japan

Abstract

Our research center started a promoting-walking activity program in the Yurin district of Kyoto in 2008, where 40–50 participants living an independent life (mean age: 75 years) were lent a pedometer, and an anti-aging medical checkup was conducted annually with functional age assessments: muscle, vascular, neural, hormone, and bone age (a project of Kenpo Juku). Students (22–23 years) visited them once a month to collect information of participants' walking and also to encourage them to walk in face-to-face meetings. The number of average steps in members (7,000–8,000 steps/day) was approximately twice as many as that of persons close to their age. The comparative study regarding self-supported degrees (persons of independence: Kenpo Juku members, persons requiring assistance, and persons requiring nursing care) in 2010 exhibited that persons of higher degrees of independence stayed young in neural age and maintained cognitive functions. A report on amyloid β 40/42 ratio (2019) substantiated this. It was suggested that a third had a low ratio and would maintain amyloid β clearance. A survey conducted from 2012 to 2014 revealed that the participants had low skin autofluorescence and were a group with weak glycative stress. Glycative stress refers to a condition in which excess aldehydes are easily produced in the body, making body proteins highly susceptible to conversion into advanced glycation endoproducts (AGEs). Trends in data between 2008–2022 of the long-term observation cases were shown regarding functional ages. Survey on medical expenses (2023) indicated that medical expenses of Kenpo Juku members were mostly lower than the national average. The face-to-face encouragement from the students increased the members' motivation to continue walking and led to the long-term continuation of the exercise habit, which may help prevent the aging of functional age.

KEY WORDS: pedometer, walking, functional age, cognitive function, amyloid β 40/42 ratio, medical expense

Introduction: To be a healthy and lively aged person

Anti-aging Medicine aims "to promote health, improve the quality of life, and achieve health and longevity". Health, youthfulness, and happiness is our goal. It doesn't mean perpetual youth and longevity, which is impossible. Health and longevity, in other words, living a life not requiring nursing care, is a goal. This study presents our projects from the Anti-Aging Medical Research Center and Glycative Stress Research Center, Graduate School of Life and Medical Sciences, Doshisha University, Kyoto, Japan.

We live in this hyper-aging society and confront the social problem of an increase of aged persons who require nursing care. Reasons for needing nursing care are as follows in descending order;

1) cerebrovascular disease
2) cognitive disorder
3) hyposthenia
4) articular disease
5) fall-related bone fracture

Consequently, physical activities are reduced.

Review meeting regarding health and welfare services for the elderly, which was reported by Ministry of Health, Labour (2004), suggested in the interim report that the goal for a model to be aimed at achievement is not "healthy person aged 65" but "active person aged 85,' which is not a fantastic story but an implementable goal.

Contact Address: Professor Yoshikazu Yonei, MD, PhD
Anti-Aging Medical Research Center / Glycative Stress Research Center,
Graduate School of Life and Medical Sciences, Doshisha University
1-3, Tatara Miyakodani, Kyotanabe-shi, Kyoto, 610-0394 Japan
TEL&FAX: +81-774-65-639 e-mail: yyonei@mail.doshisha.ac.jp
Co-author: Sekimoto I, iroha.s.niko73@gmail.com; Ogura M, m-ogura@po.kbu.ac.jp;
Yagi M, myagi@mail.doshisha.ac.jp; Yoshimura A, yoshi051500317.akari@gmail.com;
Yokota S, Himawari6s@outlook.jp

For example, walking is a basic action of humans. Walking is beneficial and easy for the elderly to do, which leads to the promotion of health and the prevention of diseases and nursing care. However, it is not easy to continue, although we know about its benefits.

Assume that grandchildren send a gift of a pedometer to their grandfather or grandmother. They would make efforts of walking at first, but in most cases after a few months the pedometer would be put away in the closet.

Activities of Kenpo Juku (2008)

Our research center started a health promotion project, centered on walking, with the elderly who lived an independent life in Yurin district, Shimogyo-ku, Kyoto[1,2]. The name of the walking club, at starting time, was Poppo Juku. That club name was changed into "Kenpo Juku" in 2014. Professor Kojiro Ishii, who became a professor of Doshisha University from Hokkaido University in 2008, and Ryo Miyazaki, who is an associate professor of Shimane University at present, devoted themselves to establish the foundation of research, as the predecessor club activity was based on Hokkaido University. "Kenpo Juku" extended its activity to an enrolled volunteer group of students in 2019. We had the 15th anniversary of establishment with virtue of support of many people in 2023.

We had had a task to be solved; how we can encourage the elderly to continue their walking.

We started students' visiting the district communication center to meet members every month. Students asked the elderly in an affectionate manner, "How many steps do you walk this month?" Then data of the number of steps for a month, which were stored in members' pedometers, were recorded in PC by students.

The target of the number of steps was decided, to avoid inducing adverse events, "to add 1,500 steps to usual steps". Members who had 3,000 steps as usual were recommended 4,000–4,500 steps as a target. Members who reached 8,000 steps were recommended to keep the status quo and not increasing steps.

Members (the elderly) were motivated to keep walking as they wanted to impress students, who were as young as their grandchildren. "We are here to watch over you", which was a message from students, was a kind of positive reinforcement on members. It was suggested that the feeling of "I am observed by someone" stimulated and encouraged members, which resulted in the continuous walking activity.

An anti-aging medical checkup was conducted as annual evaluations. The term "anti-aging" had not been widely used at that time. Members raised their spirits, saying that they were undergoing "anti-aging". The checkup results of functional ages helped members to know how they were aging; being rejuvenated, maintaining the current conditions, and having aged. They could set up a target for one year ahead based on self-judgments. These motivations of members were a key to induce behavior modification.

Anti-aging medical checkups

Data analysis of members' steps exhibited various conditions. In spite of the mean age of members, 75.0 years old (2009), they walked steps approximately 7,000–8,000 steps per day on average. They walked a far longer distance than that of Japanese average[1,2]. No adverse events were reported and they were generally in good physical condition. To eliminate ambiguity regarding health status and obtain objective results, an anti-aging medical checkup was conducted annually.

Methods

Anti-aging medical checkup evaluated physical functional age and risk factors of aging (*Fig. 1*)[3,4]. Anti-Aging QOL Common Questionnaire (AAQOL) was employed to evaluate subjective and objective symptoms and risk factors (lifestyle)[5]. AAQOL, which can be applied "in different fields to obtain neutral assessments", are used for not only medical checkups but also for measuring effectiveness respecting functional food, cosmetics, integrated medical care (acupuncture and moxibustion therapy, aroma therapy, balneotherapy, and improvement in quality of sleep), and advanced medical treatments (stem cell therapy and others). AAQOL can be downloaded from the website of Japanese Society of Anti-aging Medicine (JAAM).

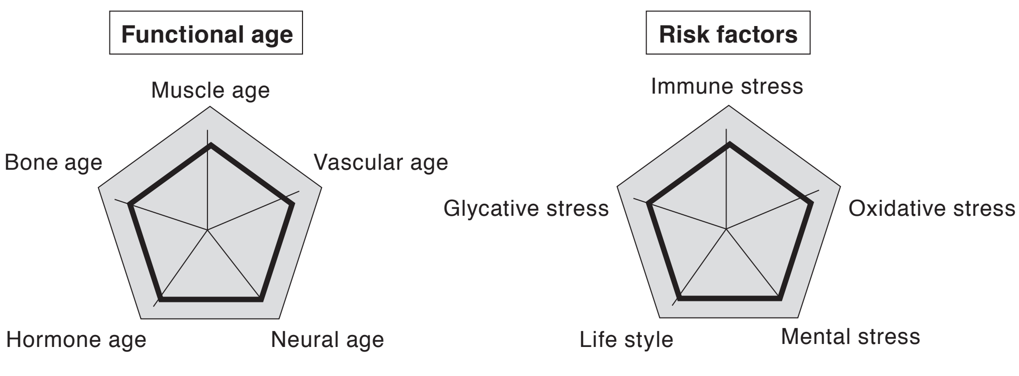

Fig. 1. **Measurement items for anti-aging medical checkups.**
Functional age is evaluated by comparing data on Japanese subjects from 2000 to 2005. It is important to determine which part of functional age is most advanced and which risk factors are most prominent. It is recommended that the most aged area and the greatest risk factor be corrected as a priority.

In questionnaires, mental and physical symptoms were divided and a five-grade evaluation was employed [5, 6]. Reevaluation was still in progress at the time of writing. ***Fig. 2*** shows assessments using Visual Analogue Scale (VAS) and Face Scale for each item respectively. Homology has been examined at present. Medical examinations by interviews were performed on lifestyle such as regular exercise habits, sleep, smoking, alcohol consumption, and the quantity of water intake. The questionnaire sheet of this study was downloaded from JAAM website: http://www.anti-aging.gr.jp/anti/clinical.phtml.

• Muscle age
Muscle age was obtained based on calculations of weight bearing index (WBI) [7], using a bioelectric impedance device (high-precision muscle mass meter, Physion MD, Physion, Kyoto, Japan). This study started to use a portable multi-frequency body composition analyzer for professional use in 2018 (Tanita Corporation, Tokyo, Japan). Muscle mass and basal metabolism were measured and sequentially, grip strength was measured by a grip dynamometer. Muscle age was obtained with references.
• Neural age
Wisconsin card sorting test (WCST) was used to measure higher brain functions [8].
• Vascular age
An acceleration plethysmogram by fingertip (DynaPulse SDP-100, Fukuda Denshi Co., Ltd., Tokyo) was used [9].
• Bone age
A supersonic bone assessment device (the independent group, the group requiring nursing care: A-1000, GE Yokokawa Systems Ltd., Tokyo).
• Hormone age
Insulin-like growth factor-I (IGF-I) and dehydroepiandrosterone-sulfate (DHEA-s) were measured [10]. Details of DHEA were described in the review by Yanase et al. [11] and in "*Wakagaeri hormone* [Rejuvenating hormone]" published by Shueisha Shinsho in 2023 [12].

Obtained data on body information were input in support software of anti-aging medical medical checkups (after several alternations, the software is converted into AAD LifeWorks). Sequentially, functional age and risk factor scores were calculated [13]. This software is applied to multiple devices and measurement methods, which enables the assessments of functional age and risk factors on an equal footing.

This study applied the rule of pirates (remove two of the strongest threats first, so remaining threats will be manageable) to defeat aging. That is, two significant items, which would be the most aged factors, out of ten biomarkers of aging, should be detected and corrected [4]. The target functional age was 80% of chronological age (or over 30 years old).

Research by Neurology Professor, Tony Wyss-Coray et al. of Stanford University (Stanford, CA, USA) suggested that when persons had a certain organ which aged faster than other organs, their risk for disease and death would increase [14]. The professor clarified that one out of 5 healthy persons, who were 50 years old or older, had at least one organ which was accelerated in aging speed.

Doctor Hägg et al. calculated three types of biological ages based on measurement values of 18 types of clinical biomarkers such as lipids in blood, fasting plasma glucose (FPG), blood pressure, pulmonary function, and body mass index (BMI) of subjects [15]. These three biological ages were Klemera-Doubal method age (KDMAge), which represented physiological age, and phenotypic age (PhenoAge), where predictive mortality was considered, and homeostatic dysregulation age (HDAge), which was computed based on regression models of biomarkers on age using healthy person samples. This study selected subjects who had no diagnosis of neurodegenerative disease (n = 325,870, mean age: 56.4, female ratio: 54.2%) from participants of UK bio-bank. Based on the data, relationships between biological ages and neurodegenerative diseases were examined. Age means, other than chronological age which represents the

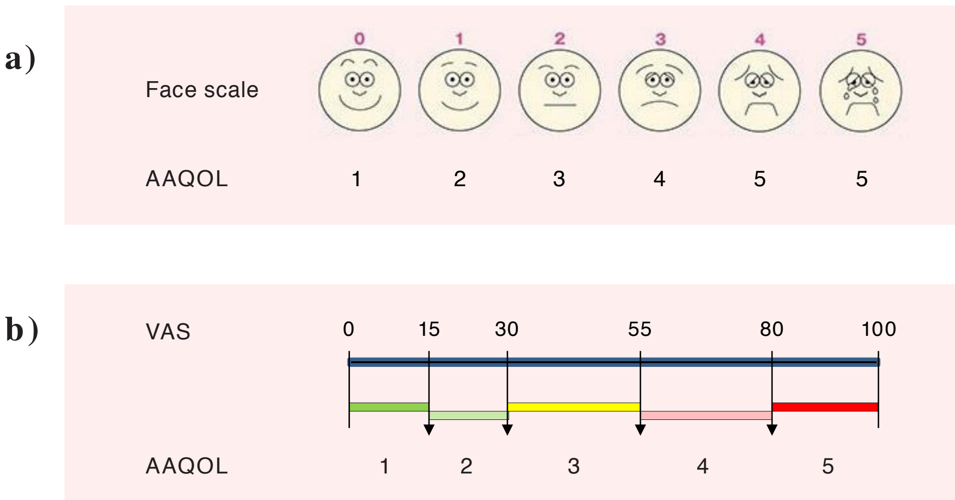

Fig. 2. **AAQOL assessment scale.**

The AAQOL scale shows the guidelines for dividing mental and physical symptoms into five levels, compared with **a)** the Face scale and **b)** the VAS. AAQOL, Anti-Aging Quality of Life common questionnaire; VAS, visual analogue scale.

number of years you've been alive, biological age, what is designated as biological or physiological age by scientists. How is biological age determined? Degree of aging and condition of health of individual differences are considered. The biological age is calculated with the length of telomeres, epigenetic clock, and values of diverse biomarkers. A new study in Sweden clarified that persons, whose biological age exceeded their chronological age, had higher risk of the onset of cognitive disorder and cerebral infarction (ischemic apoplexy).

According to a website of "Medical Care Premier" in digital Mainichi Newspaper, it shows how to estimate functional age via medical interview: https://mainichi.jp/premier/health/. This method is merely an estimated value. If you explain to patients after they have tried this method in the webisite that "the medical chekup will allow us to evaluate them with actual measurements," they will be more likely to understand the anti-aging medical checkup.

Although DNA methylation age[16] is proposed as an absolute evaluation method in functional age at present, it is not widely used and a weak point in aging cannot be determined. A comparative assessment method is simple: a curve line representing aging degrees is created on parameters and the level of age is obtained for present conditions. The target values of functional age are 80% of chronological age (age of 30 is a target for persons aged under 40). Examination values are expressed as mean ± SEM, plus 0.5–1.0.

Research Participants

Subjects, were all members of Kenpo Juku, including those who joined or left the club after December of 2008 (the commencement of this study)[1]. It is important for this project to continue the behavioral changes of the members. All participating members were provided with a pedometer and a paper with their step count information printed on it to encourage them to continue walking. There were 15 long-term observation cases (8 men, 7 women, average age 85.2 years, as of 2023) who had been examined more than 11 times since 2008.

Intervention methods

Members were lent a pedometer with two-axis acceleration sensor (Walking Style HJ-720IT, Omron Healthcare Co., Ltd., Kyoto, Japan) and provided an instruction of walking while wearing the pedometer every day during the study of interventions; they were told to continuously wear the pedometer on their lumbar except for sleep, bathing, and occasions of soaking in water. Timing of walking was not instructed and they walked according to their individual lifestyle.

Previous studies suggested that pedometer accuracy was not altered by slim figure, overweight figure or corpulent figure[17]. The pedometer recorded step data for 42 days at the longest. Collected data was recorded in PCs monthly using its dedicated software (BI-Link Professional Edition 2.0, Omron). Step data, which had a wearing time of 12 hours or longer, were adopted to eliminate data where a pedometer was forgotten.

Distribution of printed papers

Printed papers were created once a month and hand-delivered[1], motivating members to continue walking activities; these included a breakdown, an individual walking steps performance sheet and a walking steps performance sheet for all the members.

First, an individual walking steps performance sheet showed the average of the number of steps per day for the month, which was obtained with the calculation based on the collected individual monthly data. In addition, the individual performance sheet included the next-month target for the quantity of activity. The next-month target of steps was created as follows; based on past data, seasonal fluctuations and individual diseases and physical conditions were considered. Within a reasonable range, any strain would not be imposed. Furthermore, a conversion of the activity quantity from walking to suggesting specific sports, was effective to motivate the elderly.

Second, other than an individual sheet, a step performance sheet for all the members showed top five members' name and the performance of activity quantity in a descending order. Information on physical activity was provided in the accordance of the consciousness level of subjects and writing styles were intended to be simple and clear without technical terms.

Body measurement

Body measurement employed a portable multi-frequency body composition analyzer for professional use (Tanita). The measurement method of this device is 4 Compartment (4C) model analyzing body compositions via algorithm on sex, age, and body shape. Furthermore, body compositions (body adipose, lean body mass, muscle mass, and body water content) were obtained in non-invasive measurements from electric resistances by passing an electric current through the body. Blood pressure was measured by an upper-arm type automatic sphygmomanometer (Omron).

Biochemical examination of blood

Biochemical examinations measured the blood concentrations of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein cholesterol (HLD-C), fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), national glycohemoglobin standardization program (NGSP), insulin (immunoreactive insulin: IRI), insulin-like growth factor-1 (IGF-1), dehydroepiandrosterone-sulfate (DHEA-s), cortisol, uric acid, leukocyte count, erythrocyte count, hemoglobin, differential count of leukocytes, and pentosidine.

Blood of subjects was collected on the day of anti-aging medical checkup, and measurement was entrusted to LSI Medience Corporation (Tokyo).

Bone age measurement

For the measurement of bone quantity, a supersonic method (A-1000; GE Yokokawa) was used[18-20]. Bone age was calculated with the stiffness value of malleolus and % young adult mean (%YAM) as indexes.

This device employs dual-energy X-ray absorptiometry, which is excellent in accuracy and reproducibility, and has a strong correlation with the malleolus density ($r = 0.6 – 0.8$).

Stiffness values are bone density obtained with calculations using propagation velocity of ultrasonic wave and attenuation coefficient, and an index which reflects bone structures and substance. Therefore, early detection and diagnosis of osteoporosis could be enabled by the use of this device.

Neural age measurement

The higher brain function was used for the calculation of neural age and was measured by Wisconsin card sorting test (WCST)[4, 21-24]. Neural age was calculated based on the parameters: categories achieved (CA), numbers of response cards used until the first category achieved (NUCA), total errors (TE), perseverative errors of Milner (PEM), perseverative errors of Nelson (PEN), unique errors (UE), and reaction time.

Wisconsin card sorting test is a neuropsychological test on abstract behavior and shift of set, and one of the tests for frontal lobe function assessment. High brain dysfunction including the frontal lobe induces occurrences of disorders regarding language, actions, cognition, memory, and attentiveness, which cannot be explained by movement disorder, disorder of sensation, and impaired consciousness. Personal ability, behaviors, and activity affected and social activities such as communication have difficulties. Wisconsin card sorting test is applied for early detection of attention deficit hyper activity disorder (ADHD) for children and adults[25-27].

Vascular age measurement

The degree of arteriosclerosis, which was used for the calculation of vascular age, was measured using an acceleration plethysmogram (Dynapulse SDP-100, Fukuda Denshi)[28,29]. The obtained estimated vascular age represented vascular age[9].

Brain wave is a continuous blood vessel propagating wave induced by intra-arterial pressure alternation. Acceleration of pulse waveform is obtained as follows; original waves of intra-arterial pressure alternation are replaced with fingertip volume pulse waves. Differentiation is performed twice. Components of acceleration of pulse waveform are a–e wave. Estimated vascular age is calculated with SDPTG aging index (SDPTGAI) of alternations of wave height ratios in each wave of aging, arteriosclerosis, and high blood pressure.

Measurement of glycative stress

Glycative stress refers to a condition in which excess aldehydes are easily produced in the body, making proteins in the body highly susceptible to conversion into advanced glycation endoproducts (AGEs). As reported[30,31], skin autofluorescence (SAF), which is integral data of autofluorescence value derived from mainly AGEs, was measured using an AGE Reader™ (DiagnOptics, Groningen, Netherlands)[32]. Skin autofluorescence is an index of glycative stress. Glycative stress score was obtained as follows; based on the aging curve of SAF value[33], values of measurement results were input to AAD Life Works.

AGE Reader™, by irradiation of black light, measured autofluorescence of excited AGEs so that accumulation quantity of the skin can be measured in a simple and noninvasive manner.

It has been confirmed by skin biopsy for diabetic patients and dialysis patients that skin SAF is strongly correlated to the accumulation of typical AGEs including pentosidine and N^{ε}-(carboxymethyl) lysine (CML)[30]. The measurement was made at a site 10 cm distant from the elbow at right and upper dorsal part of upper arm in the posture of extending an arm horizontally to the ground and bending the elbow 90 degrees. In order to avoid the effect of sunscreen and others, the measurement was conducted after the site was disinfected with alcohol[34]. It is proven that the higher the SAF value is, the more AGEs are accumulated. It is higher in patients with type 2 diabetes and it also increases with age in the case of healthy people[35].

Measurement of oxidative stress

Oxidative stress is the state of excessive reactive oxygen species (ROS) and/or free radical. Degree of oxidative stress was obtained as follows: Japan institute of the control of aging, Nikken Seil Co., Ltd. was enlisted to conduct a Diacron-reactive oxygen metabolites (d-ROM) Test for oxidizability examinations as well as a Biological Antioxident Potential (BAP) Test as anti-oxidative activity examinations. Obtained data was provided to AAD Life Works and an oxidative stress score was calculated.

A test of d-ROM examined reactions of radical protons, which occurred after chromogen in coloring solution was oxidized by free radicals. Absorbency after the conversion of color change was measured, which was assessed by quantification of hydroperoxide.

A BAP test evaluated oxidizability of plasma, adding plasma to FeC13 and measuring color change, which was induced due to oxidation-reduction reaction via antioxidant in plasma, using a photometer.

Statistical analysis

Comparative analysis of all measurement values employed AAD Life Works (AA research Center, Doshisha University). Data visualization of aging in each test results and surveys on differences between functional age and chronological age used Excel.

In regard to correlations between the number of steps over time and results of health examinations, correlation coefficient r was calculated according to the following equation:

$$r = \frac{\frac{1}{n}\sum_{i=1}^{n}(x_i - \acute{x})(y_i - \acute{y})}{\sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - \acute{x})^2}\ \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \acute{y})^2}}$$

A paired t-test was used for the comparison between the number of steps over time and bone density. A one-side test was employed and a p-value less than 0.05 was considered statistically significant.

Public medical care expenditure

Subjects were asked to voluntarily provide information on their medical expenses for one year from January to December of 2023.

Among 36 Kenpo Juku enrolled members, 22 members (nine males and thirteen females), with a response rate of 61%, provided information on medical expenses. Further, the number of participants providing medical information of long-term observation cases was 12 among 15 (7 males and 5 females). National medical care expenditure of the fiscal year 2022 (FY2022)[36] expressed as percentage (%), being compared with adjusted values by gender, age group, and prefecture. "National medical care expenditure" is estimated cost of expenses for medical treatments covered by health insurance in medical institutions during the fiscal year. This includes costs for medical and dental treatment, pharmaceutical dispensing, hospital room and board, and in-home nursing care. What is not covered by the medical insurance system in Japan includes treatments to be evaluated such as advanced medical care, patient-selected services (extra room charge and erectile and dental metal) and assisted reproductive technology of fertility treatments.

Ethics review

The subjects were fully informed about the period, location, contents and method of the research, as well as the advantage and disadvantage expected to be caused by participating in this research. Their written consents were received. This research obtained the approval of the Ethical Committee of Doshisha University (Application Number: #0832, #14089, #17093, #21018).

Results

2010: Comparison among groups of different nursing care levels

With the purpose of the understanding of characteristics of members living independently, collected performance data are shown. Three types of subjects with data results consisting of 43 independent subjects (17 males and 26 females, age: 68.9 ± 6.3), 31 subjects requiring assistance (18 males and 13 females, age: 77.8 ± 7.2), who attended a day care giving office (Day Service Dandan, Kyoto, Japan), and 19 subjects requiring nursing care (3 males and 16 females, age: 83.7 ± 6.8)[37-40]. Hormone age assessments were not performed for group of subjects requiring assistance.

Fig. 3 and ***4*** show data results of functional age and Δfunctional age (= functional age − chronological age). Even if ages of three groups were different, the usage of Δfunctional age enabled the adjustment of chronological age differences. Group of independent subjects indicated a trend of maintaining a functional age young. Statistical analysis with adjustment suggested that Δneural had a conspicuous character where the higher nursing care degree was, the more advanced aging there was. Therefore, it was suggested that a key factor to live an independent life in advanced age was to maintain cognitive functions.

2012–2014: Alternation of functional age for a long period of time

With functional age change over time, correlations between the number of steps per day and glycative stress indexes were analyzed for 20 subjects who underwent a checkup for three consecutive years since 2012 (12 males

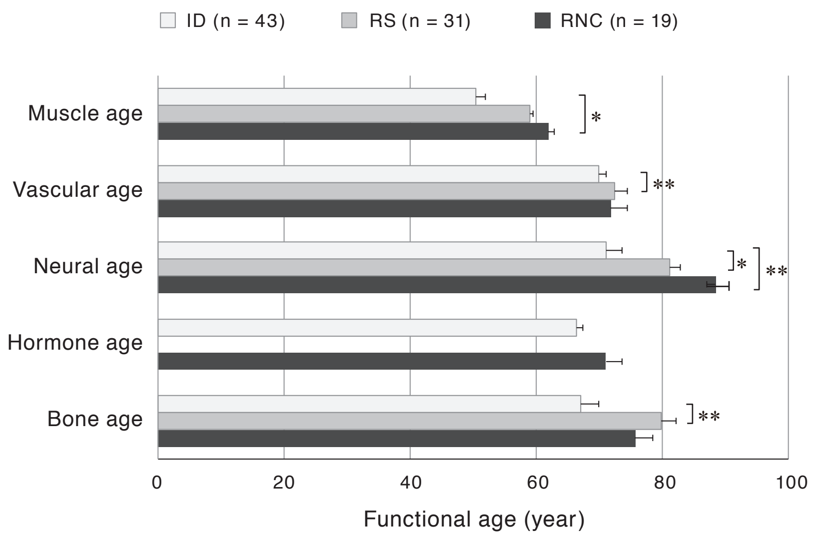

Fig. 3. **Degree of independence in daily living and functional age.**

Results are expressed as mean ± SEM, *p < 0.05, **p < 0.01, Tukey test. ID, independent living subjects (Kenpo Juku member), n = 43; RS, persons requiring support, n = 31; RNC, persons requiring nursing care, n = 19; SEM, standard error mean. Figure quoted from Reference 39.

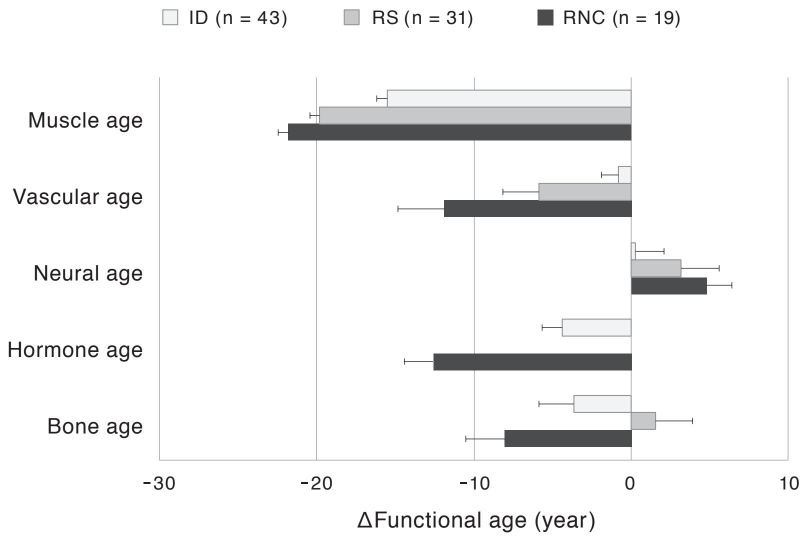

Fig. 4. **Degree of independence in daily living and ΔFunctional age.**

Results are expressed as mean ± SEM. Age adjustment can be made by comparing Δfunctional age (= functional age − chronological age). ΔNeural age increases as the level of independent living decreases. ID, independent living subjects (Kenpo Juku member), n = 43; RS, persons requiring support, n = 31; RNC, persons requiring nursing care, n = 19; SEM, standard error mean. Figure quoted from Reference 39.

and 8 females, mean age: 73.7). Average steps were 10,900 steps in 2012, 9,700 steps in 2013, and 9,300 steps in 2014. Although the number of steps slightly decreased, the number of steps of subjects, Kenpo Juku members, was twice as many as that of persons in their 70s according to Exercise and Physical Activity Guidelines of Ministry of Health and Labour Welfare (MHLW).

Trends of functional age (mean value) examinations demonstrated that all five items of functional age were maintained younger than chronological age. (***Fig. 5***) Muscle age, in particular, was in excellent condition. Neural age remained almost the same and no notable aging signs were recognized.

Glycative-stress-related indexes are shown in ***Table 1***. Analyzed results demonstrated that SAF, which represents accumulation of AGEs, and HbA1c decreased successfully. SAF values of Kenpo Juku members were significantly lower in comparison with persons who were close to members' age. Members can be placed in a group who had low glycative stress due to the exertion of daily effort in walking.

Kenpo Juku members received public medical care consisting of national medical insurance as well as lifestyle improvements. Survey on pharmacotherapy for 34 subjects (15 subjects were in a long-term observation) (2016) reported types of diseases present in descending order, 21 subjects taking hypotensive drugs, 11 subjects taking medicines for hyperlipemia, 10 subjects taking medicines for osteoporosis, nine subjects taking acid reducer, five subjects taking nonsteroidal anti inflammatory drugs, five subjects taking antidiabetic, and five subjects taking hypnotic (***Fig. 6***)[41]. These subjects did not received replacement therapy of estrogen, androgen, DHEA, somatotropin, or melatonin. Comparative examination between subjects with and without osteoporosis recognized no significant difference in Δbone age. It was suggested that patients with osteoporosis who had appropriate medical treatments maintained a good condition regarding bone density and bone age. Δvascular age was significantly higher in subjects taking hypotensive drugs than subjects without medicines (Δvascular age, with medicines: −10.1 ± 8.6, without medicines: −19.8 ± 4.1, p = 0.019). Along with development in arteriosclerosis, patients with hypertension were older in vascular age. Acceleration plethysmography by fingertip is a measurement method which sharply reflects tension angiosclerosis of the vascular wall. There is a possibility that measurement value could decrease due to a hypotensive drug. Nevertheless, subjects taking medicines were older in vascular age.

2019: Evaluation of cognitive disorder index Aβ40/42 ratio

Several causative diseases induce cognitive disorders, in descending order, Alzheimer type dementia (67.6%), cerebrovascular dementia (19.5%), Lewy body dementias (4.3%), and others. Most cases of dementia in elderly persons were combinations of these types. Advanced disorders are difficult to treat. Therefore, it is important to detect signs and symptoms and conduct preventive interventions. Measurement of neural age is considered an effective method.

Alzheimer's disease (AD) is a disease, in a term of pathophysiology, where aggregation of amyloid β (Aβ) is accumulated extracellularly and aggregation of tau protein is accumulated intracellularly. Consequently, synapse impairments and apoptosis are induced. Plasma Aβ40/42 ratio is high in the state of decreased Aβ clearance and increased Aβ accumulation. Therefore, plasma Aβ40/42 ratio is used as an index for AD risk.

The measurement in Aβ40/42 ratio of 36 subjects (age: 79.3 ± 6.4) (2019) was compared with 21 Doshisha University faculty and staff (age: 49.3 ± 12.9) as well as subjects with sleep disorders (age: 50.1 ± 4.9) (***Fig. 7***)[42, 43]. The group of Doshisha University faculty and staff consisted of recipients of anti-aging medical checkup (checkup for faculty and staff)

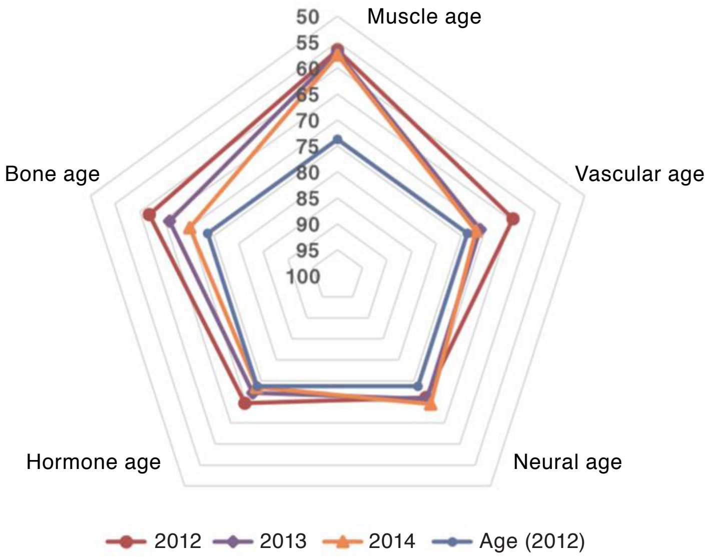

Fig. 5.
Changes in functional age over time in Kenpo Juku members.
Average age in 2012 was 73.7 years old, n = 20. During this time, chronological age increases by one year each time, and the inner pentagon becomes smaller. Figure quoted from Reference 40.

Table 1. Changes in glycative stress-related indices in Kenpo Juku members.

		2012	2013	2014	p value
TC	mg/dL	220.8 ± 33.5	212 ± 27.4	219.6 ± 37.1	0.826
HDL-C	mg/dL	70.9 ± 18.9	68.5 ± 16.8	66.5 ± 14.8	0.247
LDL-C	mg/dL	124.2 ± 23.7	119.7 ± 17.7	128.1 ± 28.5	0.641
FPG	mg/dL	94.8 ± 16.5	94.9 ± 20.5	101.1 ± 26.5	0.287
HbA1c	%	**6.4 ± 1.2**	**6.0 ± 0.7**	**5.9 ± 0.6***	**0.048**
IRI	µU/mL	4.4 ± 2.0	3.5 ± 1.4	5.0 ± 4.5	0.168
SAF		**2.47 ± 0.37**	**2.25 ± 0.28**	**2.01 ± 0.38****	**0.005**

Results are expressed as mean ± SEM, *p < 0.05, **p < 0.01, compared with 2012, n = 20, Tukey test. TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; FPG, fating plasma glucose; IRI, immunoreactive insulin; SAF; skin autofluorescence measured by AGE Reader; SEM, standard error mean. Data quoted from Reference 40.

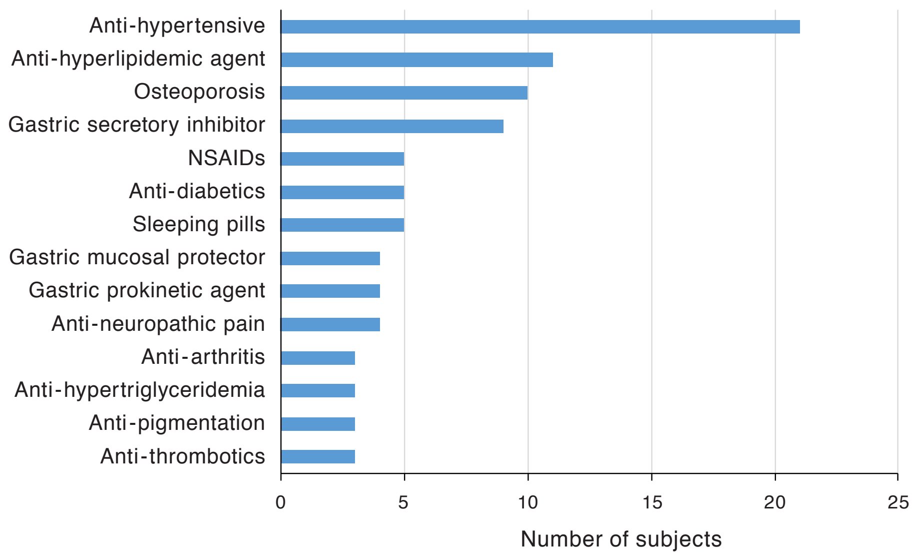

Fig. 6. **Medication status of Kenpo Juku members.**
Some members are taking multiple medications. 2016 survey, n = 34. Figure quoted from Reference 41.

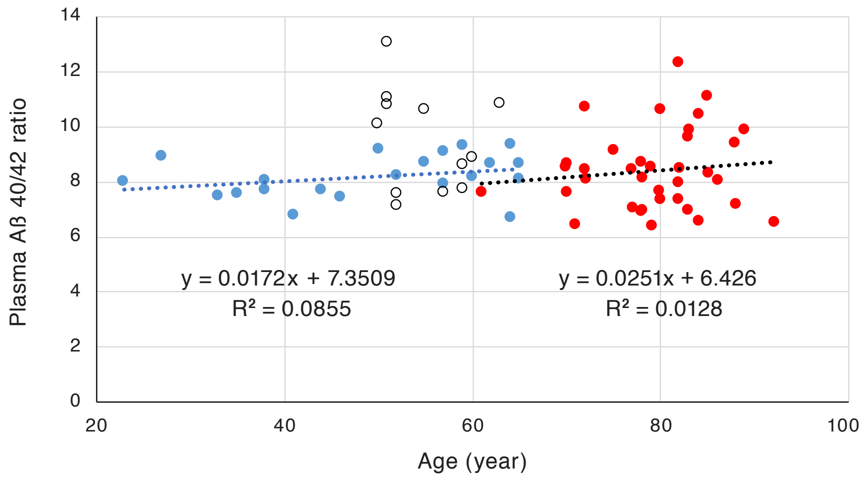

Fig. 7. **Age-related changes in plasma Aβ40/42 ratio.**
University faculty and staff (●), they are a highly health-conscious group; independent elderly in Kenpo (●), a group of people who walk about 7,000 steps a day more than the average of the same generation and have less glycative stress; subjects with sleep disorders (○), n = 12. In a comparative analysis of the average values, those with poor "sleep quality" had a significantly higher Aβ40/42 ratio than university faculty and staff and Kenpo Juku members. Figure quoted from Reference 42, 43.

and were health conscious. Aβ40/42 ratio slowly but steadily increases along with aging. In comparison with overall Japanese, regression line is expected to be shifted downward.

Aβ40/42 ratio of the members had a variation. They were polarized on the border of the regression line of age. Approximately one third maintained lower values than the regression line. From the view of correlation with glycative stress, Aβ40/42 ratio had positive correlations with HbA1C, IRI, pentosidine, and SAF (data not shown).

Walking habit is regarded as effective to prevent the progression of dementia. A cross-sectional study with 18,766 females aged 70–81 years reported that practitioners of walking had excellent cognitive functions and the decline of cognitive functions was minor [44]. Another cross-sectional study with 2,257 males aged 71–93 years reported that risks of the onset of cognitive function impairments decreased due to walking exercise [45]. It is considered that a walking habit, which induced the state of weak glycative stress, contributed to the retention of Aβ clearance.

Further, the group of subjects with poor "sleep quality" was added to this examination and was compared. Aβ40/42 ratio of subjects with a poor "sleep quality" were significantly higher than any other groups. It was suggested that the reduction of "sleep quality" resulted in a risk of decreased Aβ clearance. Consequently, synapse impairments and apoptosis are induced. Plasma Aβ40/42 ratio is high in the state of decreased Aβ clearance and increased Aβ accumulation. These findings suggest that the plasma Aβ40/42 ratio may reflect Aβ clearance.

2022: Data trends of long-term observation cases

Trends in data for the long-term observation cases (15 cases) between 2008–2022 are shown in ***Tables 2***, ***3***, and ***4***. Values of subjects and ratios related to healthy persons were respectively shown regarding BMI, BP, FPG, LDL-C, LDL-D, IGF-I, DHEA-s, and bone stiffness (%YAM). Items which were within normal range were BMI (normal range, 21–23): 73% or higher, and diastolic blood-pressure (70–85 mmHg): 64% or higher. However, normal range of systolic blood-pressure was 120–140 mmHg, and results of data were 20%–47% and there were many subjects with high blood pressure (boundary region was included). Normal range of LDL-C was 70～140 mg/dL and 80% or greater of males were within the normal range and 71% or greater of females were within the normal range. There were many males and females that were in good condition (88% or higher) regarding HDL-C (male: 40–86 mg/dL, female: 40–96 mg/dL). As for bone stiffness (%YAM, 80% or higher), many males were in good condition (75% or higher). However, only 29% or fewer of females were in good condition. Reduction in bone stiffness was remarkable among subjects taking no medicines for osteoporosis.

Regarding DHEA-s and IGF-I, subjects who exceeded the regression line were in good condition based on the collected data (***Fig. 8***). Values of DHEA-s were mostly good with both males and females (86% or higher of males and 83% or higher of females). Subjects in good conditions on IGF-I had been 75% or greater of males and 71% or greater of females until 2020. However, the ratio decreased to 50% in 2022. This was data of the group taking no hormonal replacement therapy.

To verify effects on the body by physical activities with a core of walking, a correlation analysis was conducted with the number of steps per day (***Fig. 9***). There was a positive correlation between steps and bone stiffness. However, there was no correlation recognized with DHEA-s and IGF-I. DHEA-s concentrations of subjects were maintained at high levels, in spite of advanced age, in comparison with the average of the same generation. Thus, there was a possibility that the long-term walking activity contributed to the secretion of DHEA and the maintenance of homeostasis.

Table 2. Trends in data for long-term observation cases: BMI, BP and FPG.

	n	BMI (kg/m^2)		BP (diastolic) (mmHg)		(systolic) (mmHg)		FPG (mg/dL)	
2008	12	22.6 ± 2.3	83%	77 ± 18	83%	146 ± 26	0.33	100 ± 14	83%
2009	12	22.3 ± 2.3	83%	80 ± 15	67%	149 ± 24	0.25	91 ± 14	91%
2010	13	21.9 ± 2.3	85%	81 ± 16	69%	141 ± 24	0.46	97 ± 13	69%
2011	14	22.7 ± 2.6	79%	81 ± 15	64%	150 ± 24	0.29	ND	
2012	15	22.6 ± 2.6	87%	76 ± 16	80%	145 ± 23	0.47	87 ± 11	100%
2013	15	22.7 ± 2.7	67%	78 ± 11	67%	142 ± 22	0.4	91 ± 13	93%
2014	14	21.9 ± 1.9	93%	75 ± 16	79%	144 ± 30	0.43	93 ± 15	85%
2015	15	22.7 ± 2.2	73%	78 ± 12	80%	147 ± 23	0.4	ND	
2016	15	22.4 ± 2.1	93%	78 ± 13	86%	148 ± 22	0.36	ND	
2017	14	22.8 ± 2.2	79%	79 ± 12	79%	153 ± 19	0.29	89 ± 17	93%
2018	15	22.5 ± 2.4	73%	80 ± 10	80%	152 ± 21	0.2	95 ± 17	80%
2019	15	22.4 ± 2.5	87%	79 ± 11	87%	143 ± 24	0.4	87 ± 8	100%
2020	15	22.2 ± 2.7	80%	83 ± 13	80%	162 ± 29	0.2	97 ± 13	73%
2021	13	21.4 ± 2.7	77%	74 ± 13	92%	149 ± 21	0.23	98 ± 11	77%
2022	12	22.3 ± 2.6	83%	81 ± 13	75%	156 ± 23	0.25	97 ± 13	75%

Results are expressed as mean ± SEM. Percentage indicates ratio of cases within normal range. BMI, body mass index (normal range: 20–24); BP, blood pressure (diastolic 70–85 mmHg; systolic, 120–140 mmHg); FPG, fasting plasma glucose (normal range: 80–105 mg/dL); ND, no data; SEM, standard error mean.

Table 3. Trends in data for long-term observation cases: LDL-C, LDL-D and bone stiffness.

	n (male)	n (female)	LDL-C (male) (mg/dL)		(female) (mg/dL)		HDL-C (male) (mg/dL)		(female) (mg/dL)		Bone stiffness (male) (%YAM)		(female) (%YAM)	
2008	6	6	123 ± 18	100%	119 ± 20	100%	62 ± 13	100%	70 ± 15	100%	91.0 ± 11.6	83%	69.3 ± 4.1	0%
2009	5	7	120 ± 11	100%	115 ± 11	100%	64 ± 14	100%	72 ± 18	100%	96.2 ± 6.9	100%	69.0 ± 4.6	17%
2010	6	7	121 ± 18	100%	145 ± 22	100%	70 ± 16	100%	74 ± 19	100%	87.3 ± 11.6	86%	66.9 ± 5.9	14%
2011	7	7	117 ± 18	100%	135 ± 19	100%	67 ± 14	100%	77 ± 15	100%	75.3 ± 20.4	43%	84.0 ± 14.6	25%
2012	8	7	133 ± 37	88%	116 ± 28	71%	66 ± 16	100%	74 ± 19	100%	85.8 ± 13.1	75%	60.2 ± 4.7	14%
2013	8	7	122 ± 8	100%	123 ± 23	100%	73 ± 21	100%	77 ± 20	100%	88.7 ± 11.6	63%	68.5 ± 3.8	14%
2014	7	6	112 ± 12	100%	129 ± 19	100%	73 ± 17	100%	67 ± 18	100%	79.6 ± 13.9	50%	67.5 ± 6.3	29%
2015	5	4	107 ± 25	80%	128 ± 28	100%	63 ± 16	100%	73 ± 13	100%	ND		ND	
2016	8	7	114 ± 19	100%	123 ± 31	86%	73 ± 20	88%	82 ± 21	100%	ND		ND	
2017	7	7	104 ± 9	100%	121 ± 17	100%	68 ± 15	100%	73 ± 13	100%	82.3 ± 8.5	63%	71.0 ± 5.5	14%
2018	8	7	122 ± 24	100%	125 ± 22	100%	72 ± 16	100%	73 ± 12	100%	80.5 ± 11.5	63%	65.3 ± 4.4	0%
2019	8	7	116 ± 14	100%	126 ± 23	100%	77 ± 19	100%	79 ± 18	100%	78.0 ± 12.9	63%	66.0 ± 5.0	0%
2020	8	7	112 ± 16	100%	125 ± 22	100%	77 ± 17	100%	75 ± 13	100%	82.0 ± 13.0	63%	67.7 ± 4.6	14%
2021	6	7	102 ± 11	100%	123 ± 29	86%	80 ± 15	100%	79 ± 18	100%	74.8 ± 13.5	50%	61.0 ± 4.4	14%
2022	6	6	104 ± 10	100%	123 ± 31	83%	82 ± 16	100%	76 ± 10	100%	84.5 ± 11.6	83%	66.6 ± 3.7	0%

Results are expressed as mean ± SEM. Percentage indicates ratio of cases within normal range. LDL-C, low-density lipoprotein-cholesterol (normal range; 70–140 mg/dL); HDL-C, high-density lipoprotein-cholesterol (normal range; male 40–86 mg/dL, female 40–96 mg/dL); bone stiffness measured by ultrasonografy (normal range: %YAM > 80%); YAM, young adult mean; ND, no data; SEM, standard error mean.

Table 4. Trends in data for long-term observation cases: DHEA-s and IGF-I.

	n (male)	n (female)	IGF-I (male) (ng/mL)		(female) (ng/mL)		DHEA-s (male) (µg/dL)		(female) (µg/dL)	
2008	6	6	159 ± 40	100%	131 ± 46	83%	108 ± 73	100%	46 ± 20	100%
2009	5	7	161 ± 42	100%	127 ± 24	86%	ND		ND	
2010	6	7	143 ± 27	100%	122 ± 29	100%	109 ± 71	100%	45 ± 21	100%
2011	7	7	148 ± 31	100%	159 ± 48	67%	102 ± 52	100%	129 ± 63	83%
2012	8	7	148 ± 31	100%	116 ± 33	86%	78 ± 46	100%	33 ± 17	100%
2013	8	7	104 ± 27	88%	94 ± 18	100%	91 ± 57	100%	41 ± 16	100%
2014	7	6	106 ± 23	100%	82 ± 20	83%	105 ± 80	86%	41 ± 15	100%
2015	5	4	98 ± 21	100%	92 ± 19	100%	100 ± 57	100%	53 ± 21	100%
2016	8	7	93 ± 27	75%	75 ± 14	86%	107 ± 71	100%	50 ± 18	100%
2017	7	7	104 ± 31	86%	89 ± 27	86%	90 ± 58	100%	34 ± 14	100%
2018	8	7	101 ± 37	88%	84 ± 18	71%	106 ± 60	100%	38 ± 11	100%
2019	8	7	104 ± 36	88%	78 ± 17	71%	98 ± 59	100%	40 ± 12	100%
2020	8	7	106 ± 51	75%	86 ± 22	86%	102 ± 58	100%	37 ± 9	100%
2021	6	7	ND		ND		106 ± 50	100%	51 ± 30	100%
2022	6	6	84 ± 46	50%	6 ± 16	50%	100 ± 53	100%	41 ± 14	100%

Results are expressed as mean ± SEM. Percentage indicate ratio of cases within normal range. DHEA-s, dehydroepiandrosterone-sulfate (normal range: over the regression line in **Fig. 8**); IGF-I, insulin-like growth factor-I (normal range: over the regression line in **Fig. 8**); ND, no data; SEM, standard error mean.

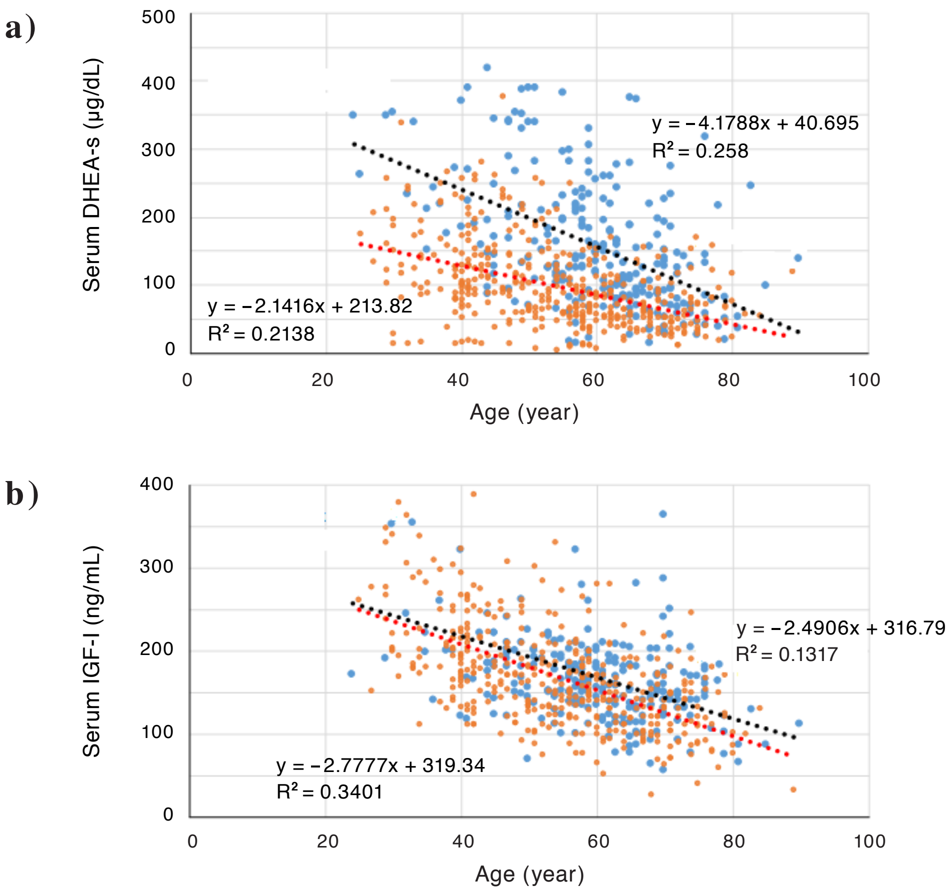

Fig. 8. **Reference data for serum DHEA-s (a) and IGF-I (b).**
n = 1,186 (●male: n = 593, ●female: n = 593). Data compiled by the Anti-Aging Medical Research Center, Doshisha University in 2022. DHEA-s, dehydroepiandrosterone-sulfate; IGF-I, insulin-like growth factor-I.

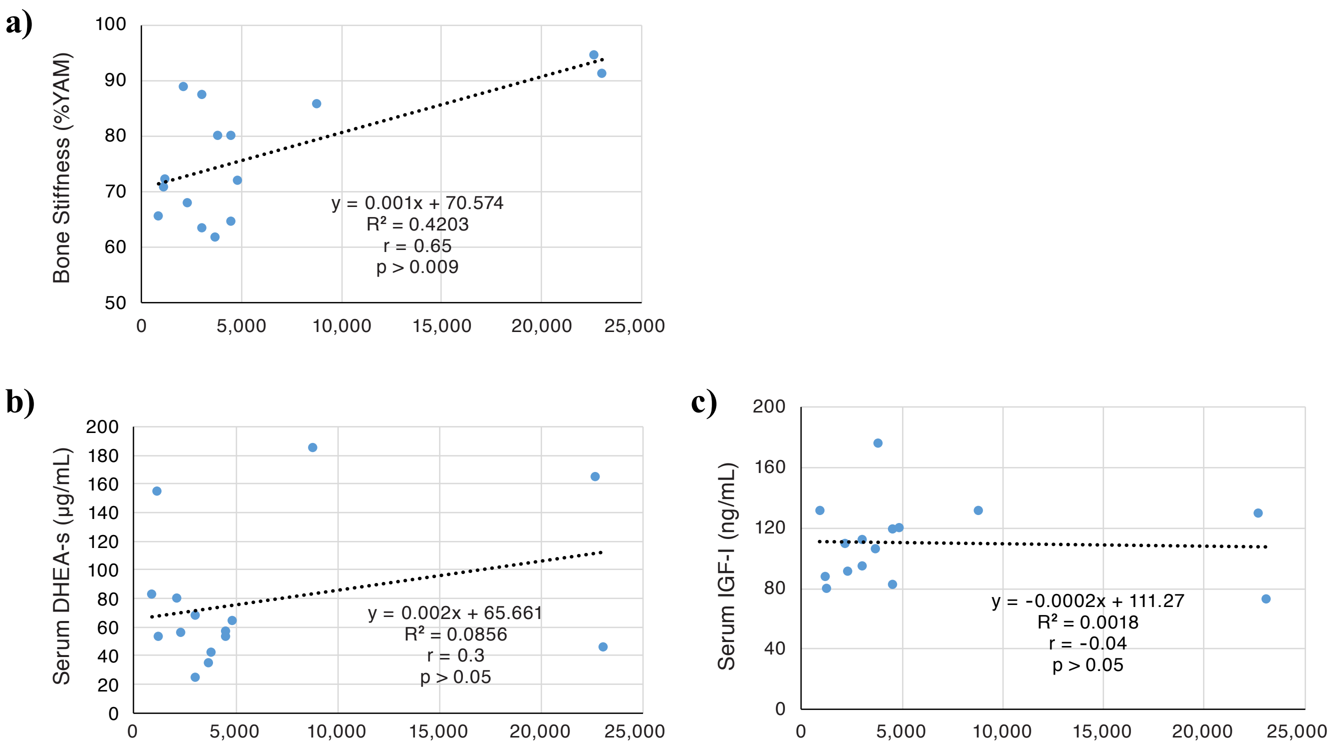

Fig. 9. **Correlation analysis with average number of steps per day.**
a) Bone stiffness, **b)** serum DHEA-s, **c)** serum IGF-I. Pearson's correlation analysis, n = 15. YAM, young adult mean; DHEA-s, dehydroepiandrosterone-sulfate; IGF-I, insulin-like growth factor-I.

Trends of individual data in functional age were shown in ***Figs. 10***, ***11***. Subjects, whose functional ages in all five items were younger than their chronological ages, were 3 males (M3, M5, and M6) and 2 females (F3 and F4). All of these cases in the long-term observation took one or more medicines of hypotensive drugs, antidiabetics, statin drugs, and medicines for osteoporosis. Their functional age in five types of ages advanced in a well-balanced fashion, which is the goal we should aim for. It has been suggested that a long-standing habit of walking, in combination with a course of medications, has positive impacts on diverse physical functions and contributes to extend healthy life expectancy.

2023: Analysis of medical expenses

A survey on medical expenses in 2023 was conducted and among 36 Kenpo Juku enrolled members, information was collected from 22 members (a response rate: 61 %). Their medical expenses were mostly lower than average expenses of Japanese (FY2022, by age group and sex) (***Table 5***)[36]. Regarding medical expenses of females, the ratio of all survey participant females (n = 13) was 96.5%. However, in the long-term observation cases, the ratio of female cases (n = 5) was 64.2%. There was a large difference. The value of 95% CI was also large, it was suggested that there were large differences depending on individual circumstances and by fiscal year. It was assumed that there were variabilities on medical expenses by fiscal year. Medical expenses of 8 cases among 12 long-observation cases were 60% or lower in comparison to average expenses. All of these cases were subjects living independently in a good health condition. We expect that walking-based health promotion interventions could contribute to the health promotion and the reduction of public medical expenses.

Conclusion

Walking-based health promotion activities have been performed in Kenpo Juku since 2008. The number of steps per day of participants was approximately twice as many as those of the same generation. The results were verified by anti-aging medical checkups. Our project was not conducted on a large scale. However, that members have continued for 15 years is a great accomplishment. The all members aimed to build up bodies that do not require nursing care. As a result, most members were in a good shape and kept living independently. It was suggested that morbidity was low and medical expense was low.

Anti-aging medical checkups played two roles through the activities. One was the collection of information. Interesting analysis results were obtained. The other was to motivate members to continue long-term health promotion activities as they had goals for the prevention of functional aging and rejuvenation.

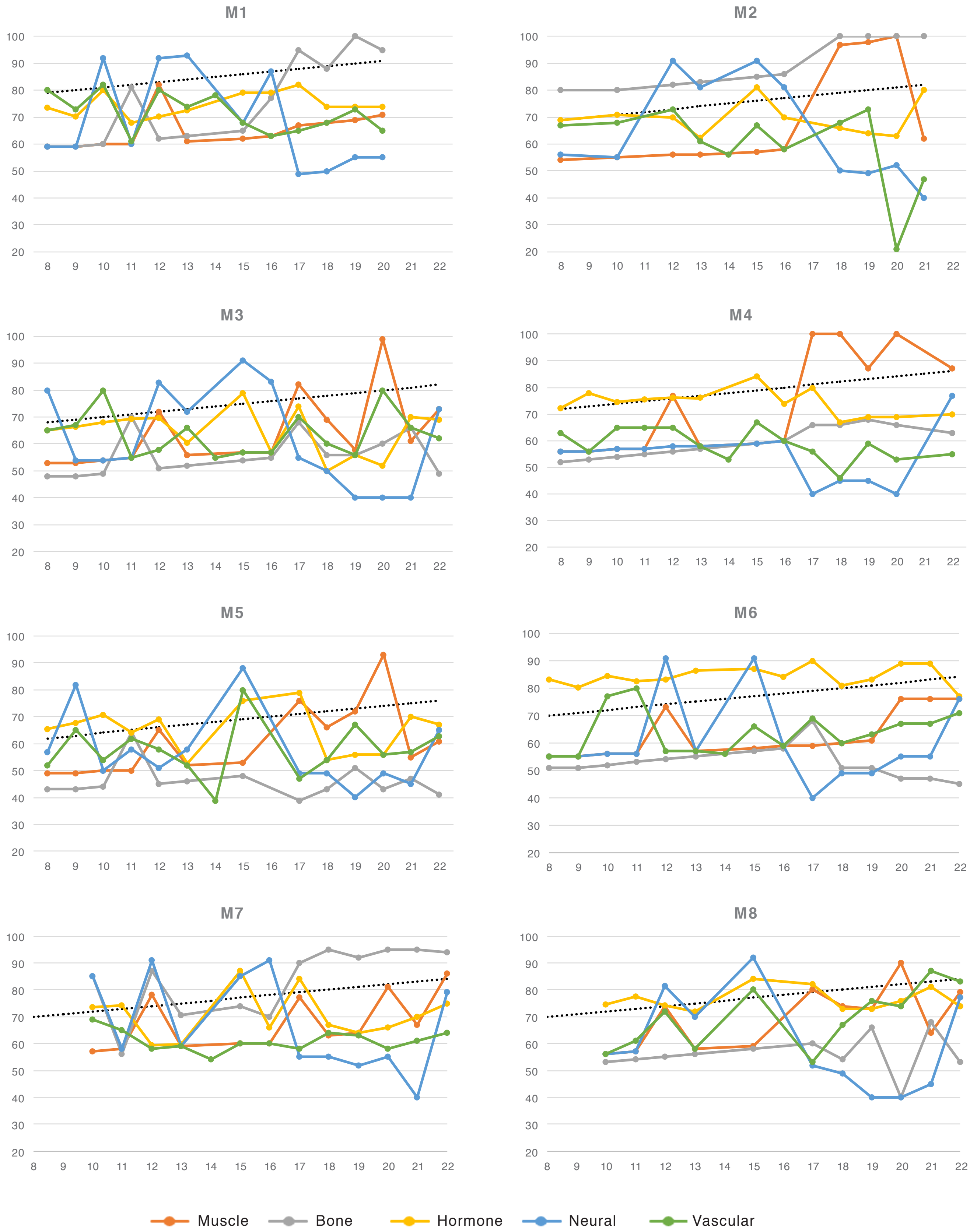

Fig. 10. **Long-term observation cases: male (n = 8).**

The vertical axis shows functional age (years), the horizontal axis shows the calendar year (*e.g.* 8 = 2008), and the dotted line shows chronological age (years).

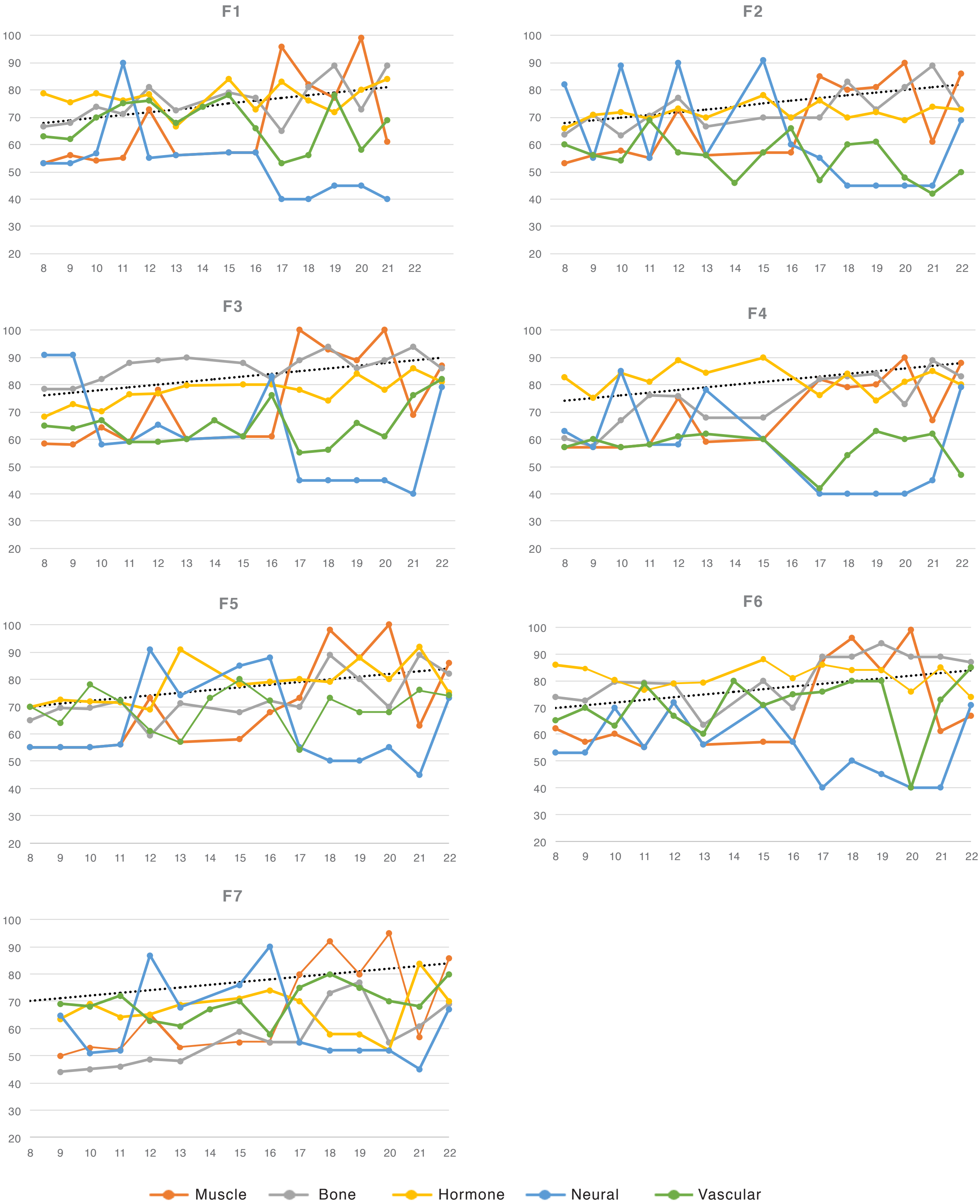

Fig. 11. **Long-term observation cases: female (n = 7).**
The vertical axis shows functional age (years), the horizontal axis shows the calendar year (*e.g.* 8 = 2008), and the dotted line shows chronological age (years).

Table 5. Survey on medical expenses in 2023.

Number of surveys or ID	Age		Medical expenses in 2022		Average expenses for Japanese		Ratio	
	mean [year]	SD	mean [Yen]	95%CI	mean [Yen]	95%CI	mean	95%CI
All survey participants								
22 (total)	84.0	6.6	683,396	208,856	968,550	86,516	74.4%	23.2%
9 (male)	86.4	5.5	534,487	229,673	1,086,991	108,840	56.4%	22.7%
13 (female)	82.4	7.0	809,395	316,177	868,331	102,122	96.5%	37.0%
Long-term observation cases								
12 (total)	85.7	4.3	619,257	281,668	1,042,808	70,202	59.1%	27.3%
7 (male)	85.0	5.1	620,701	270,702	1,098,043	88,525	55.5%	21.2%
5 (female)	86.6	3.3	617,236	608,054	965,480	78,902	64.2%	62.8%
M1	94		1,290,991		1,256,600		102.7%	
M2	84		310,373		1,031,500		30.1%	
M3	83		806,903		1,031,500		78.2%	
M4	87		219,413		1,157,200		19.0%	
M5	77		385,373		895,100		43.1%	
M6	85		651,307		1,157,200		56.3%	
M8	85		680,545		1,157,200		58.8%	
F2	83		225,600		837,400		26.9%	
F3	91		168,311		1,092,000		15.4%	
F4	89		1,814,469		966,000		187.8%	
F5	85		247,773		966,000		25.6%	
F6	85		630,027		966,000		65.2%	

The response rate of Kenpo Juku members was 61%. The ratio is expressed as a percentage of the average expenses of Japanese (adjusted by sex and age group in FY2022) provided by Ministry of Health, Labour and Welfare. SD, standard deviation; 95% CI, 95% confidence interval.

We have a hypothesis that "When weak points (the most advanced aged area in functional age) and the greatest risk factor are detected and corrected at an early stage, and aging homogeneously advances, a well-balanced, healthy lifespan is extended and a difference between average life expectancy and healthy life expectancy decrease". Our further study of multi-institutional joint research, employing anti-aging medical examinations and a health support software, AAD Life Work

https://www.dabhand.jp/customer/aad_web/, will verify the hypothesis, based on an accumulated big data analysis.

Conflict of interest

The authors claim no conflict of interest in this study.

Research funding

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (Kakenhi 23K10882).

Reference

1) Miyazaki R, Ishi K, Ichikawa H, et al. Community medicine and anti-aging: Effects of combining a long-term pedometer-based physical activity program with anti-aging medical checkups on health and anti-aging medical indicators in community-dwelling older adults (Yurin Study 1). *Anti-Aging Med*. 2010; 7: 143-152.
2) Miyazaki R, Kotani K, Tsuzaki K, et al. Effects of a year-long pedometer-based walking program on cardiovascular disease risk factors in active older people. *Asia Pac J Public Health*. 2015; 27: 155-163.
3) Yonei Y, Mizuno Y. The human dock of tomorrow: Annual health check-up for anti-aging. *Ningen Dock*. 2005; 19: 5-8.
4) Yonei Y, Takabe W, Yagi M. What does the Anti-Aging Medical Checkup show?: Data presentation. *HEP*. 2017; 44: 600-604.
5) Yonei Y, Takahashi Y, Hibino S, et al. The effects of walking with pedometers on quality of life and various symptoms and issues relating to aging. *Anti-Aging Med*. 2008; 5: 22-29.
6) Oguma Y, Iida K, Yonei Y, et al. Significance evaluation of Anti-Aging QOL Common Questionnaire. *Glycative Stress Res*. 2016; 3: 177-185.
7) Yonei Y, Miwa Y, Hibino S, et al. Japanese anthropometric reference data: Special emphasis on bioelectrical impedance analysis of muscle mass. *Anti-Aging Med*. 2008; 5: 63-72.
8) Kato G. Concept formation and transformation following frontal lobe damage: An investigation using the newly modified Wisconsin Card Sorting Test. *Journal of the Keio Medical Society* (*Keio Igaku*). 1988; 65: 861-885. (in Japanese)
9) Takazawa K, Kobayashi H, Shindo N, et al. Relationship between radial and central arterial pulse wave. *Hypertens Res*. 2007; 30: 219-228.
10) Nomoto K, Arita S, Yonei Y. Development of a model of functional endocrine age in Japanese people: Serum dehydroepiandrosterone-sulfate (DHEA-s) concentration as an index of aging. *Anti-Aging Med*. 2011; 8: 69-74.
11) Yanase T, Muta K, Nawata H. DHEA: Effects on oxidative and glycative stress and glucose metabolism. *Glycative Stress Res*. 2020; 7: 42-49.
12) Yonei Y. Rejuvenating hormone. Shueisha, Tokyo, 2023. (in Japanese)
13) Yonei Y. Diagnosis and treatment of aging. *Health Care*. 2022; 64: 585-590. (in Japanese)
14) Oh HS, Rutledge J, Nachun D, et al. Organ aging signatures in the plasma proteome track health and disease. *Nature*. 2023; 624: 164-172.
15) Mak JKL, McMurran CE, Hägg S. Clinical biomarker-based biological ageing and future risk of neurological disorders in the UK Biobank. *J Neurol Neurosurg Psychiatry*. 2023: jnnp-2023-331917.
16) Janovska J, Dalle C. Glycation and biologic clock: DNA methylation age. *Glycative Stress Res*. 2024; 11: 62-70.
17) Silcott NA, Bassett DR Jr, Thompson DL, et al. Evaluation of the Omron HJ-720ITC pedometer under free-living conditions. *Med SciSports Exerc*. 2011; 43: 1791-1797.
18) Higashi A, Ikeda J, Watanabe I, et al. Cross sectional study of the relationship between bone density to diet and life style using ultrasound bone densitometry. *Jpn J Public Health*. 1996; 43: 882-893. (in Japanese)
19) Yoshimi I, Ayagi K, Yahata Y, et al. Stiffness index of the calcaneus measured by quantitative ultrasound and menopause among Japanese women: The Hizen-Oshima Study. *Tohoku J Exp Med*. 2001; 195: 93-99.
20) Imashuku Y, Takada M, Murata K. Comparisons of bone mass measurements on various skeletal sites including quantitative ultrasonography of the calcaneus for assessing age-related losses, their correlations, and diagnostic agreement using the Japanese and WHO criteria for osteoporosis. *Radiat Med*. 2007; 25: 148-154.
21) Igarashi K, Oguni H, Osawa M, et al. Wisconsin card sorting test in children with temporal lobe epilepsy. *Brain Dev*. 2002; 24: 174-178.
22) Tsuchiya E, Oki J, Yahara N, et al. Computerized version of the Wisconsin card sorting test in children with high-functioning autistic disorder or attention-deficit/hyperactivity disorder. *Brain Dev*. 2005; 27: 233-236.
23) Matsuda N, Yuki T, Akiyama K, et al. Effects of tobacco smoking on frontal lobe function in normal healthy volunteer. *Dokkyo Journal of Medical Sciences*. 2005; 32: 81-83.
24) Nakaaki S, Murata Y, Sato J, et al. Reliability and validity of the Japanese version of the Frontal Assessment Battery in patients with the frontal variant of frontotemporal dementia. *Psychiatry Clin Neurosci*. 2007; 61: 78-83.
25) Kado Y, Sanada S, Yanagihara M, et al. Executive function in children with pervasive developmental disorder and attention-deficit/hyperactivity disorder assessed by the Keio version of the Wisconsin card sorting test. *Brain Dev*. 2012; 34: 354-359.
26) Wang Z, Jing J, Igarashi K, et al. Executive function predicts the visuospatial working memory in autism spectrum disorder and attention-deficit/hyperactivity disorder. *Autism Res*. 2018; 11: 1148-1156.
27) Kaga Y, Ohyama T, Goto Y, et al. Impairment of autonomic emotional response for executive function in children with ADHD: A multi-modal fNIRS and pupillometric study during the Wisconsin Card Sorting Test. *Brain Dev*. 2022; 44: 438-445.
28) Takada H, Okino K, Niwa Y. Anevaluation method for heart rate variability by using acceleration plethysmography. *Health Evaluation & Promotion* (*Sogokenshin*). 2004; 31: 547-551.
29) Ushiroyama T, Kajimoto Y, Sakuma K, et al. Assessment of chilly sensation in Japanese women with laser dopplerfluxmetry and acceleration plethysmogram with respect to peripheral circulation. *Bulletin of the Osaka Medical College*. 2005; 51: 76-84.
30) Meerwaldt R, Graaff R, Oomen PH, et al. Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia*. 2004; 47: 1324-1330.
31) Meerwaldt R, Hartog JW, Graaff R, et al. Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. *J Am Soc Nephrol*. 2005; 16: 3687-3693.
32) Roorda MM. Therapeutic interventions against accumulation of advanced glycation end products (AGEs). *Glycative Stress Res*. 2017; 4: 132-143.

33) Nomoto K, Yagi M, Arita S, et al. A survey of fluorescence derived from advanced glycation end products in the skin of Japanese: Differences with age and measurement location. *Anti-Aging Med*. 2012; 9: 119-124.
34) Hori M, Kishimoto S, Tezuka Y, et al. Double-blind study on effects of glucosyl ceramide in beet extract on skin elasticity and fibronectin production in human dermalfibroblasts. *Anti-Aging Med*. 2010; 7: 129-142.
35) Dyer DG, Dunn JA, Thorpe SR, et al. Accumulation of Maillard reaction products in skin collagen in diabetes and aging. *J Clin Invest*. 1993; 91: 2463-2469.
36) Ministry of Health, Labour and Welfare. Trends in medical expenses in FY2022: MEDIAS. (in Japanese) https://www.mhlw.go.jp/topics/medias/year/22/dl/iryouhi_data.pdf
37) Yabukita H, Miyazaki R, Nomoto K, et al. Characteristics of physical functions in elderly people requiring support. *Anti-Aging Med*. 2013; 10: 16-20.
38) Ishikawa M, Ishikawa S, Kamata H, et al. Efficacy of a health promotion program with facial mimetic muscle training in residents of a medical care facility for the elderly, *Anti-Aging Med*. 2010; 7: 120-128.
39) Yonei Y. Significance of anti-aging medical checkups for the elderly. *Japanese Journal of Geriatrics*. 2013; 50: 780-783. (in Japanese)
40) Kawamoto T, Takabe W, Ogura M, et al. Effect of continuous walking exercise program on the glycative stress marker in the elderly. *Glycative Stress Res*. 2017; 4: 144-157.
41) Nishizawa M, Ogura M, Takabe W, et al. Functional age and medication in the independent living elderly: Yurin study. *Glycative Stress Res*. 2017; 4: 240-249.
42) Meno M, Yokota S, Hashimoto M, et al. Plasma amyloid β 40/42 ratio and its characteristics. *Glycative Stress Res*. 2021; 8: 62-72.
43) Haasbroek K, Yagi M, Ando M, et al. Effects of mats with "A Distinctive 4-Layer 3-Dimensional Structure" on sleep quality and gut microbiota: A non-controlled open-label study. *Glycative Stress Res*. 2021; 8: 73-86.
44) Weuve J, Kang JH, Manson JE, et al. Physical activity, including walking, and cognitive function in older women. *JAMA*. 2004; 292: 1454-1461.
45) Abbott RD, White LR, Ross GW, et al. Walking and dementia in physically capable elderly men. *JAMA*. 2004; 292: 1447-1453.